

---

## 9. Insect Resistance to BT Toxins and Its Management

**Honnakerappa S. Ballari, Arun Kumar K. M.**

Assistant Professor,  
SOAS,  
Malla Reddy University,  
Hyderabad (Telangana).

### **Abstract:**

*Bacillus thuringiensis (Bt) a spore producing, gram-positive, facultative anaerobic bacteria persist in soil. The synthesis endotoxins or Cry proteins, during sporulation is its main hallmark. These proteins toxic to certain group of insects. Bt is the most frequently used bio pesticide in the world due to the enormous variety of these poisons, their effectiveness, and their comparatively low cost of manufacture. Most commonly lepidopteran and coleopteran larvae are the targets in the control of agricultural crop pests, and it is particularly useful in the development of new plant varieties carrying Bt cry genes. Even Bt can be used to successfully control populations of a number of dipteran disease vectors, which benefits human health. This book chapter objectives are to give a general overview of Bt application in the crop protection and to address the issue of the appearance of insects that are resistant to this bio pesticide. Following a discussion of the biology of this entomopathogenic microorganism, several examples of the use of commercially available Bt products as sprays or in transgenic plants will be provided. The main method for using Bt transgenic plants to stop or postpone the development of resistance in target insect populations was detailed in the last section.*

### **Keywords:**

*Bacillus thuringiensis, Toxins, Resistance, IRM.*

### **9.1 Introduction:**

The bacterium *Bacillus thurengensis* which is extensively distributed, rod-shaped, sporulating, and gram-positive, has been isolated from a variety of ecosystems, including soil, water, dead insects, silo dust, deciduous tree leaves, various conifers, insectivorous mammals, and human tissues with severe necrosis. The bacteriologist from Japan S. Ishiwata initially isolated Bt in 1901 from infected silk worms, *Bombyx mori* (L.). Then, in 1911, it was once again found by the German scientist Berliner, who isolated it from infected chrysalids of the Mediterranean flour moth *Ephestia kuehniella* (Zell. ), which were gathered at a mill in the Thuringe province (Berliner, 1915), he gave the organism the name *Bacillus thuringiensis*. Because of Bt preparations could quickly kill insect larvae in modest quantities, agronomists became intrigued by their entomopathogenic capabilities. The vast array of insecticidal proteins that Bt strains produce are effective against the larvae of a wide range of insect orders as well as, in certain circumstances, against species from different phyla. The genes producing insecticidal proteins have been effectively employed

in novel insecticidal formulations and in the development of transgenic crops, making Bt-based products the best-selling biological insecticides to date. The first formulation based on Bt was developed in France in 1938, under the name “Sporéine”, but the first well-documented industrial procedure for producing a Bt-based product dates from 1959, with the manufacture of “Bactospéine” under the first French patent for a biopesticide formulation. The first Bt-based formulation, known as "Sporéine," was created in France in 1938, but the production of "Bactospéine," the product covered by the country's first biopesticide formulation patent, did not follow a well-documented industrial process until 1959. Spore/crystal preparations derived from cultures in fermentors make up commercial Bt formulations. The preparations are dried and utilised in granulated form or as a wettable powder for spraying.  $\delta$  endotoxins are extremely diverse, resulting in a relatively constrained action spectrum for each specific toxin, and are safe for plants, animals, and practically all non-target insects to consume (Marvier *et al.*, 2007).

More than 700 cry gene sequences that produce crystal (Cry) proteins have been discovered in long before decades, and big plasmids seem to be where these genes are typically found. While many Cry proteins have beneficial pesticidal qualities that can be used to control insect pests in agriculture, other proteins generated by Bt strains as parasporal crystals have no known invertebrate target and have been referred to as parasporins (Palma *et al.*, 2014). Additionally, during the vegetative development phase, Bt isolates produce additional insecticidal proteins that are subsequently secreted into the culture media and are known as vegetative insecticidal proteins (Vip). In addition, Vip proteins are divided into four families—Vip1, Vip2, Vip3, and Vip4—based on how similar their amino acid sequences are (Warren *et al.*, 1988). As an alternative to conventional pesticides, Bt crystal and released soluble toxins have grown in importance due to their excellent host specificity. In order to discover and characterise new insecticidal proteins with various specificities, the utility of these insecticidal proteins has also prompted the quest for new Bt isolates from the most varied ecosystems. A pluripotent nature of some toxins is suggested by the fact that some of these isolates demonstrate novel and unexpected toxic actions against species other than insects.

At the end of the 19th century, various trailblazing scientists, notably Louis Pasteur, first suggested the use of entomopathogenic microorganisms for controlling the populations of insect pests. Since then, a wide variety of microbes, including bacteria, viruses, fungi, and protozoans, have been identified as prospective candidates for use in biocontrol techniques against insect pests (Riba and Silvy, 1989). These bio pesticides, which also provide the advantage of having just a little influence on the environment, have come to occupy a stable, although modest position in the insecticide market in light of the unfavourable impacts of chemical insecticides and the public health issues in tropical nations. Currently, the biopesticide market represents 2% of the approximately 600 million US dollar global crop protection business. *Bacillus thuringiensis* (Bt)-based products account for 90% of all biopesticide sales. This achievement is due to a variety of factors: The larvicidal action of Bt is rapid but sustained, Bt can be administered using ordinary equipment, and its impacts on beneficial insects and non-target organisms are minimal, among other factors, all contribute to its achievement. Biotech corporations, who started putting Bt genes into numerous crop plants, including cotton and maize, towards the end of the 1980s, have not been able to ignore the benefits of Bt.

By producing Bt toxins in diverse tissues as a result of the insertion of these genes, the plant is defended against attacks from numerous seriously harmful pests. How *Bacillus thuringiensis* is made up of bacteria from the *Bacillus cereus sensu lato* group that are able to produce a protein crystal during sporulation that contains  $\delta$ -endotoxins that have insecticidal activity. The crystalline inclusion may account for around 25% of the dry weight of the bacterium (Figure 9.1).

The bacterium *Bacillus thuringiensis* which is considerably distributed, rod-shaped, sporulating, and gram-positive, has been insulated from a variety of ecosystems, including soil, water, dead insects, silo dust, evanescent tree leaves, colorful conifers, insectivorous mammals, and mortal apkins with flinty necrosis. The bacteriologist from Japan S. Ishiwata firstly isolated Bt in 1901 from infected silk worms, *Bombyx mori* (L.). Similarly, in 1911, it was onetime over set up by the German scientist Berliner, who isolated it from infected chrysalids of the Mediterranean flour moth *Ephesia kuehniella* (Zell.), which were gathered at a plant in the Thuringe fiefdom (Berliner, 1915), he gave the organism the name *Bacillus thuringiensis*. Because of Bt medications could snappily kill nonentity naiads in modest amounts, agriculturists came intrigued by their entomopathogenic capabilities. The vast array of insecticidal proteins that Bt strains produce are effective against the naiads of a wide range of nonentity orders as well as, in certain circumstances, against species from different phyla. The genes producing insecticidal proteins have been effectively employed in new insecticidal phrasings and in the development of transgenic crops, making Bt-grounded products the best-dealing natural germicides to date. The first expression grounded on Bt was developed in France in 1938, under the name "Sporéine", but the first well-proved artificial procedure for producing a Bt-grounded product dates from 1959, with the manufacture of "Bactospéine" under the first French patent for a biopesticide expression. The first Bt-grounded expression, known as "Sporéine," was created in France in 1938, but the product of "Bactospéine," the product covered by the country's first biopesticide expression patent, didn't follow a well-proved artificial process until 1959. Spore/demitasse medications deduced from societies in fermentors make up marketable Bt phrasings. The medications are dried and utilised in grained form or as a wetttable greasepaint for scattering.  $\delta$  endotoxins are extremely different, performing in a fairly constrained action diapason for each specific poison, and are safe for plants, creatures, and virtually all non-target insects to consume (Marvier *et al.*, 2007).

More than 700 cry gene sequences that produce demitasse (Cry) proteins have been discovered in long before decades, and big plasmids feel to be where these genes are generally set up. While numerous Cry proteins have salutary pesticidal rates that can be used to control nonentity pests in husbandry, other proteins generated by Bt strains as parasporal chargers have no given brute target and have been appertained to as parasporins (Palma *et al.*, 2014). also, during the vegetative development phase, Bt isolates produce fresh insecticidal proteins that are latterly buried into the culture media and are known as vegetative insecticidal proteins (personality). In addition, personality proteins are divided into four families — Vip1, Vip2, Vip3, and Vip4 — grounded on how analogous their amino acid sequences are (Warren *et al.*, 1988). As an volition to conventional fungicides, Bt demitasse and released answerable poisons have grown in significance due to their excellent host particularity. In order to discover and characterise new insecticidal proteins with colorful particularity, the mileage of these insecticidal proteins has also urged the hunt for new Bt isolates from the most varied ecosystems.

A pluripotent nature of some poisons is suggested by the fact that some of these isolates demonstrate new and unanticipated poisonous conduct against species other than insects. At the end of the 19th century, colorful trailblazing scientists, especially Louis Pasteur, first suggested the use of entomopathogenic microorganisms for controlling the populations of nonentity pests. Since also, a wide variety of microbes, including bacteria, contagions, fungi, and protozoans, have been linked as prospective campaigners for use in biocontrol ways against nonentity pests (Riba and Silvy, 1989). These bio fungicides, which also give the advantage of having just a little influence on the terrain, have come to enthrall a stable, although modest position in the germicide request in light of the unfavourable impacts of chemical germicides and the public health issues in tropical nations. Presently, the biopesticide request represents 2 of the roughly 600 million US bone

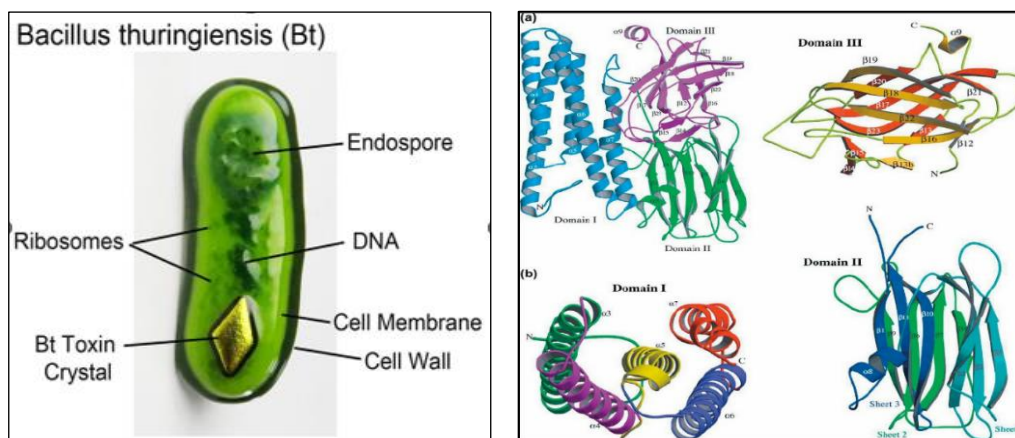
Global crop protection business. *Bacillus thuringiensis* (Bt)- grounded products regard for 90 of all biopesticide deals. This achievement is due to a variety of factors The larvicidal action of Bt is rapid-fire but sustained, Bt can be administered using ordinary outfit, and its impacts on salutary insects and non-target organisms are minimum, among other factors, all contribute to its achievement. Biotech pots, who started putting Bt genes into multitudinous crop plants, including cotton and sludge, towards the end of the 1980s, haven't been suitable to ignore the benefits of Bt. By producing Bt poisons in different apkins as a result of the insertion of these genes, the factory is defended against attacks from multitudinous seriously dangerous pests. How *Bacillus thuringiensis* is made up of bacteria from the *Bacillus cereus* sensu lato group that are suitable to produce a protein demitasse during sporulation that contains  $\delta$ - endotoxins that have insecticidal exertion. The crystalline addition may regard for around 25 of the dry weight of the bacterium (Figure 9.1).

## **9.2 Structure of Bt Toxin:**

A variety of Cry toxins, including Cry3Aa, Cry1Aa, Cry1Ac, Cry2Aa, Cry3Bb, Cry4Ba, Cry4Aa, and Cry8Ea1, have published three-dimensional structures. All Cry toxins have three structural domains (Figure 10.1) and are topologically quite similar (Donovan *et al.*, 2006; Crickmore *et al.*, 1988). Seven  $\alpha$ -helices are bundled together and joined by loops to form Domain I. The central amphipathic helix of the  $\alpha$ -helical bundle is largely preserved across all the toxins mentioned. Different Domain I mutations seem to eliminate toxicity but not binding to cellular receptors. It is unknown if these changes change the toxin molecule's overall shape, reducing its toxicity. Domain II consists of three sets of antiparallel  $\beta$  sheets, each terminating with a loop. The beta sheets form a beta-prism structure, which is centred on a hydrophobic core. Two antiparallel  $\beta$ -sheets sandwiched together to form Domain III have a "jelly-roll" structure. Strong support for Domains II and III's participation in receptor binding and insecticidal activity is provided by the results of site-directed mutagenesis and truncation studies (Crickmore *et al.*, 1988). The hydrophobic patterns found in domain I, which are thought to generate ion channels in the cell membrane, affect toxicity. As with other bacterial toxins, the domain undergoes refolding when it comes into contact with the cell membrane which helps in toxin insertion. According to several articles, toxicity is caused by the orientation of hydrophobic  $\alpha$ -4 and  $\alpha$ -5 helices that intrude into the membrane. However, these statements are not supported by in situ or in vivo research. The Cry toxins' most divergent domain, Domain II, can have an impact on host specificity if it is switched out for domains II or III of other toxins. The antiparallel  $\beta$ -sheet loops, which connect the strands, are visible at the top of the domain and are the least

conserved among the Cry toxins. It's interesting to note that the length of the apical loops in the Cry1A, Cry2A, Cry3A, Cry4A, and Cry5A toxins varies greatly. The longest loop is found in the Cry5Aa toxin, whereas the smallest is found in Cry3Aa. It is unknown how loop length affects domain organisation and function. The length of the loops unquestionably affects Domain II's configuration and, most likely, the three domains' interactions as well as the specific poisons' ability to attach to their appropriate receptors. Numerous researchers have hypothesised that shorter loops are more likely to disrupt the Domain II core -sheets' structure and, as a result, prevent Domains I and II from interacting (Ibrahim *et al.*, 2010). The loops appear to be crucial components in receptor recognition, binding, and specificity, regardless of their structural or functional significance. Figure 9.1: Toxin's three-dimensional structures (Crickmore *et al.*, 1988).

The development of channels in the cell membrane and the binding of receptors have both been linked to domain III. The insect specificity of some Cry1 toxins has changed as a result of in vitro Domain III switching. It has been proposed that domain III swapping is a mechanism of evolution and that this activity may be in charge of the creation of poisons with various specificities. Toxins having dual specificity, particularly to moths and beetles, such as CryII, are examples of substances that may have naturally undergone domain shifting (Demaagd *et al.*, 2010).



**Figure 9.1: General structure of Bt and three-dimensional structures of toxin** [5]

### 9.3 Mode of Action:

There are several models reviewed in the literature that essay to explain how cry poisons put out their clean up capacity, but only two are extensively accepted. The first one presupposition that cry poison binds to midgut receptors, oligomerizes, and inserts into the membrane to form lytic pores (Figure 9.2). The discovery of ion fluxing in encounter border membrane vesicles and synthetic lipid bilayers treated with Cry poisons is the base for the proposition that Cry poisons assemble lytic pores in the tube membrane by forming oligomers. Still, no direct substantiation has been presented for such a medium in either living cells or a nonentity. In reality, it has been demonstrated that poisons integrated into living cells' tube membranes don't produce lytic holes and aren't dangerous. Likewise, exploration on mutant Cry poisons shows that neither poison oligomers nor original

variations in the permeability of membrane vesicles are directly identified with toxin. Figure 9.2 Depicting medium of mode of action (6) Advanced alternate model (Figure 9.3) challenges the notion that Cry poison kills cells simply by bibulous lysis (Grochulski *et al.*, 1995). poison monomer rather attaches to the cadherin receptor BT- R1 and triggers the Mg2 dependent signal- transduction pathway, causing cell death. The model shows that Cry1Ab oligomers incorporated into cell membranes don't relate with cytotoxicity in living cells. Contrary to what has been suggested, poison exertion is significantly more complex than poison- convinced bibulous lysis. The largely conserved structural motif in the cadherin receptor BT- R1 is where the univalent list of the cry poison occurs to begin the complicated, dynamic process of cry poison action. In turn, a waterfall of events is touched off that leads to a form of programmed cell death appertained to as oncosis. When the Cry1Ab poison binds to the BT- R1 receptor, it triggers a chemical signal that stimulates the heterotrimeric G protein and adenylyl cyclase, along with a significant boost in cAMP conflation. Protein kinase A is actuated by cAMP, which results in a variety of cellular changes similar as cytoskel et al reorganisation and ion fluxing. Acceleration of this alternate runner pathway causes cell death by changing the chemistry of the cell. also, the poison promotes the exocytotic translocation of BT- R1 from intracellular membrane vesicles to the cell membrane as part of the payoff medium. poison- convinced signal-transduction controls the movement of the receptor, and the prosecution of cell death is directly connected with the breadth of this signalling(Zhang *et al.*, 2005).

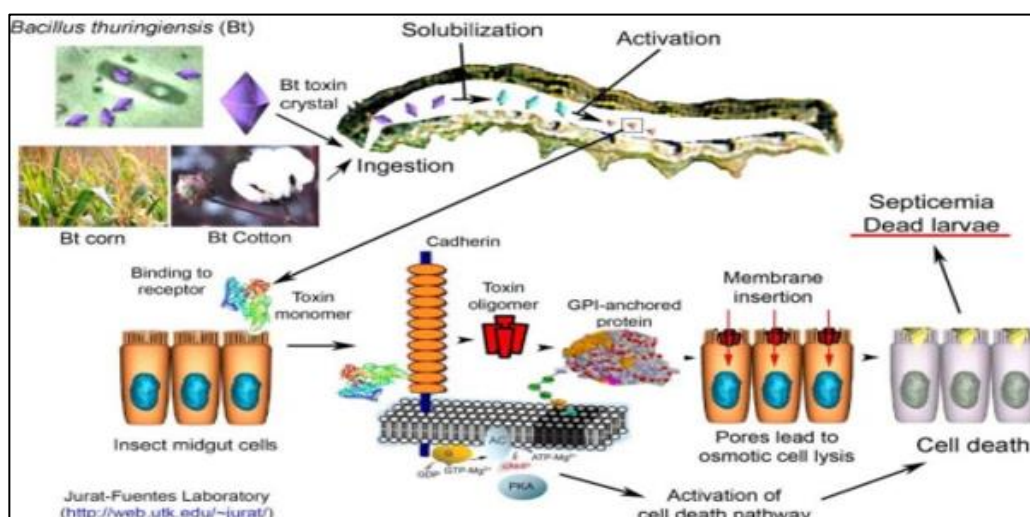


Figure 9.2: Showing Mechanism of Mode of Action (Zhang *et al.*, 2005).

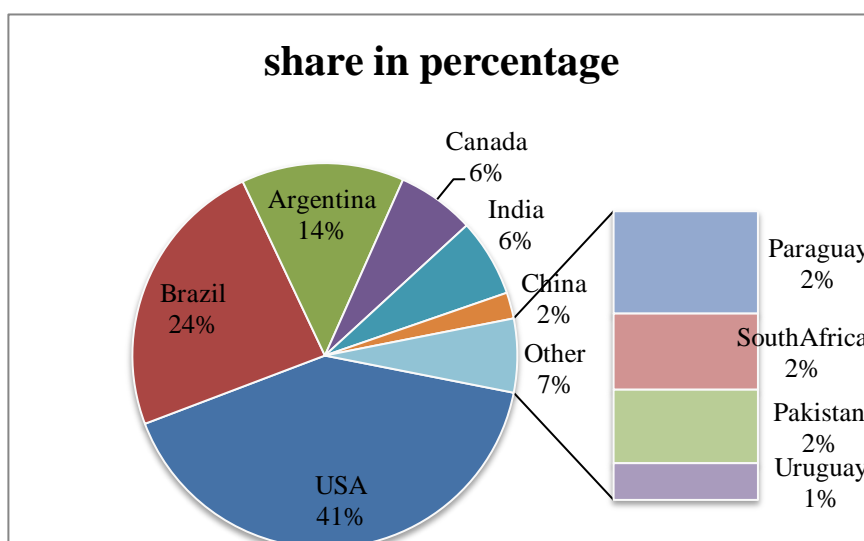
#### 9.4 Global Area Under Biotech Crops:

Biotech crops are grown globally over an area of 181.5 million hectares at an annual growth rate of 3-4% from 175.2 million hectares in 2013 to 181.5 million hectares. From 1.7 million hectares in 1996 to 181.5 million hectares in 2014, the global area of biotech crops has expanded 100-fold, making them the fastest-adopted crop technology in recent years. Regarding sustainability, resilience, and the substantial advantages it offers to both small and large farmers as well as consumers, this excellent adoption rate speaks for itself.

Twenty years after its debut, just a small number of nations still contain the vast majority of the acreage planted to GM crops. In 2014, the US cultivated 40.3% (73.1 million hectares) of the world's GM crop acreage, making it the largest cultivator and the first country to adopt GM crops (Table 9.1).

**Table 9.1: Bt Transgenic Crops Area and Its Distribution in World (Bravo *Et AL.*, 2007)**

Rank	Country	Area (mha)	Biotech crops
1	USA	73.1	Maize, soybean, cotton, canola, sugarbeet, alfalfa, papaya, squash
2	Brazil	42.2	Maize, soybean, cotton
3	Argentina	24.3	Maize, soybean, cotton
4	Canada	11.6	Canola, Maize, soybean, sugarbeet
5	India	11.6	Cotton
6	China	3.9	Cotton, papaya
7	Paraguay	3.9	Soybean, maize, cotton
8	South Africa	2.9	Soybean, maize, cotton
9	Pakistan	2.8	Cotton
10	Uruguay	1.4	Soybean, maize,
11	Bolivia	1.0	soybean
12	Philippines	0.8	Maize
13	Australia	0.7	Cotton, canola
14	Myanmar	0.3	Cotton
15	Others	<0.1	Maize, cotton, soybean, canola
		181.48m ha	



**Figure 9.3: Bt Transgenic Crops Area and Its Distribution in World (Bravo *Et AL.*, 2007)**

### 9.4.1 Major Transgenic Crops:

Ninety-nine per cent of the acreage used for GM crops globally is made up of four crops. These include canola, soy, maize and cotton. GM soybeans are grown on half of the world's GM hectares. 30% of the total global GM area is made up of GM maize, and another 14% is made up of GM cotton. 5% of the global GM hectares are made up of GM canola (Table 9.2). The development of new constructions of Bt toxin genes with promoters to be expressed in monocots or dicots in diverse tissues of the plant, including the integration of a native Bt gene into the chloroplast genome of tobacco, was made possible by advancements in biotechnological techniques. Since the toxin gene does not need to be altered for increased production because the chloroplast genome is bacterial in origin. This method offers fresh opportunities for Bt plant breeding in the future, even though it is not yet commercially available (James, 2015).

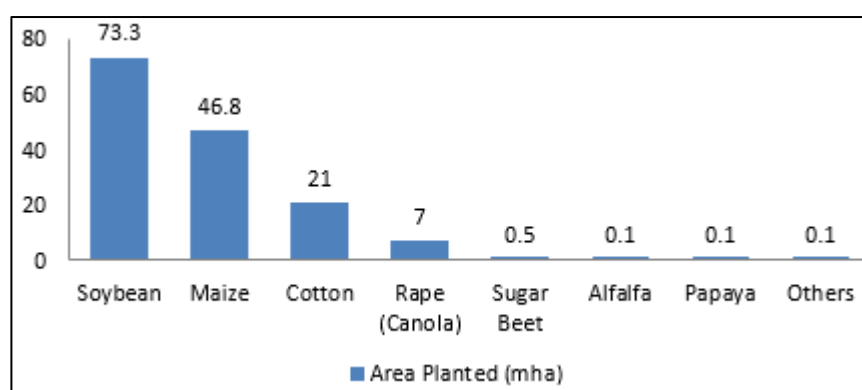


Figure 9.4: Major Transgenic Crops

### 9.4.2 GM Transgenic Crops Expressing Bt Toxins

Crop plants have been treated with Bt toxins to protect them against several types of insect pests. By the number of employees, a succinct summary was provided (MacBride et al., 1995) in Table 9.3. Bt toxins are currently being used to modify a variety of crops, including vegetables, forage crops, root crops, cereals, and trees, to provide protection against insects (Jouanin et al., 1998).

Table 9.3: Transgenic Plants Expressing Bt Toxins in different important crops (Shelton et al., 2000)

Crop	Gene	Target pest
Cotton	<i>cryIAb/cryIAc</i>	Bollworms
Corn	<i>cryIAb</i>	European corn borer
Potato	<i>cry3a</i>	Colorado potato beetle
Rice	<i>cryIAb/cryIAc</i>	Stem borers and leaf folders
Tomato	Cry1 Ac	Fruit borer



Crop	Gene	Target pest
Brinjal	<i>cryIAb/cryIB</i>	Shoot and fruit borer
Canola	<i>cryIAc</i>	Diamondback moth
Soybean	<i>cryIAc</i>	Soybean looper
Corn	<i>cryIAb/cryIA</i>	European corn borer
Potato	<i>cryIAb</i>	Tuber moth

### 9.5 Insects Resistance Against Bt Crops:

Resistance is a heritable alteration in a pest population's sensitivity that is reflected in a product's repeated inability to provide the desired level of control (Fontes *et al.*, 2002). Due to the intense temporal and geographical selection pressure of Bt toxins regulated by a single gene, the potential for rapid evolution of insect resistance is the most urgent concern relating to the practical application of transgenic plants in agricultural systems (Groot *et al.*, 2001). In the lab, roughly 17 insect species have already developed resistance to Bt, but only one has demonstrated broad resistance in the field. It is widely believed that resistance will eventually appear in Bt-plants (Ferre and Van, 2002).

The development of resistance will put the use of related Bt bio-pesticides at risk for all users, including those not using transgenic technologies, in addition to the costs associated with the loss of the product and the development of alternative control strategies [either transgenic or conventional] (Andow,2002).

Many researchers came to the conclusion that field-evolved resistance in some populations of 5 of 13 species of important pests by 2010 compared with only one such species in 2005 (McGaughey *et al.*, 1998) in Table 9.4 after conducting 77 studies in eight countries. Increases in the area planted to Bt crops, the number of pest populations exposed to Bt crops, and the cumulative duration of exposure are among the factors causing this rise in recorded occurrences of resistance.

The United States, which accounts for over half of the global Bt crop area each year, is home to three of the five resistant pests. The other two resistant pests originate from South Africa and India. Four of the five resistant pests are caterpillars; the fifth is an insidious beetle called western corn rootworm (*Diabrotica virgifera virgifera*).

**Table 9.4: Present status of resistance to Bt plants (McGaughey *et al.*,1998)**

Pest	Country	Gene	Crop	Year(i)	Year(r)	period
<i>Helicoverpazea</i>	USA	Cry1Ac	cotton	1996	2002	6 years
<i>Spodopterafrugiperda</i>	Puerto Rico	Cry1F	maize	2003	2007	4 years
<i>Busseolafusca</i>	South Africa	Cry1Ab	maize	1998	2004	6 years
<i>Pectinophoragossypiella</i>	India	Cry1Ac	cotton	2002	2009	7 years
<i>Dibarotica virgifera virgifera</i>	USA	Cry3Bb1	maize	2010	2013	3 years

## **9.6 Risk of Resistance for Bt:**

Insects can adapt to Bt proteins, just like they can to most insecticides. This risk may be increased in Bt by the following factors:

- Bt proteins are expressed at high levels in most or all plant tissues;
- The proteins are produced by the plant continually during the growing season
- Some of the major target pests, such as European corn borer, corn rootworm, and pink bollworm, feed almost exclusively on corn or cotton.

These factors can increase insect exposure to the controlling toxins (Bt protein) and hence, increase selection pressure for resistance. That means that if the toxin kills susceptible insects, those that survive and reproduce are more likely to be resistant to the toxin.

## **9.7 Insect Resistance Management:**

The commercialization of Bt crops—transgenic plants that express Bt proteins—for the management of insect pests is widespread. The threat that insect resistance poses to the continued use of Bt plant protectants has resulted in the development of the idea of managing insect resistance. IRM has acquired prominence since it is thought to be crucial to the sustainable use of Bt crops, which are genetically modified. It might be described as a strategy that delays the emergence of insect resistance to pesticides in the target pest populations. Insect resistance management (IRM) is an important part of stewarding this valuable technology. IRM requirements for Bt crops, however, fluctuate between nations because to variations in insect biology, farming methods, and experience.

The size and diversity of the agricultural systems in nations with small-scale farming systems present both potential and considerable obstacles for IRM. IRM initiatives in these nations should, to the greatest extent possible, be implemented through the technology suppliers rather than by pushing individual farmers to use revolutionary techniques. Alternative crop and non-crop hosts should be taken into account in appropriate IRM methods as sources of unstructured refuge, especially for highly polyphagous pests like the cotton bollworm *H. armigera*.

### **9.7.1 Refuges:**

Refuges are host plants that do not have the particular insect protective trait, allowing some of the target pest population to avoid exposure and preserve the population's vulnerability to the trait. Recessive Bt resistance requires both copies of the receptor gene to be lost or altered in order to develop resistance. Because the RS genotypes don't do well under survival conditions, refuge is more effective the less prevalent Bt resistance is.

The initially extremely uncommon RR genotypes are what promote the development of resistance, but for a very long time, they can only mate with the RS kinds. Planting refuges reduces the fitness difference between genotypes that are more and less resistant, which ultimately slows the evolution of resistance.

### **A. Refuge Approaches:**

- **Structured refuges** are an area of the farm that is solely used to grow non-Bt crops. These refuges are sown as separate fields (blocks), rows along the perimeter of Bt fields, or rows inside Bt fields. The size (as a proportion of the related Bt crop) and closeness to the Bt field(s) are the two most important factors for a structured refuge. In order for vulnerable insects from the refuge and resistant insects from the Bt fields to interact and reproduce, refuges must be able to produce a significant quantity of susceptible insects and present close enough to the Bt field.
- **Seed blends** (refuge-in-the-bag) seed mixtures combine Bt seed with non-Bt seed (refuge) in a single seed bag. The benefit of seed mixtures is that growers don't have to plan the planting of a separate refuge, guaranteeing refuge compliance. To present, some Bt maize PIP products have received approval for seed mixtures. For more information on the FIFRA Scientific Advisory Panel (SAP) sessions that the EPA has held on seed blends, check the links provided below under "Information Sources."
- **Natural refuge** refers to uncultivated plants, weeds, or natural hosts that might act as a supply of vulnerable insects. Such a refuge may be successful if the targeted pest(s) feed on a variety of plant hosts and are not restricted to the Bt crop. Only as an IRM technique for Bt cotton in the Southeast of the United States has natural refuge been approved. For more information, see the SAP links mentioned below under "Information Sources." The EPA sponsored a SAP meeting on natural refuge in 2006.

### **9.7.2 Multigene Strategy (Pyramided Plants):**

**Pyramiding** A specific example of gene stacking in which two or more genes combined in a single genotype give at least two mechanisms of action against the same target pest(s). to create crops that express a minimum of two poisonous chemicals that act separately, preventing the spread of resistance from one to the other.

With the release of Bollgard II, this strategy, known as gene pyramiding, was made commercially viable. a transgenic cotton plant that expresses both the Cry1Ac and Cry2Ab variants of the Bt protein. In that they bind to various midgut receptors in the insect, the two proteins function independently of one another.

Insects that are homozygous for numerous resistance genes are much more uncommon (Table 10.5) than those that are homozygous for only one resistance gene.

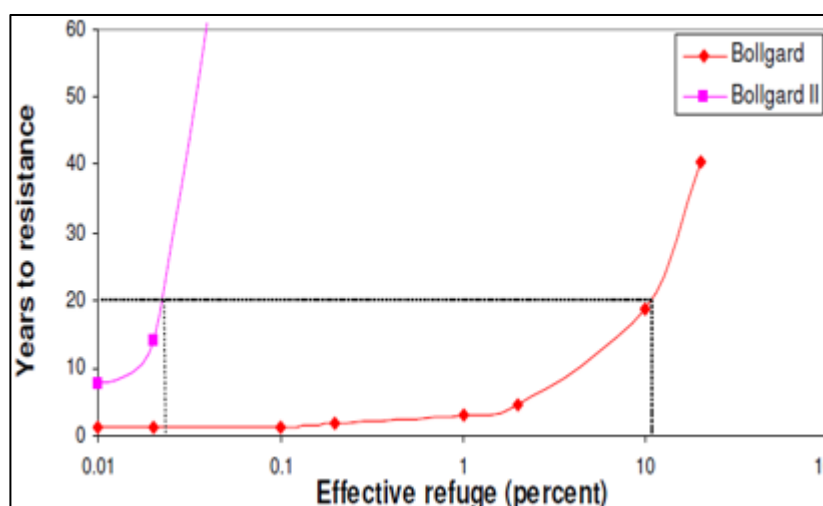
A species cannot easily evolve resistance to both toxins because that would require two simultaneous, independent mutations in genes encoding the receptors (Shelton *et al.*, 2000).

Successful resistance management is demonstrated by the low percentage of resistant people even after prolonged exposure to Bt cotton over many years (Jackson *et al.*, 2003).

Two Bt proteins, Cry1Ac and Cry2Ab2, are used in products like Bollgard II cotton to suppress lepidopteran pests. Since their modes of action are different, both proteins are more efficient than single-Bt products against the pests they are intended to control (Figure 9.4).

**Table 9.5: Bt toxin pyramids used proactively and separately from one-toxin plants or remedially and concurrent with one-toxin plants (Jackson *et al.*, 2003)**

Pest	Crop	Country	Toxins in pyramid	Resistance detected
<b>Proactive and separate from one-toxin plants</b>				
<i>H. armigera</i>	Cotton	Australia	Cry1Ac, Cry2Ab	None
<i>H. punctigera</i>	Cotton	Australia	Cry1Ac, Cry2Ab	None
<b>Remedial and concurrent with one-toxin plants</b>				
<i>D. virgifera</i>	Corn	USA	Cry3Bb, Cry34/35Ab	Cry3Bb
<i>H. zea</i>	Cotton	USA	Cry1Ac, Cry2Ab	Cry1Ac
<i>H. zea</i>	Cotton	USA	Cry1Ac, Cry1F	Cry1Ac
<i>P. gossypiella</i>	Cotton	India	Cry1Ac, Cry2Ab	Cry1Ac
<i>S. frugiperda</i>	Corn	USA	Cry1F, Cry1A.105b, Cry2Ab	Cry1F



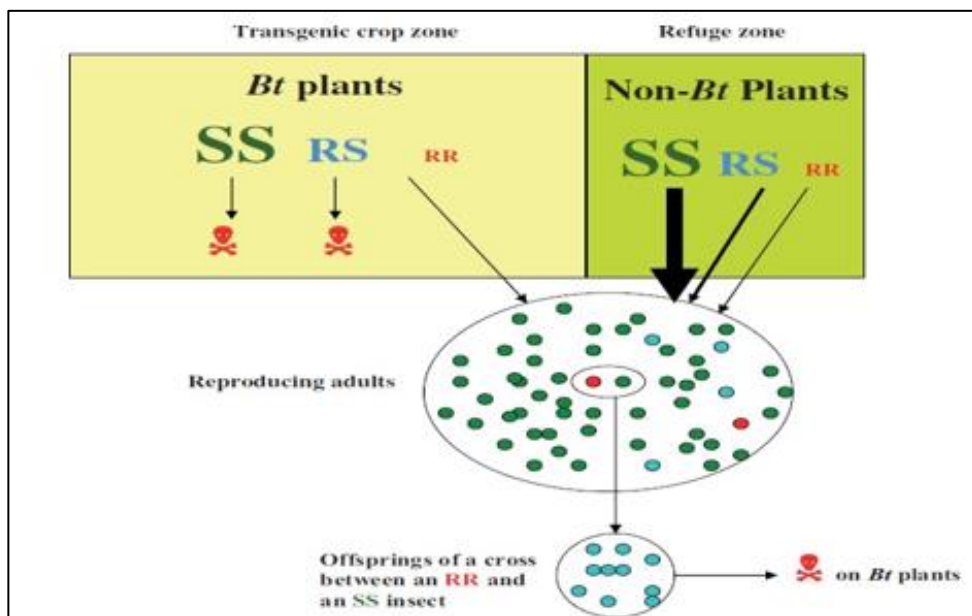
**Figure 9.4: Effect of Products with Two Bt Proteins on rate of resistance (Tabashnik *et al.*, 2013)**

### 9.7.3 High Dose Strategy:

A suitable resistance operation strategy is needed, according to the EPA and the 1998 Science Advisory Panel Subpanel, to help the emergence of insect resistance to the Bt proteins generated in transgenic crop plants. The 1998 Subpanel agreed that programmes for managing resistance should be grounded on the employment of both a high dose of Bt and structured refuges made to offer an acceptable number of adult insects that are susceptible to the substance. According to the high dose/ refuge method, there are three genotypes of Bt-resistant organisms' susceptible homozygotes (SS), heterozygotes (RS), and resistant homozygotes (RR). This is grounded on the supposition that Bt resistance is sheepish and is handed by a single locus with two alleles. also, it's assumed that resistant and susceptible grown-ups would constantly copulate at arbitrary and that there will be a low original resistance allele frequency.

Only a many extremely uncommon RR individualities would immaculately tolerate a big dose of Bt crop. The Bt toxin will affect those with both SS and RS. A structured refuge is a non-Bt area in a farmer's field or group of fields that supports the generation of susceptible (SS) insects that may aimlessly copulate with uncommon resistant (RR) insects who survive the Bt crop to induce susceptible RS heterozygotes that will be destroyed by the Bt crop. Insect populations will no longer contain resistant (R) alleles as a result, which will stop the evolution of resistance (Figure 9.5).

It has been discovered that a strategy to bring transgenic plants that synthesise extremely high degrees of insecticidal proteins is particularly efficacious in arresting or delaying the development of resistant insects.



**Figure 9.5: Schematic representation of the “high dose- refuge” (HDR) strategy. The success of the HDR program depends on resistance subsisting a exquisite and modest attribute and the genetically modified plants producing a dosage of poison sufficient to kill all homozygous susceptible entities (SS-green) and all heterozygous entities with for both resistance and vulnerability alleles (RS-blue) (Graham, 2010).**

## 9.8 Why IRM For Bt?

The effectiveness of Bt PIPs (Plant Incorporated Proteins) and the preservation of their substantial agricultural and environmental benefits are highly valued by EPA. In order to prevent the development of resistance in the target pests, the Agency is dedicated to maintaining adequate oversight of these products. The EPA has enforced the implementation of an Insect Resistance Management (IRM) strategy for each commercially registered Bt PIP in order to combat the threat of resistance. IRM aims to postpone the emergence of resistance for as long as possible, but it's vital to remember that resistance may not be completely prevented from developing.

## **9.9 Conclusion:**

In addition to reducing the need for pesticides and their expense, bt crops have provided a powerful tool for farmers and the environment in the fight against plant pests. Additionally, increased usage of transgenic crops for insect control is likely to involve additional cultivars with blends of two or more Bt toxins, including novel Bt toxins as VIP. However, modified Bt toxins that have undergone genetic engineering can kill insects that are resistant to traditional Bt toxins. With a wider variety of genetically modified crops and pest-targeting insect species, there is a likelihood that the adoption of transgenic crops will rise in developing countries.

Additionally, by integrating more effectively noticed patterns of field evolved resistance into future resistance management tactics, it may be possible to reduce the negative effects of present and upcoming generations of transgenic crops while maximising their advantages. To grow more crops, it is vital to utilise all possibility with the least amount of sacrifice. Bt insect resistance technology can benefit crops, farmers, and consumers alike when used in conjunction with good agricultural practises.

## **9.10 References:**

1. Andow DA. Resisting resistance to Bt-corn. In: Letourneau, D.K.; Burrows, B.E. ed. *Genetically modified organisms. Assessing environmental and human health effects.* CRC Press, Boca Raton, 2002, 99-124.
2. Bravo A, Gill SS, Soberón M. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon.* 2007; 49:423-435
3. Crickmore N, Zeigler DR, Feitelson J, Schnepf E, Van RJ, Lereclus D. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol Mol Biol Rev.* 1998; 62:807-813
4. Demaagd RA, Weemen-Hendriks M, Stiekema W, Bosch D. *Bacillus thuringiensis* delta-endotoxin Cry1C domain III can function as a specificity determinant for *Spodoptera exigua* in different, but not all, Cry1- Cry1C hybrids. *Appl Environ Microbiol.* 2000; 66:1559- 1563.
5. Donovan WP, Engleman JT, Donovan JC, Baum JA, Bunkers GJ, Chi DJ *et al.* Discovery and characterization of Sip1A: A novel secreted protein from *Bacillus thuringiensis* with activity against coleopteran larvae. *Appl. Microbiol. Biotechnol.* 2006; 72:713-719.
6. Ferre J, Van RJ. *Biochemistry and Genetics of Insect Resistance to Bacillus thuringiensis.* *Annual Review Entomology.* 2002; 47:501-533.
7. Fontes EMG, Carmen SS, Sujii PER, Panizzi AR. The Environmental Effects of Genetically Modified Crops Resistant to Insects. *Neotropical Entomology.* 2002; 31:497-513
8. Graham M. *Insect Resistance Management Strategies for Bt Crops in Small Scale Farming Systems* Head, Monsanto Company, St. Louis, USA, 2010, 1-34.
9. Grochulski P, Masson L, Borisova S, Puztai-Carey M, Schwartz JL, Brousseau R. *Bacillus thuringiensis* CryIA (a) insecticidal toxin: crystal structure and channel formation. *J Mol Biol.* 1995; 254:447-464.
10. Groot AT, Dicke M. Transgenic crops in an agroecological context: Multitrophic effects

- of insect-resistant plants. Wageningen University Press, the Netherlands. 2001, 76.
11. Ibrahim MA, Griko N, Junker M, Bulla LA, Bacillus thuringiensis A genomics and proteomics perspective., *Bioeng Bugs*. 2010; 1(1):31-50.
  12. Ishiwata S. (1901) On a kind of severe flacherie (sotto disease), *Dainihon Sanshi Kaiho* 114, 1–5.
  13. Jackson RE, Bradley JR, Van Duyn JW. Performance of feral and Cry1Ac-selected *Helicoverpa zea* (Lepidoptera: Noctuidae) strains on transgenic cottons expressing one or two *Bacillus thuringiensis* spp. Kurstaki proteins under greenhouse conditions. *J Entomol. Sci.* 2003; 39:46-55.
  14. James C. Global status of commercialized biotech/GM crops: lessons from the laboratory and field. *Journal of Economic Entomology*. 2015; 96:1031-1038
  15. Jouanin L, Bonade M, Girard C, Morrot G, Gibaud M, The design and implementation of insect resistance management programs for Bt crops. *Plant Sci.* 1998; 131(1):1-11.
  16. MacBride GB, Loftis JC, Adkins NC. What do significance tests really tell us about the environment? *Environ. Manage.* 1995; 17:423-432
  17. Marvier M., McCreedy C., Regetz J., Kareiva P. (2007) Meta-analysis of effects of Bt cotton and maize on nontarget invertebrates, *Science* 316, 1475–1477.
  18. McGaughey WH, Gould F, Gelernter W. Bt resistance management. *Nature Biotechnology*. 1998; 16:144-146
  19. Palma L, Munoz D, Berry C, Murillo J, Caballero P. *Bacillus thuringiensis* Toxins: An Overview of Their Biocidal Activity. *Toxins*. 2014; 6:3296-3325
  20. Riba G., Silvy C. (1989) *Combattre les ravageurs des cultures enjeux et perspectives*, INRA, Paris.
  21. Shelton AM, Tang JD, Rousch RT, Metz TD, Earle ED. Field tests on managing resistance to Bt-engineered plants. *Nature Biotechnol.* 2000; 18:339-342.
  22. Shelton AM, Tang JD, Rousch RT, Metz TD, Earle ED. Field tests on managing resistance to Bt-engineered plants. *Nature Biotechnol.* 2000; 18:339-342
  23. Tabashnik BE, Brevault T, Carriere Y. Insect resistance to Bt crops: Lessons from the first billion acres. *Nature Biotechnology*. 2013; 31:510-521.
  24. Warren GW, Koziel MG, Mullins MA, Nye GJ, Carr B, Desai NM *et al.* Auxiliary Proteins for Enhancing the Insecticidal Activity of Pesticidal Proteins. U.S. Patent. 1998; 5:770-696
  25. Zhang X, Candas M, Griko NB, Rose-Young L, Bulla LA. Cytotoxicity of *Bacillus thuringiensis* Cry1Ab toxin depends on specific binding of the toxin to the cadherin receptor BT-R1 expressed in insect cells. *Cell Death Differ.* 2005; 12:1407-1416.