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Advances of transgenic *Bt*-crops in insect pest management: An overview

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Abstract

The application of transgenic technology has resulted in useful GM insect-resistant varieties by genetic engineering (GE). Crops expressing *Cry* toxins derived from *Bacillus thuringiensis* (*Bt*) have been planted globally, and are a vital tool for pest control. The use of notable *Bt* crops, such as *Bt*-maize and *Bt*-cotton, have resulted in significant reductions of insecticide use and clear benefits on the environment and farmer health. Consequently, *Bt*-crops can be a useful component of integrated pest management (IPM) systems to protect the crop from targeted pests. Development, commercial use, benefits and risks associated with the adoption of GM insect-resistant crops and future of transgenic *Bt*-varieties to mediate crop protection against insect pests have been discussed in this review.

Keywords: Transgenic technology; *Bt*-genes; insect-resistance; *Bt*-crops; pest control

Introduction

The introduction of transgenic technology has added a new era to pest control and becoming vital component of integrated pest management worldwide [1]. In the past two decades, transgenic technology has been developed to generate insect-resistant crops for reducing both yield loss and pesticide utilization [2]. *Bacillus thuringiensis (Bt)* insect-resistant crops are one of the most outstanding achievements in plant transgenic technology, which have achieved significant success economically and ecologically. *Bt* is a potent insecticide containing crystal protein endotoxin produced by some strains of soil bacterium *B. thuringiensis*. The *Bt*-crystal (*Cry*) insecticidal protein (δ-endotoxin) genes are highly selective and represent a class of numerous proteins with insecticidal action on larvae from various orders: *Cry1* and *Cry2* are toxic for lepidopteran pests, *Cry2A* for lepidopterans and dipteran pests, and *Cry3* for coleopteran pests [3]. *Bt-cry* protein is toxic to insects, but non-toxic to humans and animals. The first generation of insect-resistant crops that were commercialized expressed single *Bt-Cry* genes, each produce a single *Bt*-toxin active against important lepidopteran pests and kill a narrow set of target pests, which poses a relatively high risk that insect will evolve resistance to the toxin. This narrow range of action and concerns about the evolution of pest resistance accelerated to the development of *Bt*-crops producing more than one toxin. In the second and third generations, scientists have mitigated this risk by stacking or pyramiding different genes such as multiple but different *Cry* genes and *Cry* genes combined with other insecticidal proteins, which target different receptors in insect pests but also provide resistance to a wider range of pests and delay evolution of resistance in pests [2, 4]. In comparison to single-toxin *Bt*-crops, multi-toxin *Bt*-crops can be more effectively control pests and reduce crop damage, which may increase environmental and economic benefits [5].

Bt-insect-resistant GM crops have been planted on a cumulative total area of over 560 million hectares worldwide since 1996 [6]. Of the 28 countries growing transgenic crops, 20 were developing and the remaining 8 were developed countries. A total of 17.3 million farmers grew transgenic crops in 2012; over 90% were small resource–poor farmers from developing countries [7].

The first *Bt*-crops developed each produce a single *Bt* toxin active against important lepidopteran pests and are still used in some countries. Each of the toxins in *Bt*-crops kills a narrow set of target pests [8, 9]. The first insect-resistant GM crops were tobacco produced in 1987 [10]. Furthermore, GM crop of japonica rice and indica rice was produced in 1988 and 1990 respectively.

Bt-crops not only provide an effective alternative tool for controlling target insects [11], but also provide many social, environmental, and economic benefits, such as reducing the use of chemical insecticides, benefiting the environment and human health, and increasing farm income [12, 13, 14, 15, 16, 17]. For example, the direct global farm income benefit from *Bt*-cotton was $ 2.9 billion in 2008. Within this, 65% of the farm income gain has derived from yield gains (less pest damage) and the balance (35%) from reduced expenditure on crop protection (spraying of insecticides) [13]. The advantages of future applications of transgenic technology could be even much bigger and could contribute significantly to global food security and poverty alleviation [18].

Nevertheless, as with any technology, there have been questions about the potential risks transgenic crops might have on the environment. One of the major ecological concerns regarding the environmental risks of insect-resistant GM crops is their potential effects on non-target organisms (NTOs) [19].

This review discusses application of transgenic technology in pest control by giving an emphasize on transgenic *Bt*-crops. Development, commercial use, benefits and risks associated with the adoption of GM insect-resistant crops and future of transgenic *Bt*-varieties to mediate crop protection against insect pests have been discussed in this review.

Development and commercialization of transgenic *Bt*-crops

Transgenic crops are produced through different transformation techniques including a modified *Ti* plasmid system in *Agrobacterium tumefaciens* and direct gene transfer including (polyethlyne glycol) PEG-induced DNA uptake, microinjection of DNA into cultured cells, electroporation and microprojectile bombardment. Efficient methods of gene cloning, transformation, plant regeneration, availability of new gene constructs, improved vector systems based on *Ti* and *Ri* plasmids of Agrobacterium, appropriate organ-specific promoters for gene expression, series of selectable marker genes and a large number of cloned [20]. By these methods; transgenic crops have been produced in several major crop plants such as cotton, maize, rice, etc.

Development of transgenic biotechnology has promoted the commercialization of GM crops to a great extent [21]. So far, *Bt*-maize and *Bt*-cotton are the only insect-resistant GM crops for commercial planting. *Bt*-genes (*Cry1Ac, Cry1Ab*, *Cry2Ab,* and *Cry1F)* of cotton were commercialized in 11 countries in 2009 [22], and the total planting area reached 15 million hectares, which comprised approximately half of all the cotton grown in the world in 2009 [23]. China and India are the two major cotton-growing countries. To delay the development of pest resistance, *Bt*-cotton varieties containing two different *Cry* proteins (Bollgard II and Wide Strike) have been gradually adopted by some countries in recent years. On the other hand, maize transformed with *Bt*-genes (*Cry1Ab, Cry1F, Cry3Bb1, VIP3A, Cry34Ab1/Cry35Ab, and Cry2Ab*) was commercially planted in 16 countries worldwide in 2009, and the total planting area reached 35.3 million hectares. In 2010, *Bt*-maize was grown on 39 million hectares, an increase of 3.0 million hectares, or a year-over year growth rate of 10% [24]. The USA and Canada are the only two countries to grow triple-stack maize with one gene for the European corn borer, a second for root worm, and a third for herbicide tolerance. It seems that the growth of biotech maize stacked with double and triple genes versus single genes is typical of the shift in all countries that deploy stacked genes in maize [25].

In 2009, China's Ministry of Agriculture issued its first two biosafety certificates for commercial production of two *Bt*-rice lines (*cry1Ab*/*Ac* Huahui No. 1 and *cry1Ab*/*Ac Bt* Shanyou 63) against leaffolders and yellow stem borers for Hubei Province. However insect-resistant GM rice in China has not yet fully commercialized up to now [26].

Crop Varieties Transformed with *Bt*-Genes

Many crops, such as cotton, maize, potato, tomato and rice, have been genetically transformed with genes derived from soil bacteria *Bt*-coding for proteins that are highly active against many important pests as shown in Table 1. The use of non-*Bt* insect-resistance traits with different modes of action, such as protease inhibitors or lectins, solely or in combination with *Bt*, has long been advocated as a means of delaying selection for resistant pest [3]. In the long term, new transgenic crops expressing novel *Cry* or other insecticidal proteins, stacked genes, or fusion proteins will increase in importance [25].

Table 1: *Bt*-transgenic plants expressing genes for insect resistance [3, 25] with slight modification

Plant	Gene	Resistance to
Tobacco	Magi6 peptide	*Spodoptera frugiperda*
Tobacco	*Cry1A*	*Helicoverpa zea*
Tomato	*Cry1A*	Pinworm
Potato	*Cry1Ab,* *Cry1Ac, Cry5*	Potato tuber moth
Potato	*Cry3A*	Colorado potato beetle
Cotton	*Cry1A*	Pink bollworm
Cotton	*Cry1Ac*	*Pectinophora gossippiella*
Maize	*Cry1A*	European corn borer
Rice	*Cry1Ab*	Lepidopteron
Rice (Indica, Minghui 63)	*Cry2A*	Yellow Stem Borers
Rice (Indica, Minghui 63)	*Cry1Ac,* *Cry2A, Cry9c*	Yellow Stem Borers and Asiatic Stem Borer

Contribution of transgenic crops

Economic benefits

Transgenic crops have contributed to economic gains at the farm level of about US$ 98.2 billion during the 16-year period (1996-2011), of which 51% were due to reduced production costs (less ploughing, less labour and fewer pesticide sprays), and 49% due to substantial yield gains of 328 million tons. Thus, in addition to higher yield, the benefits to farmers of transgenic crops include the lower input costs in terms of pesticide use, and ease of crop management [7].

Pest reduction

Another benefit of the deployment of *Bt*-crops has been the reduction of pest insect populations on a regional scale. In China it has been found that the area-wide suppression of cotton bollworm associated with *Bt*-cotton, also with benefits to non-*Bt* crops [11]. Human health benefits have also been documented for *Bt*-crops [27].

Biodiversity conservation

Transgenic crops have helped conserving biodiversity by saving 108.7 million ha of land, which would probably have been required to produce 328 million tons of additional food, feed and fiber produced by these crops during the period 1996 to 2011. The reduction in pesticide use has resulted in increased biodiversity within *Bt*-fields compared with non-*Bt* fields treated with conventional insecticides [28]. One benefit of higher biodiversity may be the improved natural control of other pests, which has been reported in the case of aphids in cotton in China. The use of insecticides in non-*Bt* cotton fields reduces the populations of aphid predators, while allowing those predators to survive in *Bt*-fields; therefore, improved control of aphids by natural predators is seen in *Bt*-fields [29].

Pesticides reduction

The accumulative reduction in pesticides for the period 1996 to 2011 was estimated at 473 million kg of active ingredient, a saving of 8.9% in pesticides. In 2011 alone, there was a reduction of 37 million kg, equivalent to a saving of 8.5% in pesticides. The reduction in pesticide usage would lead to reduced exposure of farm labor to pesticides, reduction in harmful effects of pesticides on non-target organisms, and reduced amounts of pesticide residues in food and food products. The additional benefits to farmers would be to control insect pests which have become resistant to commonly used pesticides, and reduction in crop protection costs [7].

Poverty alleviation

Transgenic cotton alone has helped to alleviate poverty by making significant contribution to the income of about 16 million small resource-poor farmers in 2012. This can be enhanced substantially in the remaining years of the second decade of commercialization, principally with transgenic cotton, maize and rice [30].

These factors are likely to have substantial impact on the livelihood of farmers in both developed and developing countries. In many developing countries, small-scale farmers suffer pest-related yield losses because of technical and economic constraints. Insect-resistant GM crops can contribute to increased yields and agricultural growth in such situations. The advantages of future applications could be even much bigger. Transgenic crops can contribute significantly to global food security and poverty alleviation [18].

Ecological aspects of *Bt*-crops

The most serious threat to the continued efficacy of *Bt*-crops is the evolution of resistance to *Bt* in target pests. A recent analysis of 24 cases, with each case involving responses of one pest species in one country to a single *Bt*-toxin, shows that the practical impacts of field-evolved resistance can vary from none to severe, depending on the magnitude, frequency and spatial distribution of resistance [31]. Despite the widespread adoption of *Bt*-crops, there are still a range of unanswered questions concerning longer term agro-ecosystem interactions. For instance, insect species that are not susceptible to the expressed toxin can develop into secondary pests and cause significant damage to the crop [32]. Secondary pests which before were of minor importance, might now find favourable conditions and become major pests [33]. Two main drivers may trigger an outbreak of secondary pest species with the use of *Bt*-crops are: (i) a reduction in natural enemy populations; or (ii) a decrease in interspecific competition with the target pest.

Reduction of natural enemies

Natural enemies include predators, parasitoids and pathogens. A major concern related to the growing of *Bt*-crops is their potential impact on the abundance of natural enemies. The impact of *Bt*-toxins on natural enemies can occur through direct and/or indirect effects [34]. Direct impacts might occur due to the ingestion of the insecticidal. *Bt*-proteins were passed from the *Cry3Bb Bt*-maize plant to the predator (*Harmonia axyridis*, a common coccinellid) via prey consumption (*Rhopalosiphum maidis*, the corn leaf aphid and *Rhopalosiphum padi*, the bird cherry-oat aphid), which significantly reduced their life span [35]. Furthermore, although not yet demonstrated in the context of *Bt*-crops, there is also concern regarding toxin bioaccumulation through the food chain, possibly driving cascade effects within the ecosystem [36]. Indirect effects might manifest through reductions in prey/host populations or in the nutritional quality of the prey. Impacts of the toxin on herbivores may manifest at a sub-lethal level which can affect life parameters such as lifespan and fecundity [37]. There is evidence that the low nutritional quality of prey items after they have ingested *Bt*-proteins has a significant impact on the performance, development and even survival of natural enemies [38, 35]. Moreover, high mortality rates in the target species may cause a reduction in specialist natural enemies, which themselves can be important prey for generalist predators [35]. Additionally, prey species in general might migrate to non-*Bt* fields in search of preferable food resources [39].

Species replacement

It is possible that when a primary pest is successfully controlled by a *Bt*-toxin, a non-susceptible species starts to utilize the newly available ecological resource [40]. A good example of species replacement is the western bean cutworm (WBC) [*Striacosta albicosta* (Smith)] a noctuid moth native to West and Central America. In the mid-1990s, the WBC began an expansion of range size that correlated with the introduction of transgenic maize. It has now effectively established itself as a major Lepidopteran pest of maize crops in some areas of the Corn Belt in the US and Canada [41]. This secondary pest shows low susceptibility to most transgenic maize currently commercialized [42]. Transgenic crops expressing *Cry1Ab* and *Cry9C* toxins have larger populations of WBC compared to conventional maize [41]. It is possible that changes in cultural practices (e.g. conservation tillage and reduced insecticide use) due to the widespread adoption of *Bt*-maize across these areas might have contributed to the WBC's rapid expansion [43]. In *Bt*-cotton in the USA, stink bug pests, specifically *Nezara viridula* L. and *Euschistus servus* S., have recently become a severe problem in the absence of the target pests *H. zea* and *Heliothis virescens* [44]. As *Bt* growing expands worldwide, it is of critical importance to examine the key species–susceptible and non-susceptible pests– which might compete for resources within the same transgenic crop.

Impact of secondary pests on Bt-cotton as an example

From the worldwide 24.3 million hectares cropped with *Bt*-cotton, India, China and USA account for 11.0, 4.2 and 4.1 million hectares, respectively, with the adoption rate varying between 90% and 95%. The *Bt*-cotton hectarage in Africa is increasing, for instance Burkina Faso and Sudan cropped 50% and 300% more *Bt*-cotton, respectively, compared with 2012 [6]. In China, in some areas where the bollworm incidence is higher, the adoption is close to 100% [45]. The drop in insecticide use and the ineffectiveness of *Bt*-cotton against these secondary pests has led to a reversal of the ecological role of cotton [46, 47]. Conventional cotton had been a population sink for the mirid bug secondary pest, while nowadays *Bt*-cotton fields are a source of these pests [33]. In the USA, for example, in the mid southern and southeastern cotton-producing regions, there has been a significant increase in the number of insects considered as secondary cotton pests, such as aphids, leafhoppers, mirid plant bugs and stinkbugs [23].

Future of transgenic technology in insect control

Transgenic crops have clearly increased profitability for farmers in developed and developing countries. The introduction of foreign genes conferring insect resistance to crop plants has been a major success in terms of levels of protection afforded by expression of *Bt*-toxins. The first generation of *Bt*-crops has been extraordinarily successful, with a few examples of pest populations evolving resistance. These crops are already being supplanted with second-generation varieties with more resilient traits generated by stacking and pyramiding resistance genes. Even so, this is not the time to be complacent and the search for more efficacious and potent strains must continue [2]. The next generation of insect-resistant GM plants has designed to delay/prevent the onset of resistance and thus provide more durable levels of crop protection. The goal of achieving multi-mechanistic resistance in crops is increasingly achievable with agricultural biotechnology. New *Bt*-strains are reported on a regular basis, especially now proteomics methods can be used to screen for novel toxins on a large scale. Field evaluation of transgenic crops containing insecticidal genes is vital as a component of the overall process of creating and deploying insect resistant transgenic plants that are useful and sustainable.

Conclusion

Transgenic crops are an additional tool to supplement conventional pest resistance programs. It is important to integrate transgenic technology into the on-going insect pest management (IPM) programs. Long term impact of transgenic crops on development of resistance to insects, environmental and public concerns and biosafety issues should be carefully examined before release of transgenic crops for commercial planting. The ideal transgenic technology should be commercially feasible, environmentally benign (biodegradable), and easy to use in diverse agro-ecosystems as well as show a wide-spectrum of activity against the crop pests. It should also be harmless to the natural enemies, target the sites in insects that have developed resistance to the conventional pesticides, flexible enough to allow ready deployment of alternatives (if and when the resistance is developed by the pest), and preferably produce acute rather than chronic effects on the target insects.

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