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# *Bacillus thuringiensis* and its use in agriculture

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**Abstract** : *Bacillus thuringiensis* (Bt) is an ubiquitous Gram positive, spore forming bacterium that forms parasporal crystal inclusions. These proteinaceous inclusions are called crystal (Cry) proteins or delta-endotoxins, which are toxic to insects. Hence preparations of Bt are being used as bioinsecticides for the past four decades in commercial agriculture and forest management for the control of certain insect species belonging to the orders of Lepidoptera, Diptera and Coleoptera. Bt is a major source for transfer of genes into plants to impart insect resistance. Since 1996 millions of hectares are grown with insect resistant transgenic plants containing Bt genes, in different parts of the world. Commercial release of transgenic Bt cotton in India is expected soon. (*Key Words* : *Bacillus thuringiensis*, Biopesticide, Transgenic plants)

Chemical pesticides though extremely effective in controlling pest population suffer from disadvantages. They have a broad toxic range and potentially toxic to non-target organisms. Alternatively biological control of insects has become more popular and provides an alternative opportunity to reduce insect damage with several clear advantages over chemical pesticides. The *Bacillus thuringiensis* products account for 60-90 per cent of total biopesticide market (Feitelson *et al.* 1992). This bacterium was first isolated in Japan in 1901 by Ishiwata from diseased *Bombyx mori* larvae. A decade later, Ernst Berliner (1915) isolated a similar organism from diseased granary populations of *Ephestia kuehniella* Zell (Mediterranean flour moth) larvae obtained from Thueringen, Germany, and named the bacterium *Bacillus thruingiensis* (Bt). Application of Bt was started in 1928 when a Bt strain isolated from *Ephestia* sp. was tested on European corn borer, *Ostrinia nubilalis* (Kumar *et al.* 1996). In 1960, stimulated by the growing concern over the use of chemical insecticides, a Bt strain was commercialized for the first time and marketed as "Thuricide". Slowly, the first strain was replaced by a more potent strain (HD-1) isolated by Dulmage (1970). In earlier days, it was believed that Bt was mostly active against lepidopterans. Later Bt strains were isolated with toxicity against other insect orders such as Diptera, Coleoptera, Homoptera and against nematodes, mites and protozoa. These findings ultimately let to more screening programs and now there are thousands of Bt isolates in various collections. The advancement in genetic engineering and molecular biology in the early eighties led to the cloning of Bt crystal protein (*cry* ) gene for the first time in 1981 (Schnepf and Whiteley, 1981). Since then, more than 100 *cry* genes have been successfully cloned and added to the growing list (Wasano *et al.* 2001). Initially several *cry* genes were expressed in plant colonizing microorganisms to target the stem and root dwelling insect pest. Today, the most efficient way to deliver *cry* genes seems to be the development of transgenic plants expressing them (Kannaiyan, 2000; De Cosa *et al.* 2001).

## Bt subspecies

Bt is a gram positive, spore forming bacteria that exists in a diverse locations, such as the soil, plant surfaces, insect cadavers and in grain storage dusts (de Maagd *et al.* 1999). During the sporulation stage of its life cycle, Bt produces one or more proteinaceous crystal inclusions, possessing varying degrees of insecticidal activity (Bulla *et al.* 1977). The presence of these parasporal crystals in the sporangia and their insecticidal activity has been the unique character available for differentiating Bt from its closely related species, *Bacillus cereus*. In early days, Bt strains were classified into sub species based mainly on morphological and biochemical characters. Although several methods were tried for classification, serotyping using 'H' flagellar antigen remains the most widely used, simplest and practical method to classify Bt strains (de Barjac and Frachon, 1990). In 1993, Bouroque and co workers reported that there might be variation in the biochemical characters, plasmid patterns, shape, stability and the insecticidal activity of the Bt isolates even if placed within the same serotypes. A list of 80 Bt serotypes based on H Serotyping is given in Table 1 (Lecadet *et al.* 1998).

## Insecticidal crystal proteins of Bt

The insecticidal crystal proteins are formed during sporulation stage of the bacterium's life cycle. Crystal formation can be observed microscopically during the later part of stage II. In Bt H-14, electron microscopic studies revealed the presence of nascent inclusions even at stage -I (Abdel-Hameed *et al.* 1990).

Bipyramidal crystals synthesised in host cells are typically about 1.1 µm long and 0.5 µm wide (Oeda et al. 1989). Bipyramidal crystals show a greater frequency of toxicity than all the other types. Most of the lepidopteran active isolates contain such inclusions (Attamthom *et al.* 1995); cuboidal crystals are active against lepidopteran and dipteran larvae or lepidopteran larvae alone (Yamamato and Mclauzhtin, 1981); spherical and irregular crystals are mostly mosquitocidal, often active against certain coleopteran species (Krieg *et al.* 1983). Irregular cyrstals also include those with very little or no identified toxicity (Zelanzy *et al.* 1994. Based on Cry protein composition, the crystals have various forms. Most Cry1 type proteins form bipyramidal crystals, Cry2 type assume cuboidal form and the Cry3 proteins form flat rhomboidal crystals. The crystals formed by Bt *israelensis* are typically spherical. The crystal toxin is insoluble in water or inorganic solvents, but soluble in alkaline solvents. Cry1 proteins are soluble at pH 9.5, while the Cry 2 proteins are soluble at a pH of about 12. Similarly, Cry4A, Cry5B and Cyt toxins are soluble at pH 9.5, while the Cry4D toxin requires a pH 12. The Cry3A toxin on the other hand, dissolves at pH's below 4 and above 9.5. The crystals can also be dissolved at neutral pH in the presence of detergents and denaturing agents like urea, B-mercaptoethanol, DTT and SDS (Gill *et al.* 1992). Separation of crystals from the spores and cell debris involves isopycnic centrifugation in sucrose or caesium chloride gradients, zonal gradient centrifugation using. In addition, relatively large scale and rapid purification of crystals from a broad range of Bt strains is possible by linear and discountinuous gradients using Renongrafin-76 (Sharpe *et al.* 1975). Bt toxins usually termed protoxin are activated by the insect midgut proteases to yield the toxic fragment. Gill *et al.* (1992) reported that the larger protoxin of about 130 - 140 kDa undergoes proteolysis yielding a toxic fragment of 60 - 70 kDa derived from N-terminal half of the protoxin. Structurally the activated toxin can be divided into three structural regions: 1) N-terminal region, which is the toxic domain (amino acid sequence 1 - 279) consisting of several conserved hydrophobic regions. 2) A conserved C-terminal region (amino acid sequence 461 - 695) and 3) a variable region between these two region that contains most of the amino acid differences.

Hofte and Whiteley (1989) investigated sequences among a number of toxins and found five well conserved regions and designated as blocks from one to five. According to this it was predicted that the crystal toxin, consists of three domains. This has been confirmed by X-ray crystallographic studies (Li *et al.* 1991; Grochulski *et al.* 1995). The long hydrophobic and amphipathic helices of domain I suggest that this domain might be responsible for the formation of lytic pores in the intestinal epithelium of the target organism. The involvement of Domain II in receptor binding was supported by site directed mutagenesis and segment swapping experiments (Jurat-Fuentes and Adang, 2001), The β Sandwich structure of domain III could play a number of roles. The main function consisting of maintaining the structural integrity of the toxin molecule, mostly it shields the molecule from proteases during proteolysis within the gut of the target pest (Li *et al.* 1991). The Cry protein when ingested by the insect is first converted from to active toxin protein by proteolysis in the alkaline mid gut region (Lightwood *et al.* 2000), which then diffuses through peritrophic membrane and binds to high affinity receptors present on the midgut epithelium. When the activated toxin binds to the specific receptors, the interaction of receptor-toxin becomes irreversible and generates pores on the membrane. the formation of pores eventually disturbs the ionic gradient, further leading to the swelling of microvilli and destruction of epithelial membrane, leading to the cell death.

## Classification of *cry* genes

The first attempt to systematically classify Bt crystal protein genes was undertaken by Hofte and Whiteley (1989). The systematic designations followed by Hofte and Whiteley were as follows. The principal toxicity spectrum was denoted by Roman letters (from I to IV) grouped under different classes. Within the class major and minor amino acid differences were denoted by upper and lower case letter, respectively (eg : *cry IAa* ). Since, Bt genes encoding cytolytic proteins were totally unrelated to *cry* genes, they were designated as *cyt* genes. In general the term *'cry'* (first given by Heid *et al.* 1982) was followed to designate the genes encoding crystal proteins and Cry for toxins. The *cryI* genes code for lepidoteran specific bipyramidal crystal proteins having molecular range of 130 - 140 kDa. The *cryII* class of genes encode about 65-71 kDa proteins which form cubiodal inclusions during sporulation. The Cry II protein are toxic to either lepidopteran on lepidopteran and dipteran larvae. The *cryIII* class encodes 73 kDa coleopteran specific proteins. The *cry*IV class of genes is composed of a mixed group of dipteran - specific crystal protein genes encoding polypeptides with predicted molecular mass of 135, 128, 74 and 72 kDa respectively. The *cry V* class of genes encode a protein having molecular weight of about 80 kDa. They show toxicity towards coleopteran and lepidopteran larvae. The *cryVI* class on the other

hand are reported to exhibit activity against nematodes (Feitelson *et al.* 1992).

Recently, Crickmore *et al.* (1998) revised the nomenclature for the *cry* and *cyt* genes. In this newly revised system, a broad definition was given to Cry Protein: a parasporal inclusion (crystal) protein from Bt that exhibits some experimentally vertifiable toxic effect to a target organism, or any protein that has obvious sequence similarity to a known Cry protein. Similary Cyt protein denotes a parasporal inclusion (Crystal) protein from Bt that exhibit haemolytic activity, or any protein that has obvious sequence similarity to a known Cyt protein. The known Cry and Cyt proteins now fall into 30 sets including Cyt1, Cyt2 and CryI through Cry28. Some of the important features of revised nomenclature are :

I. The *cry* genes whose products share <45% amino acid sequence homology are characterized by different Arabic numbers, designated as primary ranks (eg. *cry1*, *cry2* etc.).

II. The *cry* genes of the same primary rank whose products show < 78% amino acid sequence homology are differentiated by secondary ranks by using uppercase letters (eg.*cryIA cryIB* etc.,).

III. The *cry* genes having same primary and secondary ranks whose products share less than 95% amino acid sequence homology receive separate tertiary rank, designated by lowercase letters (eg. *cryIAa, cryIAb* etc.,)

IV. The *cry* genes whose products are different in amino acid sequence, but are more than 95% identical to each other are given separate quaternary ranks by another Arabic number (eg. *cry1Aa1,Cry1AQ2* etc.,)

**Cloning of *cry* genes**

The majority for *cry* genes are localized in the self replicating plasmids, having a size ranging from 140 to 150 Mda. Further investigation showed that in some strains (Bt var. *entomocidus*, Bt var. *aizawai*) *cry* genes are localized on the chromosome (Udayasuriyan *et al.* 1996; Sekar *et al.* 1987). The main objective of cloning *cry* gene using recombinant DNA methodologies is to improve Bt strains available for better production and to improve the toxicity. The first ever report on cloning of Bt crystal protein gene was reported in 1981. Schenpf and Whiteley (1981) cloned a crystal protein gene from Bt subsp. *kurstaki* and expressed in *E.coli*. The recombinant strains of *E.coli* synthesised a 130 kDa protein that showed positive reaction with antibody raised against a peptide of the same size from Bt crystals. In 1993, Kalman *et al.* cloned a new *cry IC* gene (*cryICb*) from Bt *gallariae*, based on distinct eletrophoretic mobility of PCR products amplified using specific primers. Shin *et al.* (1995) cloned two *cry*V type genes, *cryVI* and *cryV465*, from Bt *kurstaki* HD-1 and Bt subsp *entomocidus* BP 465 respectively and determined their nucleotide sequence. The cloned CryVI protein was toxic to *Plutella xylostella* Linn. and *Bombyx mori*, whereas the Cry 465 protein showed toxicity towards *Plutella xylostella* only. Delecluse *et al.* (1995) cloned a mosquitocidal gene, designated *cry* 11B, encoding a 81 kDa crystal protein in Bt subsp *jegathesan*. The sequence of Cry 11B protein showed high homology towards Cry 11A toxin (CryIVD) from Bt subsp. *israelensis*.

A novel mosquitocidal protein gene, *cry20Aa* was cloned from Bt subsp *fukuokaensis*. The gene product was naturally truncated and had a molecular weight of 86 kDa. The amino acid comparisons showed *cry20Aa* to be an entirely different protein (Lee and Gill, 1997). Two new crystal protein genes, *cry19A* and orf2, were isolated from Bt subsp. *Jegathesan* encoding a 74.7 kDa protein and 60kDa protein respectively. The orf2 amino acid sequence is very much similar to carboxy terminus of Cry4 proteins (Rosso and Delecluse, 1997). Sasaki *et al.* (1997) cloned a *cry2A*- type gene from Bt serovar *sotto* SKWOI - 102-06 and designated as *cry2* (SKW). The cloned protein was toxic to *Bombyx mori*. The CryIIa was active on both *Plutella xylostella* and *Bombyx mori*. A new CryIId protein was toxic to *Plutella xylostella* as Cry1Ia but less active on *Bombyx mori* (Choi *et al.* 2000). The Cry2Aa of a new Bt strain (47-8) was effective against *Helicoverpa armigera*, cotton bollworm (Lenin *et al.* 2001).

**Expression of *cry* genes in recombinant bacteria**

For many years conjugation and transduction have been used to transfer recombinant plasmids. At present a variety of *E.coli*- Bt shuttle vectors have been constructed to facilitate the introduction of cloned *cry* genes in Bt. In addition to these shuttle vectors, integrational vectors have been used to insert cloned *cry* genes into resident plasmids or into the chromosome (Kalman *et al.* 1995), by homologous recombination. In several cases, the cloned *cry* gene transfer into Bt host strain has resulted in improved spectrum for toxicity (Kalman *et al.* 1995). In recent times, enhancing the stability of crystal proteins under field conditions has had a great attention. To combat this, scientists went for DNA manipulations and expressed the cloned cry genes in other microorganisms, preferably plant associating microbes.

The first report of such an expression was established, when Monsanto scientists successfully expressed a *cry1Ab* gene into a root colonizing bacterium *Pseudomonas fluorescens*. After that numerous attempts were made to introduce *cry* genes into a variety of organisms, for improved environmental delivery of Cry proteins (Bora *et al.* 1994; Lampel *et al.* 1994; Udayasuriyan *et al.* 1995; Herrera *et al.* 1997).

**Development of Bt transgenic plants**

The first reported use of the Bt gene expressed in plants for insect control occurred in 1987 (Barton *et al.* 1987; Vaeck *et al.* 1987; ). Since the first release in 1996, the increase in area under transgenic Bt crops from about 1m ha to 11.8 mha in 1999 testifies the economic advantage (besides a substantially lower application of synthetic pesticides) that farmers have experienced. Nevertheless, commercial cultivation of Bt crops have been limited to the mainland USA (excluding Hawaii), southern parts of Australia and China. It is important to learn how to manage widespread adoption of Bt crops without leading to rapid emergence of resistance in target insects (Manjunath and Mohan, 2000; Raguraman and Uthamasamy, 2001).

The first field test of transgenic upland cotton, *G. hirsutum* L., containing a gene that encodes for δ-endotoxin from Bt was conducted in 1989 (Jenkins *et al.* 1991). The expression of a site specific modification in the coding sequences (DNA), use of improved promoters in *Agrobacterium* Ti plasmid transformation vectors, and the usage of a truncated gene (Perlak *et al.*,1990) has shown 100-fold increase in toxic protein expression, in plant tissues than the wild type. In plants, the modified *cry1A (b)* and *cry1A (c)* genes expressed toxic protein upto 0.05 to 0.10 per cent total soluble proteins in leaves. In 1996, Bt-cotton (Bollgard ™, Monsanto) was released to protect tobacco budworm, and to a lesser extent cotton bollworm and pink bollworm. These transgenic cotton harbouring a Bt *cry1Ac* gene were able to increase average yield by 14 per cent with a reduction in insecticide use to 300,000 gallons in 1997 *(http://www.monsanto.com)*. *Ingard*® and Starlink ® are the popular Bt cotton varieties in Australia grown in 30,000 ha during 1996-98 (Uthamasamy and Reguraman, 2000). The transgenic potato containing the *cry3A* gene is commercialised with Colorado potato beetle as the target pest (Perlak *et al.* 1993). A modified *cry1Ab* gene from Bt has been inserted into rice (*O. sativa* L. *Japonica*) cultivar and confers resistance to two major rice pests, rice leaf folder *Cnaphalocrosis medinalis* (Guenee) and striped stem borer, *Chilosupressalis* (Walker) (Fujimoto *et al.* 1996). Now there are numerous reports that describe rice transformation with *B. thuringiensis* genes including *cry1Ab* and *cry1Ac* (Nayak *et al.* 1997; Maqbool and Christou, 1999; Bentur *et al.* 2000a). Insect resistant transgenic tomato, *Lycopersicon spp* was developed in 1987, was found show tolerance towards tobacco hornworm, budworm and cotton bollworm (Fischhoff *et al.* 1987). They also showed a truncated version of the gene from the HD-1 strain was more effective than the full length expression for transformation. Tomato plants were transformed to express the δ-endotoxin gene from Bt subsp *tenebrionis* using *Agrobacterium* method. The transformed plants were found to harbour a 74 kDa protein, which showed resistance to Colorado beetle. Using microprojectile bombardment of immature embryos, scientists at CIBA biotechnology successfully placed a synthetic gene coding a truncated version of the *cry1A(b)* protein derived from Bt into corn plants. The transformed plants showed resistance to corn borer *Ostrinia furnacalis* (Guenee). The evaluation of transgenic corn lines in field showed stable integration of the introduced gene conferring resistance to the target pest (Wang *et al.* 1995). The number of plants that have been transformed with Bt genes is increasing day by day. At least 10 *cry* genes encoding different Bt toxins have been expressed in 26 different plant species (Table 2).

**Insect resistance to Cry proteins**

The increasing use of chemical insecticides led to the development of resistance and resurgence among insect populations. This led to the switching over of chemical insecticides to Bt-based products and bioformulations. In 1985, McGaughey reported that Indian meal moth population from grain storage bins treated with Bt formulation showed high $LC_{50}$ values relative to populations in untreated bins. Later resistance to Bt insecticides was reported from field population *Plutella xylostella* (Tabashnik *et al.* 1990). Recently de Maagd *et al.* (1999) showed that Bt toxins are not an exception for insects to develop resistance. Globally the development of resistance to *P. xylostella* towards Bt subsp *kurstaki* has been reported from Florida (Shelton *et al.* 1993), Japan (Hama *et al.* 1992), The Philippines (Ferre *et al.* 1991) and China. Recently laboratory studies reveal 76 folds increase in resistance towards Cry1Ac for *Helicoverpa armigera* (Hubner) by the end of the 10th generation (Kranthi *et al.* 2000). Due to the escalation of resistance to Bt toxins in recent times, scientists were forced to undertake studies for understanding the mechanism involved in resistance development in insects. A variety of studies conducted on different resistant populations shows that the

primary reason of resistance lies in the lowering of the affinity of the toxin to the brush border membrane (Ferre *et al.* 1991). In contrast, studies made by Gould *et al.* (1992) and Tabashnik (1994) demonstrated cross resistance to Bt toxins. The reasons for this cross resistance can be attributed to proteolysis of protoxins of decreased solubilization of crystals in the midgut of the larvae. The inheritance of resistance to Bt toxins is also studied (Bentur *et al.* 2000b).

**Resistance management strategies**

Several strategies were proposed (Alstad and Andow, 1995; Kumar *et al.* 1996; McGaughey *et al.* 1998 and Gatehouse, 1999). The following are important.

I. The first step in resistance management is to establish the target pest's baseline susceptibility to insecticidal protein. Once a baseline has been established, regular monitoring of susceptible insects may indicate early stages of resistance among insect populations.

II. The use of multiple toxin genes (pyramiding genes) with different modes of action so that cross - resistance is unlikely to occur. For example: Two *cry* genes for toxins with different receptors, or a *cry* gene in combination with an altogether different gene.

III. Use of temporal or spatial refuges. Rotation of Bt crops with non-transgenic plants would slow down development of resistance, particularly if resistance is not stable in the insect population (de Maagd *et al.* 1999).

IV. Another approach advocated by Denholm and Rowland (1992) is high does strategy. A high does can be defined as that which consistently kills heterozygotes allowing homozygotes to survive. Because homozygous resistant individuals are at very low frequency early in the evolution of resistance, suitable refuges may provide a continuous source of susceptible individuals.

V. Yet another problem is the constitutive expression of Bt genes has led to significant selection pressure on pest population. The use of tissue specific promoters would decrease selection pressure by allowing pests to feed unharmed on economically less important parts of the plant (Vaeck *et al.* 1987 and Wong *et al.* 1992). The spatial, temporal and inducible expression of Bt genes in transgenic plants has thus become a attractive strategy of resistance management (Gujar *et al.* 2000).

**Search for new Bt proteins**

A few kind of insecticidal crystal protein genes (*crylAb and crylAc*) of Bt are now widely used to develop insect resistant transgenic crops. Despite an earlier view that insects would not develop resistance to Bt toxins, now it is realized that insect resistance to Bt toxins can evolve under situations of continuous exposure and/or selection pressure. This has caused a great concern for continuous use of transgenic plants with a single kind of Bt toxin. Due to differential binding specificity to the insect midgut membrane vesicle, a new Bt toxin may be effective in avoiding or at least delaying the development of insect resistance by alternate or combinatorial use. Commercial Bt-based bioinsecticides, are usually the formulations of spores and crystalline inclusions that are released upon lysis of Bt during its stationary phase of growth. The products are applied @10-50 g or about $10^{20}$ molecules per acre. The molecular potency of Bt toxins is higher compared to that of other pesticides, 300 times higher than synthetic pyrethroids or 80,000 times higher than organophosphates (Feitelson *et al.*, 1992). Most of the Bt product is based on subsp. *kurstaki* strain HD-1. It is effective on over 200 crops and against more than 55 lepidopteran species (Justin *et al.* 1988; Rabindra and Jayaraj, 1988). Subsp. *tenebrionis* and/or *san diego* are effective against coleopteran insects, such as Colorada potato beetle (Table 3).

About 75% of Bt market is in North America and Far East (Lambert and Peferoen, 1992). Efforts are needed to expand the use of Bt in other parts of the world in order that the use of hazardous chemical pesticides is reduced. There is great need for higher potency Bt toxins to have effective control over less susceptible and/or hidden pests such as *H. armigera*, and *S. litura* (Rabindra et al. 1994; Perlak *et al.* 1990; Whitlock *et al.* 1991). Loss due to a single polyphagous pest (*H. armigera*) alone is estimated as about Rs. 1000 crores per year in India (Singh, 1996). Variation of single amino acid in the Bt toxin can remarkably influence the level of toxicity (Udayasuriyan *et al.* 1994; Rajamohan et al. 1995). The discovery rate of new Bt toxin is more because of its genome diversity. Therefore, it is reasonable to search for new Bt toxins which are more effective against insect pests of Indian crops. Our studies on screening of indigenous Bt strains for toxicity and molecular chracterization of new Bt strains revealed difference in nature and composition of Bt toxins, between native and standard Bt strains. Hence, cloning of toxin genes from new Bt strains and their characterization are essential to expand the use of Bt and for the management of resistance development in insects.

## Conclusions

Due to the various constraints that has emerged with the use of chemical pesticides such as, development of resistance and resurgence in insects, residual toxicity, secondary outbreak of minor insect pests and biological magnification in the ecosystem, scientists are now looking for new avenues to control or manage insect pests. One of the earliest solutions that was answered to the above mentioned problems was the use of biological agents in pest control. Among the various options that are avilable as on date, use of soil bacterium, the Bt has a great potential for use as a biopesticide. In environmental impact terms, the narrow spectrum of biopesticide such as Bt has been seen as an advantage. However, this has proved to be a double-edged sword in that, in commercial terms, a broader spectrum of activity is usually required. In practice, these demands are not mutually exclusive, and the recent explosion of research into Bt biology has yielded novel isolates (and an increasing number of *cry* genes) with activities well beyond that associated with Bt until recently. As long as it can be demonstrated that the toxicological activity of these novel isolates does not extend to non-target (beneficial) species, i.e., that they retain the environmental and safety advantages traditionally associated with Bt, then an extension in the sepctrum of activity of selected Bt isolates can be exploited commercially. Various mutant forms of insecticidal proteins with improved biological activity will be created in the future by fusing diverse domains and *in vitro* mutagenesis of genes that codes for these biological agents. Bt transgenic plants hold great promise as an important new tool in integrated pest management programs. This technology allows the crop plant to deliver its own means of protection against insect attack. The expected result is a very specific and direct biological control method that is environmentally sound and that can be expected to reduce the need for manual and chemical inputs by the grower. Commercial bioinsecticide formulations are generally ineffective in controlling cotton bollworm and European corn borer in which topical applications of the powder do not reach the inside of the plant tissue where the insects bore and feed. Such transgenic crops provide farmers a means of controlling a serious insect pest that is not easily controlled by current chemical pesticides. However total reliance on any one method (or tool) for pest control will inevitably lead to rapid buildup of resistance in the pest populations. Thus strategies are needed which integrate the use of a wide range of crop protection agents, e.g., synthetic, naturally-occurring and behaviour modifying chemicals together with biorational, microbial and cultural methods.

**Table 1.** Serovars of *Bacillus thuringiensis*

| S.No | Serovar | S.No | Serovar | S.No | Serovar |
|---|---|---|---|---|---|
| 1. | *thuringiensis* | 28. | yosoo | 55. | *jinghongiensis* |
| 2. | *finitimus* | 29. | *tochgiensis* | 56. | *guiyangiensis* |
| 3. | *alesti* | 30. | *yunnanensis* | 57. | *higo* |
| 4. | *kurstaki* | 31. | *pondicheriensis* | 58. | *roskildiensis* |
| 5. | *sumiyoshiensis* | 32. | *colmeri* | 59. | *chanpaisis* |
| 6. | *fukuokaensis* | 33. | *shandongiensis* | 60. | *wratislaviensis* |
| 7. | *sotto* | 34. | *japonensis* | 61. | *balearica* |
| 8. | *kenyae* | 35. | *neoleonensis* | 62. | *muju* |
| 9. | *galleriae* | 36. | *novosibirsk* | 63. | *navarrensis* |
| 10. | *canadensis* | 37. | *coreanensis* | 64. | *xiaguangiensis* |
| 11. | *entomocidus* | 38. | *silo* | 65. | *kim* |
| 12. | *aizawai* | 39. | *mexicanensis* | 66. | *asturiensis* |
| 13. | *morrisoni* | 40. | *monterrey* | 67. | *poloniensis* |
| 14. | *ostriniae* | 41. | *jagathesan* | 68. | *palmanyolensis* |
| 15. | *nigeriensis* | 42. | *amagiensis* | 69. | *rongseni* |
| 16. | *tolworthi* | 43. | *medellin* | 70. | *pirenaica* |
| 17. | *darmstadiensis* | 44. | *toguchini* | 71. | *argentinensis* |
| 18. | *londrina* | 45. | *cameroun* | 72. | *iberica* |
| 19. | *toumanoff.* | 46. | *leesis* | 73. | *pingluonsis* |
| 20. | *kyushuensis* | 47. | *konkukian* | 74. | *sylvestriensis* |
| 21. | *thompsoni* | 48. | *seoulensis* | 75. | *zhaodongensis* |
| 22. | *pakistani* | 49. | *malaysiensis* | 76. | *bolivia* |
| 23. | *israelensis* | 50. | *andaluciensis* | 77. | *azorensis* |
| 24. | *dakota* | 51. | *oswaldocruzi* | 78. | *pulsiensis* |
| 25. | *indiana* | 52. | *brasiliensis* | 79. | *graciosensis* |
| 26. | *tohokuensis* | 53. | *huazhongensis* | 80. | *vazensis* |
| 27. | *kumamotoensis* | 54. | *sooncheon* | | |

(Based on Lecadet *et al.* 1998.)

Table 2. Transgenic plants expressing crystal protein genes from *Bt*

| Sl. No. | Crystal proteins | Target insects | Transformed plants |
|---|---|---|---|
| 1. | Cry1Aa | Lepidoptera | cranberry, poplar, rutabaga |
| 2. | Cry1Ab | Lepidoptera | Apple, cotton, maize, poplar, potato, rice, tobacco, tomato, white clover, white spruce |
| 3. | Cry1Ac | Lepidoptera | Apple, broccoli, cabbage, cotton, grapevine, oilseed rape, peanut, rice soybean, tobacco, tomato, walnut |
| 4. | Cry1Ba | Lepidoptera | White clover |
| 5. | Cry1Ca | Lepidoptera | Alfalfa, arabidopsis, tobacco |
| 6. | Cry1H | Lepidoptera | Maize |
| 7. | Cry2Aa | Lepidoptera | Eggplant, potato, tobacco |
| 8. | Cry3A | Coleoptera | Cotton, rice |
| 9. | Cry6A | Coleoptera | Alfalfa |
| 10. | Cry9A | Lepidoptera | Maize |
| 11. | Bt | unspecified | Juneberry, hawthorn, pear, sugar cane |

(From Schuler *et al.* 1998)

Table 3. Natural and genetically modified Bt products registered for agricultural use*

| BT STRAIN | COMPANY | PRODUCT | TARGET INSECTS |
|---|---|---|---|
| (a) Natural | | | |
| *kurstaki* HD-1 | Abbot, USA | Biobit, dipel, Foray | Lepidoptera |
| *kurstaki* HD-1 | Thermo Trilogy, USA | Javelin, Steward, Thuricide | Lepidoptera |
| *aizawai* | Abbot | Florbac, Xentari | Lepidoptera |
| *tenebrionis* | Abbot | Novodar | Coleoptera |
| *tenebrionis* | Thermal Trilogy | Trident | Coleoptera |
| *galleriae* | SPIC, India | Spicturin | Lepidoptera |
| YB-1520 | Huazhong Agric. University, China | Mainfeng pesticide | Lepidoptera |
| (b) Genetically modified | | | |
| *aizawai* & *kurstaki* | Thermo Trilogy | Agree, Design | Lepidoptera |
| *kurstaki* | Ecogen, USA | Condor, Cutlass Crymax, Leptinox | Lepidoptera |
| *kurstaki* | Ecogen | Raven | Lepidoptera |
| Bt toxin encapsulated in *Pseudomonas fluorescens* | Mycogen, USA | MVP | Coleoptera |
| | | MATTCH | Lepidoptera |
| | | MTRACK | Lepidoptera |
| | | Cellcap® | Coleoptera |

*Based on Navon (2000)

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