African Journal of Biotechnology Vol. 9(14), pp. 2022-2031, 5 April, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

*Review*

# *Bacillus thuringiensis* and its application in agriculture

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Accepted 25 February, 2010

**Presently, a number of approaches to pest control via genetic engineering have been developed and genetically engineered crops expressing insecticidal characteristics are under cultivation for the last 15 years. Use of *Bacillus thuringiensis* genes encoding δ endotoxins with insecticidal characteristics is the major approach and a number of such *B. thuringiensis* genes have been expressed in crops with variable level of efficiency. It is very crucial to achieve adequate level of *B. thuringiensis* gene expression to have durable resistance against target insect pests. As with many aspects of genetic engineering, politics can impact on the success of a project involving the development of *B. thuringiensis* transgenic crops, irrespective of its apparent social, economic or environmental benefits. Public education will be essential to ensure the widespread adoption of genetic adoption technologies in agriculture, and scientists will have to play an active role in this process.**

**Key words:** *Bacillus thuringiensis*, endotoxins, crop plants.

## INTRODUCTION

Insects constitute major part of plant pests. Hill (1987) listed ten orders of insects based on their damage to crop. The most important insects are *Lepidoptera*, *Coleoptera*, *Homoptera*, *Diptera* and *Orthoptera*. Ranking first, *Lepidoptera*, normally at its larval stage are responsible for serious crop damage. Most of the *Bacillus thuringiensis* toxins can be extremely toxic to *Lepidoptera* larvae; they are becoming more and more attractive in genetic engineering for plant insect resistance. *B. thuringiensis*, commonly known as *Bt*, is a bacterium that occurs naturally in soil. It has been used as a biological pesticide for more than 50 years (Musser and Shelton, 2003; Carriere et al., 2003; Quid and Zilberman, 2003). It is a gram positive bacterium, discovered in 1901 by Ishiwata from diseased silkworm (*Bombyx mori)* larvae, which produces proteinaceous crystalline inclusion bodies upon sporulation. Berliner (1915) isolated it from diseased larvae of *Ephetia kuhniella* and designated it as *B. thuringiensis*. Further research on *B. thuringiensis* by Steinhaus (1951) led to renewed interest in biopesticides, and as a result, more potent products such as Thuricide® and Dipel® were introduced. There are several subspecies of this bacterium, which are effective against *Lepidopteran*, *Coleopteran* and *Dipteran* insects. Formulations based on *B. thuringiensis* occupy the key position, accounting for nearly 90% of the total biopesticides (Neale, 1997). It has been used in the field for the past 50 years.

The insecticidal proteins produced in the crystal form constitute two different families, *Cry* and *Cyt*, which have been further classified on the basis of amino acid identity into about 300 *Cry* and 22 *Cyt* sub-groups to date *(http://epunix.biolos.susx.ac.uk/home/Neil_Crickmore/B. thuringiensis/toxins.html)*.

*B. thuringeinsis* var. *morrisoni* and *Bacillus israelensis* carry four genes that encode mosquito and black fly toxins, *Cry IVA*, *Cry IVB*, *Cry IVC* and *Cry IVD* (Bechtel and Bulla, 1976). The identification of kurstaki strain provided a boost for the commercialization of *B. thuringiensis.* The HD1 strain identified by Dulmage (1981) is the most important *B. thuringiensis* product in the market. The problems associated with shelf life, potency and the presence of viable spores have been overcome using modern tools in microbiology and genetic engineering. Genes encoding for δ- endotoxins have been cloned since the 1980s (Schneph and Whitley, 1981) and the expression of the first introduced genes in tobacco and tomato provided the first examples of genetically modified plants with resistance to insects (Barton et al., 1987, Vaeck et al., 1987).

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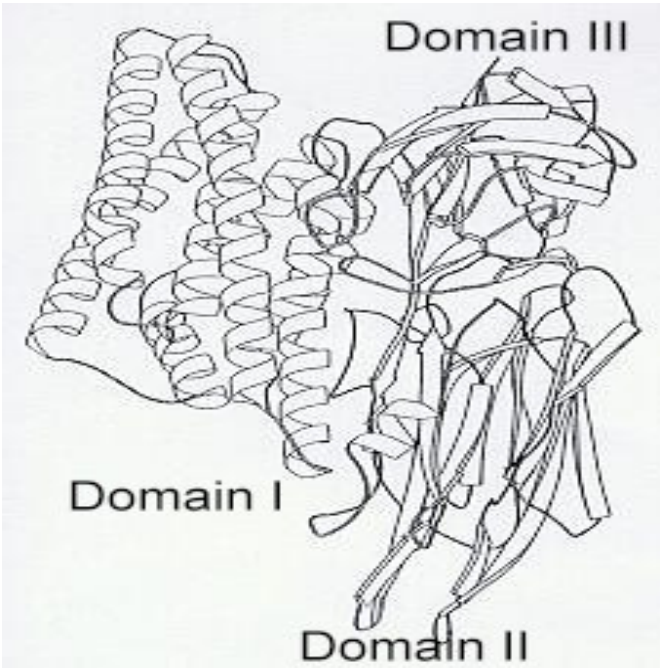


**Figure 1.** Different domains involved in the toxicity of *B. thuringiensis* toxin in the mid-gut of targeted insect. Source: Sharma et al., 2000.

## GENETIC MANIPULATION OF *B. THURINGIENSIS*

The genetic manipulation of *Cry* genes in *B. thuringiensis* offers promising means of improving the efficacy and cost effectiveness of *B. thuringiensis* based biopesticide products. Certain combinations of *Cry* proteins have been proposed to exhibit synergistic toxicity towards *Lepidopteran* and *Dipteran* pests (Roush, 1997). In addition, the presence of spores can also synergize the activity of Cry proteins against *Lepidopteran* pests, and may forestall the development of insect resistance to *Cry* proteins (Liu et al., 1998). Other factors may contribute to the entomopathogenic character of *B. thuringiensis* including the vegetative insecticidal proteins (*VIPs*), $\alpha$-endotoxins and a variety of secondary metabolites, including Zwittermycin may be amenable to genetic manipulation (Glare and Callaghan, 2000; National Academy of Sciences, 2000; Aronson and Shai, 2001; National Agricultural Biotechnology Council, 2001; Shelton et al., 2002).

## MODE OF ACTION

The crystalline proteins get solubilised in the mid-gut at high pH, releasing proteins called $\delta$- endotoxins. The toxin portioned is derived from the N-terminal half of the protoxin, while the C-terminal portion is involved in the formation of parasporal inclusion bodies and is usually hydrolysed into small peptides (Choma et al., 1990).

The main target for *B. thuringiensis* toxin is insect mid-gut (Knowles, 1994). The crystalline protoxins are inactive, until they are solubilised by the gut ptoteaes (Milne and Kaplan, 1993), which cleave nearly 500 aminoacids from the C-terminus of 130 kDa protoxin and 28 aminoacids from the N-terminus, leaving 55 - 65 kDa protease resistant active core comprising the N-terminal half of the protoxin (Hoftey and Whitley, 1989). The toxic protein fragment can be divided into three domains (Figure 1). Domain I is involved in pore formation, domain II determines the receptor binding and domain III serves in the protection of toxin from proteases. The 70 kDa *Cry II*, *Cry III* and *Cry IVD* proteins are naturally occurring truncated forms.

The mechanism of *B. thuringiensis* toxicity has been reviewed in details by Knowles (1994). The crystal proteins are specifically toxic to insect pest depending on their classification group (Olsen and Daly, 2000; Stewart et al., 2001; Adamczyk et al., 2001a, b; Gore et al., 2001, 2002). Essentially, the active toxin first binds to glyco-protein receptors in the brush border membrane of susceptible insect's mid-gut epithelium. These receptors, in fact, play a key role in determining susceptibility/ resistance to a particular *B. thuringiensis* toxin and their nature is under intense investigation (Alcantara et al., 2004), with a number of mid-gut integral membrane glyco-proteins, including amino-peptidase and a cadheirn-like protein identified (Yaoi et al., 1997). Following binding, the toxin rapidly and irreversibly inserts into the cell membrane. Insertion results in the formation of pores

which leads to epithelial cell lysis as a result of most probably, selective cation permeability (English and Slatin, 1992). This cytolysis leads to gut paralysis, cessation of feeding and finally (typically after 1 - 3 days) death from starvation and/ or septicaemia.

Differences in the extent of solubilization of different toxins may explain the differences in the toxicity of various proteins (Meenakshisundaram and Gujar, 1998). Decreased solubility could be one of the potential mechanisms for insect resistance to *B. thuringiensis* proteins. In cotton bollworm (*Helicoverpa zea*), *CryIIA* is less soluble than *Cry1Ac* and fails to bind a saturable binding component in the mid-gut brush border membrane (English et al., 1994). The unique mode of *CryIIA* may provide a useful tool for management of resistance to *B. thuringiensis* toxins (Maqbool et al., 1998). Although the binding of the *Cry* toxins to the receptors determines the species sensitivity to various toxins, yet there are distinct exceptions, for example, *Cry1Ac* binds well to the ligand bands of beet armyworm (*Spodoptera exigua*) brush border membrane proteins, but there is very little toxicity to this insect (Garczyynski et al., 1991). *Cry1Ab* is more toxic to the gypsy moth than *Cry1Ac*, but does not bind well with the receptors in the brush border membrane (Wolfersberger, 1990).

## DIVERSIFICATION OF GENES ENCODING δ-ENDOTOXINS

Due to the crystalline nature of proteins encoded by the *B. thuringiensis* genes, the term "*Cry"* is used in gene and protein nomenclature. Initially, each newly characterized gene or protein received an arbitrary designation from its discoverer such as *kurhdI* (Gieser et al., 1986), Bta (Sanchis et al., 1989), bt1, bt2 (Hofte et al., 1986) etc. Later, the toxin genes earlier mentioned were classified into four types, based on insect specificity and sequence homology (Hoftey and Whitley, 1989). *Cry I* type genes encode proteins of 130 kDa, and are usually specific to *Lepidopteran* larvae, *Cry II* genes encode for 70 kDa proteins specific to *Lepidopteran* and *Dipteran* larvae. *Cry III* genes encode for 70 kDa proteins specific to *Coleopteran* larvae and *Cry IV* genes are specific to *Dipteran* larvae. The system was further extended to include type *V* genes that encode for 81 kDa proteins effective against *Lepidopteran* and *Coleopteran* larvae (Tailor et al., 1992). As more *B. thuringiensis* were cloned in more detail, some inconsistencies were encountered in this scheme. For example, *Cry I*-type proteins were designated as lepidopteran-specific, however *CryIAb* and *CryIC* both exhibit dual activity against *Dipteran* and *Lepidopteran* larvae (Smith et al., 1996). *CryIB* is toxic to *Dipteran*, *Colepteran* and *Lepidopteran* larvae (Zhong et al., 2000). Therefore, a new classification system, based exclusively on aminoacid identity, was proposed and is currently being used (Crickmore et al., 1998). *Cry* and Cyt were assigned to a class, if their sequence similarity is greater than 45% than other members of the class. In this system, Roman numericals have been exchanged for Arabic numerals in the primary rank. Each toxin will be assigned a unique name with all four ranks that is, primary, secondary, tertiary and quaternary (for example, *Cry23Aa1*). The inclusion of third and fourth rank may be optional. The new toxin could, therefore, simply be referred to as *Cry23A*. Proteins with same primary rank often affect the same order of insect. Those with different secondary and tertiary rank may have altered potency and targeting within an order. At the tertiary rank, differences can be due to dispersed point mutations. The quaternary rank was established to group alleles of genes coding for known toxins that differ only slightly. The ranks (primary, secondary and tertiary) represent approximately 45, 78 and 95% amino acid identity.

The *B. thuringiensis* δ- endotoxins are now known to constitute a family of related proteins for which about 300 *Cry* genes have been described so far ((http://epunix. biolos.susx.ac.uk/home/Neil_Crickmore/B. thuringiensis/ toxins.html), with specificities to *Lepidoptera*, *Coleoptera*, *Homoptera*, *Diptera* and *Orthoptera* and there is continuous addition of newly isolated genes to this list each day. More than 150 of these *Cry* toxins have been cloned and tested for their toxicity on various insect species (Saraswathy and Polumetla, 2004) and the list is expanding.

About 40% of the currently identified *B. thuringiensis* toxins are not active on insects, due to various reasons like low solubility in the insect gut environment, lack of binding to brush border membrane vesicles (BBMV) in the larval midgut and presence of protease cleavage sites (Saraswathy and Polumetla, 2004). Knowledge of δ-endotoxins can be utilized to make these inactive toxins active by protein engineering.

## SPECIFICITY AND DURABILITY

The high degree of specificity of *B. thuringiensis* proteins is often cited as one of the benefits of their use over synthetic pesticides (Hilder and Boulter, 1999). However, most crops are not subjected to attack by a single pest species but rather by an entire complex of different pests. For example, cotton, although grown under a number of different cropping systems, is subject to losses from a surprising similar pest complex worldwide, principally heliothines, mites, aphids, spider mites and thrips (Lutterell et al., 1994). Many of these pests are not susceptible to any known *B. thuringiensiss*. The value of transgenics protected against, for example heliothines is likely to be much reduced if chemical pesticides still have to be regularly applied to control, for instance, whitefly. There is a need to identify insect control genes for these currently unsusceptible pests. Because transgene products are essentially confined within the host plant, they are

intrinsically specific to those pests which are heinous enough to eat those plants. So, whereas there is a trend in favor of highly selective, narrow spectrum compounds for use as chemical pesticides, with transgenics it may be argued that a broader spectrum of activity is desirable, provided this does not include beneficial insects as well (Hilder and Hamilton, 1994).

Of relevance to the durability and specificity of resistance is the question of regulation of expression of transgenes by the use of appropriate promoters. In most cases, insect resistance genes have been inserted with constitutive promoters such as *CaMV35S*, maize ubiquitin or rice actin 1 (Tu et al., 2000; Maqbool et al., 2001; Husnain et al., 2002; Ramesh et al., 2004) , which direct expression in most plant tissues. It has been suggested that limiting the time and place of expression by the use of tissue specific e.g. *PHA-L* promoter for seed-specific expression or *RSuS-1* promoter for phloem-specific expression (Liu et al., 2002; Ramesh et al., 2004) or inducible e.g. potato *pin*2 wound-induced promoters (Duan et al., 1996) might contribute to the management of resistance in the pest and unfavorable interactions with beneficial insects. This tends to be presented as a self-evident truth, rather than a reasoned argument. Of primary importance for pest control is that there is a reliable, spatial expression of the insect control protein (ICP) at the site on which the pest feeds and at the stage when it is most vulnerable. In most cases, these criteria are met by constitutive promoters. It is important to note that phloem expression from *CaMV35S* matches that from *RSuS-1* in dicots (Hilder and Boulter, 1999). Constitutive expression also allows broader-spectrum ICPs to be targeted at different components of the pest complex. The greatest risk of resistance build-up would probably arise in the case of prolonged exposure to ineffective levels of the transgene product, a situation which would hardly be tolerated by farmers who in practice would surely adopt additional (different) control measures (deployment of which would in fact reduce the risk of resistance development). It has also been suggested that restricted expression might minimize any yield penalty' associated with transgene expression (Xu et al., 1993; Schuler et al., 1998). This would become a serious consideration if any such penalty were demonstrated. There are other certain situations where specific promoters would have clear advantages, for example, for root-feeding cyst nematodes which modify expression at their feeding sites and tend to inactivate general promoters, although even here *CaMV35S* appears to work (Atkinson, 1993), for sap-sucking pests for which the transgenes like GNA must be effectively expressed in vascular tissues (Ramesh et al., 2004). In an investigation taken by the author currently, transgenic rice harboring the *B. thuringiensis* gene driven by the *RSuS-1* promoter demonstrated an effective level of resistance against the targeted insect pests. This would probably minimize the concerns of regulatory agencies or rice consumers for the presence of transgenic protein in the seed.

## ACHIEVING ADEQUATE EXPRESSION LEVELS OF *B. THURINGIENSIS* IN CROP PLANTS

The initial approach to express *B. thuringiensis* genes in plants was simply to place the bacterial coding regions between highly active promoter functional in plants and a region providing transcriptional termination and poly-adenylation functions (Barton et al., 1987; Fischoff et al., 1987). In most cases, the promoter was the region of cauliflower mosaic virus responsible for the transcription of the abundant 35S RNA. The 3' ends of the chimeric *B. thuringiensis* genes usually come from the *T-DNA* genes of the *Agrobacrerium tumefaciens*. The early examination of transgenic tobacco or tomato gave very poor expression of *B. thuringiensis* and consequently very poor protection against insect predation. Protection was then improved when only the toxic N-terminal of the protein was expressed in plants, the data looked considerably better (Fischoff et al., 1987), but the protein level was still relatively lower. In the same year, the Belgian group at plant genetic systems (PGS) achieved a substantial increase in *B. thuringiensis* expression levels by exploiting a coupled selection system that linked the expression of *B. thuringiensis* with that of an antibiotic resistance gene (Vaeck et al., 1987). The transformed plants were selected for high levels of antibiotic resistance, and then co-selected for high levels of expression of the *B. thuringiensis* gene. Because *B. thuringiensis* normally produces a protoxin that is proteolytically processed in the susceptible insect, the additional protein fragments fused to the C-terminal of *B. thuringiensis* made little difference to the toxicity of the fusion protein. Proteins levels were still relatively low, but reasonable control of *Manduca sexta* was achieved.

During the 1990s, researchers made a significant breakthrough in the expression levels of *B. thuringiensis* (Perlak et al., 1990; Wunn et al., 1996; Cheng et al., 1998; Khanna and Raina, 2002). The *Cry* genes have a high *A/T*-content compared with plant genes (typically values are 60 - 70% for *B. thuringiensis* genes and 40 - 50% for plant genes). As a consequence, *Cry* gene codon usage is inefficient in plants, and the *A/T*-rich regions may contain transcriptional termination (polyadenylation) sites, cryptic mRNA splice sites, and mRNA instability motifs (*ATTTA*). The effects of different degrees of gene modification were investigated in the *Cry IA(b)* and *Cry IA(c) (*Perlak et al., 1991). Removing of the polyadenylation sites and *ATTTA* sequences, gave a total of 356 of the 615 codons and raised the levels (up to 0.2 - 0.3% of total soluble protein) 100-fold higher than the level for unmodified genes. These effects were initially observed in transgenic tobacco, tomato and cotton (Perlak et al., 1990, 1991). Later, the same effects were detected in rice (Wun et al., 1996; Cheng et al., 1998; Khanna and

Raina, 2002), potato (Perlak et al., 1993) and corn (Koziel et al., 1993). It was further revealed that not only the presence of rare codons, but some other factors were also primarily responsible for the low expression of wild type genes (van Aarssen, 1995). The unmodified gene sequences interfere with transcript accumulation probably as a result of mRNA splicing thus leading to the presence of three cryptic plant introns. Codon optimization further increased the expression levels.

Even higher levels of expression have been achieved by transforming tobacco and oilseed rape chloroplasts with an unmodified, full length, *CryIAc and Cry1Aa10* coding regions, respectively (McBride et al., 1995; Hou et al., 2003). As the transcriptional and translational machinery of plastids is similar to that of bacterial, modification of *Cry IA(c)* was not considered to be necessary. The effects of amplification of integrate gene in plastids resulted in *B. thuringiensis* protein levels of 3 - 5% of total soluble protein. This level of expression even provided protection against relatively *Cry IAc* tolerant plant pests.

Over the years there has been a gradual development of insect resistance against *B. thuringiensis* toxins which is particularly related to level of *B. thuringiensis* protein toxin. Resistance in the field has been detected in three *lepiopteran* species (*H. zea* in USA, *Spodoptera frugipedra* in Puerto Rico and *B. fusca* in South Africa) upto 2007 (Tabashnik, 2008). The use of *B. thuringiensis* genes in pyramided stocks would be more beneficial rather than using them singly or sequentially. Pyramided gene technology would be anticipated to last 150 - 250 years and single genes used sequentially, 6 - 9 years (Roush, 1997). The role of refuges is also important to be considered (Tabashnik, 2008). It has been experienced that lack of high dose of *B. thuringiensis* toxin for *Helicoverpa zea* in USA may have favored its faster evolution of resistance. But, if the high dose standard is not met, increasing the abundance of refuges relative to *B. thuringiensis* crops can delay resistance. For *Helicoverpa zea*, higher refuge abundance was associated with slower resistance evolution in North Carolina compared to Arkansas and Mississippi (Tabashnik et al., 2008).

## FIELD TRIAL TESTING OF *B. THURINGIENSIS* CROPS

The global area of genetically modified crops has grown to 125 million ha in year 2008, up from 114.3 million ha in 2007 (James, 2008). Transgenic crops for *B. thuringiensis* genes share 19.1 million ha that accounts for 15% of total area (125 million ha). The first field trial with genetically engineered plants expressing *B. thuringiensis* toxin was conducted in 1986 with tobacco (James, 2000, 2002). Since then, transgenic corn, tomato and cotton have been field tested in USA, Argentina and Australia. In 1996, *B. thuringiensis* crops occupied 1.2 million ha (James 2000). Delannay et al. (1989) evaluated transgenic tomatoes expressing *B. thuringiensis* insect control protein under field conditions in 1987 and 1988. The transgenic plants showed very limited feeding damage after infestation with the tobacco hornworm (*M. sexta*) whereas control plants showed heavy feeding damage and were almost completely defoliated within two weeks. Significant control of tomato fruit worm and tomato pinworm was also observed. Bioassay showed that transgenic plants produced 1 ng of *B. thuringiensis* protein per mg of soluble protein. Koziel et al. (1993) produced transgenic maize plants and obtained high levels of resistance to *Ostrinia nubilalis Hubner* (European corn borer). A synthetic gene encoding a truncated version of the *Cry IA(b)* protein derived from *B. thuringiensis* was introduced. Modification of the native *Cry IA(b*) coding region and increasing guanine-cytosine content from 38% to 65% greatly enhanced its expression in maize. The first field trials with *B. thuringiensis* transgenic cotton were conducted in USA in 1988 (Jenknis et al., 1991). The *Cry IA* proteins expressed in *B. thuringiensis* cotton and corn have been extensively tested for toxicological analysis in the laboratory and field. These studies (Huang et al., 1999; Thomas et al., 1995; McGaughey et al., 1998) strongly support the specific activity spectrums of *B. thuringiensis* proteins, which are largely mediated by the gut conditions required for activation of the proteins, and by the need for specific binding to receptors in the mid-gut before toxicity is demonstrated.

*B. thuringiensis* proteins have been commercialized in cotton (expressing the *Cry IA(c)*, maize (*Cry IAb*), and potato (*Cry3A*). The *Cry IAc* protein in *B. thuringiensis* cotton provides insecticidal activity against many *Lepidopteran* species. However, there were no effective controls for *O. nubilalis Hubner* (European corn borers). Potatoes have been engineered for control of *Leptinotarsa decemlineata Say* (Colorado potato beetle), a significant pest in many production areas (Duncan et al., 2001). Chitkowski et al. (2003), conducted a field trial of transgenic cotton involving Bollgard II (Monsanto 15985), which expresses two *B. thuringiensis Berliner* proteins (*Cry1Ac + Cry2Ab*) and Bollgard (DP50B), which expresses only one *B. thuringiensis* protein (*Cry1Ac*). This study demonstrated that the dual-toxin Bollgard II genotype is highly effective against lepidopterous pests that are not adequately controlled by the current single-toxin Bollgard varieties.

More *B. thuringiensis* crops are under development, including rice, sorghum, lupins, peas and other legumes, and several tree crops (Duan et al., 1996; James, 2000; Oritz et al., 2000). *B. thuringiensis* cotton varieties have been developed and commercialized as Bollgard (Bryant et al., 1999; Edge et al., 2001) in the USA, China, South Africa, and Argentina, and as Ingard (Pyke and Fitt, 1998; Wilson et al., 1998; Finnegan et al., 1998) in Australia. Some of the successful cases for the development of *B. thuringiensis* transgenic crops so far have been illustrated

**Table 1.** Successful examples to show *B. thuringiensis* genes (originated from *Bacillus thuringiensis*) integration for insect resistance in rice.

| Gene | Target pest | References |
|---|---|---|
| Cry 1A(b) | Striped stem borer and leaf folder | Fujimoto et al. (1993) |
| Cry 1A(b) | Yellow stem borer and striped stem borer | Wunn et al. (1996) |
| Cry 1A(b) | Yellow stem borer and striped stem borer | Ghareyazie et al. (1997) |
| Cry 1A(b) | Yellow stem borer | Datta et al. (2002) |
| Cry 1A(b) | Yellow stem borer | Alam et al. (1999) |
| Cry 1A(b)/ Cry 1A(c) | Leaffolder and yellow stem borer | Tu et al. (2000) |
| Cry 1A(b)/ Cry 1A(c) | Yellow stem borer | Ramesh et al. (2004) |
| Cry 1A(c) | Yellow stem borer | Nayak et al. (1997) |
| Cry 1A(c) | Yellow stem borer | Khanna and Raina (2002) |
| Cry 1A(c) | Striped stem borer | Liu et al. (2002) |
| Cry 2A | Leaffolder and yellow stem borer | Maqbool et al. (1998) |
| Cry 2A/ Cry 1A(c) | Leaffolder and yellow stem borer | Maqbool et al. (2001) |
| Cry 1Ie | Corn borer | Liu et al., 2004 |

**Table 2**. Development of some other *B. thuringiensis* transgenic crops for insect resistance.

| Crop target | Gene | Target pest | References |
|---|---|---|---|
| Corn | *Cry 1A(b)* | European corn borer | Koziel et al. (1993) |
| Soybean | *Cry 1A(c)* | Bollworm and Bud worm | Stewart (1996) |
| Tobacco | *Cry 2aa2* | Cotton bollworm | De Cosa et al. (2001) |
| Sugar cane | *Cry 1A(b)* | Stem borer | Arencibia et al. (1997) |
| Potato | *Cry 5 B. thuringiensis* | Potato tuber moth | Douches et al. (1998) |
| Alfalfa | *Cry 1C* | Leaf worm | Strizhov et al. (1996) |
| Tomato | *B. thuringiensis (k)* | Tobacco hornworm, tomato pink worm and tomato fruit worm | Dellannay et al. (1989) |
| Brassica | *Cry 1A(c)* | Pod borer | Stewart (1996) |
| Cotton | *Cry 1A(b)/(c)* | Lepidoptera | Stewart(2001), Chitkowski et al. (2003) |
| | *Cry 2A* | Pink Bollworm | Tabashnik et al. (2002) |

in Tables 1 and 2.

Tu et al. (2000) produced transgenic indica rice CMS restorer line Minghui 63 (T5I-I) expressing a *B. thuringiensis* fusion gene derived from *Cry IAb* and *CryIAc* under the control of the rice actin 1 promoter. The level of *B. thuringiensis* fusion protein detected was 20 ng/mg soluble protein. Field testing of the transgenic rice lines showed high protection to leaf folder (*Cnaphalocrocis medinalis Guene*) and *Scirpophaga incertulas Walker* (yellow stem borer). The percentage of plants with whiteheads (stem borer injury) was significantly lower on the *B. thuringiensis* Shanyou63 (11%) as compared to control Shanyou63 (44%) plants. Similarly transgenic plants showed no leaf folder attack (0.0%) as compared with non-transgenic (Shanyou63) where, 60% of the plants were affected by rice leaf folder (RLF). Ye et al. (2003), demonstrated high level of stable resistance in transgenic japonica rice lines KMD1 and KMD2, with *Cry IA(b)* gene to rice leaffolder (*C. medinalis Guenee*) under field conditions for three years in Zhejiang Province, China. Both KMD1 and KMD2 exhibited high and stable resistance against natural infestation by the leaffolder, and showed no symptoms of damaged leaves throughout the growing season. In contrast, the untransformed parental control line (Xiushui-11) showed RLF damage not only in untreated plots, but also in plots treated once with chemical insecticides. The results demonstrated that both lines have potential for protecting rice from the leaffolder damage.

Bashir et al. (2004) reported the first field trial of different transgenic lines of indica Basmati rice B-370, expressing *Cry1Ac* and *Cry2A* genes, in Pakistan. Different transgenic lines were grown under field conditions for two consecutive years. Transgenic lines exhibited inherent ability to protect rice plants from target insects. Natural infestations of rice skipper and rice leaf folder were also observed and transgenic plants were statistically superior to their untransformed counterparts. The transgenic lines had no effect on non-target insects belonging to orders other than *Diptera* and *Lepidoptera*

and germination of subsequently grown three local varieties of wheat. Chances of gene spread were calculated at a level of 0.18% cross pollination in the experimental rice lines.

Douches et al. (2004), in their investigation of field and storage testing of *B. thuringiensis* potato harboring *Cry5* for resistance to tuber worm conducted over a period of five years from 1997 - 2001, observed that the *Cry5* transgenic lines were proved to be resistant in the field with 99 - 100% free of tuber damage. In the year 2001 storage study, these lines were also 90% free of tuber worm damage. It was suggested that the expression of the *Cry5* gene in the potato tuber and foliage will provide the seed producer and grower a tool in which to reduce potato tuberworm damage to the tuber crop in the field and storage.

A filed trial of Monsato's "YieldGard" *B. thuringiensis* Transgenic corn expressing *Cry 1A(c)* was conducted in China (He et al., 2003) for resistance evaluation to Asian corn borer. Damage ratings and number of larvae surviving per plant indicated that *B. thuringiensis* corn was highly resistant to Asian corn borer. Therefore, "YieldGard" offers the potential for season-long protection against first and second-generation of Asian corn borer.

Speese et al. (2005) conducted the field evaluation of *B. thuringiensis* sweet corn over different growing conditions/ locations and pest pressure in Virginia, USA. The results showed that when the insect pressure was low to moderate, *B. thuringiensis* sweet corn did not require any supplementary application of insecticide. However, when the pressure was extremely high, it only needed 1 - 2 applications compared to non-transgenic isolines which required 6 - 9 applications under such conditions.

## THE FUTURE OF TRANSGENIC *B. THURINGIENSIS* GENE EXPRESSING PLANTS

Although it is very clear that the transformation technology has advanced to the stage where dramatic protection from insect attack can be demonstrated in a range of important crops including cotton, soybean oilseed rape, maize and rice, yet two main factors remain to be assessed and tested in the marketplace. The first one is the durability of insect tolerance based on *B. thuringiensis* genes. The use of transgenic plants will have little value if the important insect pests become resistant to *B. thuringiensis* after only a couple of years, and a considerable research and thought will have to go into the deployment of transgenic crops in agricultural systems in the short term, so that the resistance is delayed or prevented. As indicate previously, this may be achieved using combinations of pyramiding different sorts of insect resistance genes together in the same plant or in different plants in rotation. The stacked trait products were by far the fastest growing trait between 2007 and 2008 (James, 2008). Double stacks with pest resistance were also the fastest growing component in Phillipines doubling from 25% of Biotech maize in 2007 to 57% in 2008. A total of 10 countries (USA, Canada, Phillipines, Australia, Mexico, South Africa, Honduras, Chile, Colombia and Argentina) planted biotech crops with stacked traits in 2008. Biotech maize with eight genes named SmartStax™ is expected to be release in the USA in 2010 with resistance to several insect pests and herbicide tolerant traits. A number of *B. thuringiensis* stacked gene product of cotton are likely to be available in the near future (upto 2014). Among them are Monsanto's Bollgard-III® (*CryIAc*, *Cry2Ab* and *Vip3A*), Bayer's TwinkLink® (*Cry1Ab* and *Cry2Ae*) and Syngenta's VipCot® (*Cry1Ab* and *Vip3A*).

Second factor is achieving public acceptance for transgenic crops. This may not be difficult for fibre transgenic crops like cotton, but will probably require considerable public education for food crops like tomato, potato, rice etc, despite the good toxicology data already existing for *B. thuringiensis*. As with many aspects of genetic engineering, politics can impact on the success of a project involving the development of *B. thuringiensis* transgenic crops, irrespective of its apparent social, economic or environmental benefits. Public education will be essential to ensure the widespread adoption of genetic adoption technologies in agriculture, and scientists will have to play an active role in this process.

*B. thuringiensis* toxins and their genes are a unique resource for agricultural systems and it is considered that the most cost effective and environmentally appropriate form of package for this biological insecticide is the seeds that the farmer buys and plants. Techniques exist for producing such seeds and this goal may be realized in the foreseeable future.

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