African Journal of Biotechnology Vol. 10(23), pp. 4677-4681, 1 June, 2011
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 ©2011 Academic Journals

Review

The trends and future of biotechnology crops for insect pest control

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Accepted 13 January, 2011

Biotech crops, including those that are genetically modified (GM) with *Bacillus thuringiensis* (Bt) endotoxins for insect resistance, have been cultivated commercially and adopted in steadily increasing numbers of countries over the past 14 years. This review discusses the current status of insect resistant transgenic crops and the often raised concern that its resilience is limited and that its efficacy will be compromised by insect resistance. We consider this trait as it is currently deployed in fields across the world as well as potential candidates that are at various stages of development along the pathway between the laboratory and deregulation. Future trends and prospects for biotechnological applications to mediate crop protection against insects are also considered. These include strategies employing stacked genes, modified Bt toxins, vegetative insecticidal proteins, lectins, endogenous resistance mechanisms as well as novel approaches. In addition, the benefits and risks associated with the adoption of GM insect resistant crops, especially for developing countries and resource-poor smallholder farmers are also discussed.

Key words: *Bacillus thuringiensis* (Bt), endotoxins, Cry proteins, transgenic crops, insect resistance.

INTRODUCTION

In addition to genetically modified (GM) crops, biotech crops include all crops developed through modern biotechnology, also using mutagenesis (Patade and Suprasanna, 2008) and marker assisted breeding (Varshney et al., 2005). However, for the purposes of this review, biotech crops refer to GM crops.

There is a need to increase food production considerably in the foreseeable future to meet the food and feed demands of the world, requiring higher production, particularly in developing countries in Asia, Africa and Latin America. This demand has to be met primarily through yield increases on existing cultivated lands in order to be environmentally sustainable and cost effective (Edwards and Gatehouse, 2007; James, 2009). One way to increase yields is to minimize losses due to pests, which destroy on average 14 to 25% of the total global agricultural production. These losses are most significant in food crops since crop protection is less efficient in food crops than cash crops (Oerke and Dehne, 2004). In 2003, losses were estimated to be 37% for rice, 40% for potatoes, 31% for maize, 26% for soybean and 28% for wheat (Oerke, 2006; Oerke and Dehne, 2004). The costs of pesticides, estimated at more than US $10 billion per annum, need to be added to these figures, and the fact that pesticides often affect non-target organisms and leave harmful residues should also be considered (Sharma et al., 2000).

GLOBAL STATUS OF BIOTECH CROPS

Biotech crops have been grown commercially since 1996. In 2009, global production reached 134 million (M) ha in 25 countries (James, 2009). The nine industrialized coun-

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Abbreviations: GM, Genetically modified; **Bt,** *Bacillus thuringiensis*; **Cry,** crystal; **Cyt,** cytolitic; **Vips,** vegetative insecticidal proteins.

tries contributing to this figure, still cultivated a larger area of GM crops than the 16 developing countries, but the gap was closing as more developing country farmers experienced the benefits of planting biotech crops first hand, thereby enabling this to become the fastest adoption of any crop technology in recent years, with a growth rate of approximately 8% per annum (James, 2009). These high adoption levels are due to the economic and environmental benefits experienced by farmers in both industrial and developing countries.

James (2009) further reported that by 2009, 725 approvals for commercial cultivation had been granted for 155 events in 24 crops. Of the approximately 14 M farmers that cultivated biotech crops last year, more than 90% were small-scale and resource poor farmers, most of them growing *Bacillus thringiensis* (Bt) cotton, followed by Bt maize.

ADOPTION OF Bt CROPS IN DEVELOPING COUNTRIES

In India, Bt cotton was first planted in 2002 by 54,000 farmers on 50,000 ha (James, 2003). By 2009, 5.6 M small- and resource-poor farmers were cultivating it on 8.4 M ha of which 90% of the farmers had replanted the crop and this represents 87% of all the cotton planted in the country (James, 2003). A similar picture emerged from China where 7.1 M farmers grew Bt cotton on 3.8 M ha; this constituted 69% of all planted cotton in the country in 2008 (James, 2009). On average, farmers gained 10% higher yield, applied 60% fewer sprays and earned $220 p/ha more. Argentina and Brazil grew mostly herbicide resistant soybean, however, in Argentina Bt maize and cotton were planted on 2.8 M ha (worth $482M) and 0.4 M ha (earning $19.7M), respectively, and in Brazil, Bt cotton was planted on 0.5 M ha. This was done mainly on large farms.

In South Africa, both Bt cotton (more than 85% of the country's crop) and Bt maize are grown. This is the only country to date where white Bt maize, 0.9 M ha representing 67% of the country's total production, was planted for food (James, 2007). By the end of 2009, China also approved Bt rice and GM phytase maize for commercial cultivation (James, 2009). These crops were developed by public institutions and their cultivation should have substantial impacts within China in the coming years, both with regard to human and animal nutrition as well as policy and decision-making processes related to GM crops.

BENEFITS FROM Bt CROPS

To date, a large collection of more than 200 Bt proteins showing differing levels of toxicity to selected insects have been identified in various strains of the bacterium *B. thringiensis* (Bravo et al., 2007; Gatehouse, 2008). Bt proteins have been used as a safe but expensive biopesticide for over 40 years. They are non-toxic to vertebrates unlike synthetic pesticides, and are very specific to particular insect pests. This is also the case with Bt transgenic crops. In contrast to Bt technologies, synthetic pesticides often kill non-target pests and their predators, in addition to the target pest. Bt crops are also particularly suitable for small-scale farmers since no equipment and pesticide knowledge are needed for cultivation and these crops reduce exposure of farmers to insecticides, especially for those using hand sprayers (Heldt, 2006; Qaim and Janvry, 2005). In this context, the cultivation of Bt maize has reduced yield losses due to root worms and stem borers substantially without resorting to the more toxic organophosphate insecticides. For maize, it is estimated that Bt varieties can substitute 40–50% of the insecticides currently in use (Heldt, 2006). For cotton, conventional varieties require 2–30 sprays per season, which are drastically reduced with Bt varieties. This benefits both the environment and labourers' health, especially in developing countries where pesticides are mainly applied with knapsack sprayers, like in China (Huang and Wang, 2002).

Another benefit of Bt maize is that it accumulates less mycotoxins from opportunistic fungi that infect damaged kernels (Munkvold et al., 1999). Healthier cobs without insect damage are less likely to be infected by fungi, which produce mycotoxins that are harmful, and often lethal, to humans and livestock (Miller et al., 2006). Bt crops also increase incomes through higher yields of healthier grain which is emphasized by the continued increasing adoption. This holds true for both small holder farms and large farms. In addition, there has been no documented proof of any negative impact on non-target insects in Bt fields (Christou et al., 2006).

INSECT RESISTANCE TO Bt TOXINS

The widespread adoption of Bt crops increases the risk that the target insects will develop resistance (Bates et al., 2005). This technology has already exceeded the predicted time span that typically passes in the field before resistance to most conventional neurotoxic pesticides emerges. This is the case, despite what has been hailed as one of the world's largest pressures for selection for resistance (Bates et al., 2005; Tabashnik et al., 2008). This lack of observed resistance is ascribed to (i) The fitness cost to resistant individuals, especially those obtained in laboratory studies, which are not able to survive in the field; (ii) the low frequency of resistance alleles; (iii) dilution of resistant alleles with susceptible alleles through mating with insects that have fed on non-Bt crops or that have not developed resistance yet and (iv) the high toxin dose that is delivered by commercialized Bt crops.

The risk of insect resistance is further mitigated with the refuge strategy on large commercial farms, or by the abundance of non-Bt host plants in small-holder agriculture in developing countries where intercropping with other crops that are also hosts to the target pest is common (Bates et al., 2005; Christou et al., 2006; Gressel, 2005). Expression of multiple Cry genes in transgenic crops is a more recent strategy to combat resistance. Examples are the expression of Cry1Ac and Cry2Ab in cotton, which confers simultaneous resistance to *Helicoverpa zea, Spodoptera frugiperda* and *Spodoptera exigua*. These genes each target different receptors in the pests and therefore require multiple mutations for resistance to develop (Zhao et al., 2003).

Since 2005, however, indications of field resistance have been observed and documented. Fall armyworm, *S. frugiperda*, showed resistance to Cry1F in maize in Puerto Rico and was voluntarily discontinued (Matten et al., 2008). There is an ongoing controversy whether the cotton bollworm, *H. zea* is becoming resistant in the USA against Cry1Ac in cotton and maize (Moar et al., 2008; Tabashnik et al., 2008). Such resistance has been documented in isolated cases and indicates that widespread resistance to crops expressing only Cry1Ac may not be far away (Tabashnik et al., 2008). In another study, resistance of *Heliothis virescens* to multiple Cry proteins was demonstrated in the laboratory (Wierenga et al., 1996). However, laboratory results cannot be extrapolated directly to the field. Even though it may be possible to select resistant populations in the laboratory, similar resistance have not been observed in the field, probably because the environmental conditions affecting the fitness of the insects cannot be mimicked in the laboratory (Christou et al., 2006; Tabashnik et al., 2009; Wierenga et al., 1996).

CURRENT STATUS OF Bt TECHNOLOGY FOR INSECT RESISTANCE

*B. thuringiensis (*Bt *)* is a soil bacterium that produces a diverse group of insecticidal protein toxins with narrow specificity towards different insects. These toxins, called Crystal (Cry) and Cytolitic (Cyt) proteins are accumulated in crystalline inclusion bodies in the bacteria. Another class of toxins from these bacteria is expressed during bacterial growth and is known as vegetative insecticidal proteins or Vips (Gatehouse, 2008; Schnepf et al., 1998).

Cry proteins are pro-toxins that are activated by host proteases in the insect gut. They have been extensively studied and consist of three domains (Bravo et al., 2007). Of these, domains II and III determine the insect specificity and interact with specific receptors located on the insect mid-gut surface that leads to oligomerization of the toxin molecules into a pre-pore structure that can insert into the host membrane. Domain I then facilitates insertion into the target membrane to form a trans-membrane pore. Once the pore has formed, ionic leakage destroys the cells and kills the insect (Bravo et al., 2007). Generally, the toxin needs to be expressed at concentrations of more than 0.2% of total soluble protein in the appropriate tissue in a transgenic plant to be effective (Gatehouse, 2008).

The first generation of insect resistant crops that were commercialized expressed single Bt Cry genes, which poses a relatively high risk that insects will evolve resistance to the toxin. In the second and third generations, scientists have mitigated this risk through 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 (Christou et al., 2006; Gatehouse, 2008). Alternatively, synthetic variants of Cry genes has been employed as in the case of MON863 which expresses a synthetic Bt *kumamotoensis* Cry3Bb1 gene against corn rootworm, which is eight times more effective than the native, non-modified version (Vaughn et al., 2005). Therefore multiple mutations/adaptations need to be made by target pests in order to develop resistance to this robust new generation of insect resistant crops.

FUTURE OF GM PEST CONTROL

Constitutive expression of Bt genes has been very successful, but in some cases tissue specific expression is a better option, for example in epidermal cells, which first come under attack from insects or in the phloem for sap sucking insects. It has been reported that expression can be regulated using transcription factors or chemical induction and with this technique, it is possible to create within plant refuges where parts of the plants do not express the genes and act as non-GM refuge (Christou et al., 2006). Plastid expression, such as in chloroplasts, is also an important target for future Bt crops (Bock, 2007). Higher levels of toxin, up to 3–5% of total leaf protein, are accumulated in chloroplasts since the plastid genome is bacterial in origin as are Bt genes (McBride et al., 1995). Since cytoplasmic plastids are predominantly maternally inherited, it will reduce the chances for gene flow through pollen.

Improved insect resistance has also been achieved through the employment of multiple resistance genes in a single plant, also known as gene stacking or gene pyramiding. A number of these products are already under commercial cultivation. A whole new generation of such crops is under development aimed at preventing or slowing down development of resistance. These include the expression of multiple Cry genes targeting one pest (Christou et al., 2006; Gatehouse, 2008). The development of hybrid Cry proteins through domain swapping to enhance both toxicity and host range is another development discussed by Gatehouse (2008). Cry genes are also combined with plant lectins to target several pests, for example the snowdrop (*Galanthus nivalis*) lectin, fused to the Cry gene, delivers proteins to the haemolymph of lepidopteran larvae. Other examples are of the SFII spider neurotoxin fusion protein that is lethal to lepidopteran larvae and garlic leaf lectin against peach potato aphid (Christou et al., 2006). Fusion proteins of Cry1A with the galactose-binding domain of the ricin B-chain increase the number of binding domains for Cry1A (Mehlo et al., 2005). Recently, transgenic maize was obtained with six insect resistance genes against corn rootworm and lepidopteran pests and two herbicide tolerance genes which can provide a “one stop solution” to pest and weed problems through gene stacking (Grainnet, 2007).

Employment of novel Bt insecticidal proteins other than the three-domain Cry proteins are also being developed such as binary toxins of Cry34/35 and Vip1/2 toxins against corn rootworm (*Diabrotica virgifera)* as an alternative to the synthetic Cry3Bb1 discussed previously. In addition, single chain vegetative insecticidal proteins such as Vip3 have been found to have a broader range of toxicity and can be further improved with protein engineering (Gatehouse, 2008).

PROTEIN ENGINEERING IN Bt TOXINS

The structural similarity of all three-domain Cry proteins led to the idea of protein engineering to exchange domains amongst these proteins (Naimov et al., 2003). Combining domains from different proteins will create new cry proteins with novel specificity and a new opportunity to manage insect resistance. Site-directed mutagenesis in Cry toxins instead of “domain swapping” is also being attempted since it was found that such mutations in the loop regions of domain II increase the toxicity of Cry3A to gypsy moth 40 fold and that of Cry3Bb against rootworm 8 fold (Wu et al., 2000). Another approach, that of removing the α-1 helix of domain I, resulted in a protein that did not require binding to cadherin to oligomerize and was toxic to resistant insects (Gatehouse, 2008).

NON-Bt APPROACHES

Resistance to pests can also be achieved by exploiting plant defense mechanisms such as proteinase inhibitors, especially for storage pests. There are, however, concerns about the effect of mammals and humans ingesting relatively large amounts of these proteins such as α-amylase (Gatehouse, 2008). Another defense mechanism that can be harnessed is secondary metabolism compounds such as the cyanogenic glycoside dhurrin from sorghum which is induced by tissue damage or volatile communication compounds that deter insect colonization or attract natural enemies of insect pests (Gatehouse, 2008).

Another novel approach is exploiting the large number of potential insecticidal proteins produced by *Photorhabdus luminescens*, a nematode symbiotic bacterium. One of these proteins, Toxin A (when expressed at only 0.07% of total protein) is effective against tobacco hornworm and corn rootworm (French-Constant et al., 2007).

PROSPECTS

There is no argument that transgenic insect resistant technologies have been and still are a major scientific success. However, accessibility of these products is relatively restricted, especially in developing countries, due to vocal opposition to GM technology and lack of regulatory mechanisms within which to deploy them (Paarlberg, 2008a; Paarlberg, 2002). Often, the potential economic returns of a new product are insufficient for commercialization and the high cost of commercialization makes it difficult even for public institutions to develop products for farmers in the developing world. That is one of the reasons that the current available GM crops are mostly limited to products of large commercial companies. A recent exception, which holds great potential for the future deployment of publicly developed GM crops is the approval of Bt rice and phytase maize in China (James, 2009). However, this progress is offset by the recent restraint to commercial release of Bt brinjal in India due to public concern.

Especially in Africa, regulatory systems are still poorly developed compared to that of developing countries in Asia, which becomes particularly apparent in view of the global adoption of GMO crops (James, 2009; Paarlberg, 2008b). Outside of South Africa, only Burkina Faso and Egypt allow commercial cultivation of GM crops. Can this continent afford to miss out any longer on the benefits of these crops due to regulatory constraints?

CONCLUSION

Bt technologies continue to be effective and relevant, even 14 years after the deployment of the first GM crops, and novel ways are constantly being employed to ensure that this technology remains effective. It provides economic and environmental benefits, both proven and potential. However, in the developing world, a change in attitude by governments, non-governmental organizations and the public at large is needed for insect-resistant transgenic crops to be able to benefit all the world's population, not just a few.

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