Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(1): 941-948
© 2017 JEZS
Received: 06-11-2016
Accepted: 07-12-2016

Aijaz Ahmad Sheikh
Division of Entomology
Sheri-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K), India

Muneeb Ahmad Wani
Division of Floriculture & Landscape Architecture
Sheri-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K), India-190025]

Parveena Bano
Division of Entomology
Sheri-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K), India

Sajad Un Nabi
ICAR-CITH Old Air field Rangreth Srinagar (J&K), India

Tariq Ahmad Bhat
Division of vegetable science
Sheri-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K), India

Mohammad Amin Bhat
Division of Soil Science CCKV Hissar Haryana India

Mohd Saleem Dar
Division of Plant Pathology
Sheri-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K), India

Correspondence
Aijaz Ahmad Sheikh
Division of Entomology
Sheri-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K), India

An overview on resistance of insect pests against Bt Crops

Aijaz Ahmad Sheikh, Muneeb Ahmad Wani, Parveena Bano, Sajad Un Nabi, Tariq Ahmad Bhat, Mohammad Amin Bhat and Mohd Saleem Dar

Abstract
Bacillus thuringiensis (Bt) is a ubiquitous, rod-shaped and sporulating bacterium that produces a wide variety of insecticidal proteins active against larvae of very diverse insect orders. Once ingested by insects, these crystals are solubilized in the midgut, then proteolytically activated by midgut proteases and bound to specific receptors located in the insect cell membrane leading to cell disruption and insect death. The process of genetic transformation allows genes to be transferred from one organism/source to another, products developed through this procedure are known as genetically modified organisms (GMOs) or transgenic/biotech crops. Cry1A family is the most commonly used Bt toxins, especially Cry1Ac in transgenic Bt cotton and Cry1Ab in transgenic Bt corn. The genetically engineered insect-resistant crops (Bt crops) were first commercially grown in 1996 and adopted in different countries. The area of Bt crops planted each year continues to increase, with 181.48 million hectares grown in more than two dozen countries in 2015. The reason behind this widespread adoption of GM varieties is that it causes reduced purchases of costly inputs such as pesticide, while increases farm income along with the benefits to the environment. Genetic modification of plants has helped the agricultural system flourish; insects are beginning to evolve resistance to the Bt crops. Unfortunately, the field population of pests evolved resistance to different Bt toxins and the number of resistant species is going to increase, which is threatening to the continuous success of Bt crops. The number of resistant species has been increased worldwide, 13 cases of field-developed resistance to 5 Bt toxins in transgenic corn and cotton have been reported. Therefore, understanding of the molecular and genetic basis of resistance to Bt could help in designing a suitable management approach to delay the resistance development in the insect pests. To delay the onset of resistance, it is essential that farmers understand and implement Insect Resistance Management (IRM) practices. The tactics available for sustainable deployment of insect resistance genes in transgenic crops can be grouped into four strategies. These are not essentially mutually exclusive. Two or more strategies can be combined together by deploying one or several genes (Gene strategies), produced at high dose of the endotoxin (Dose strategies) and may be grown along with refuges, as mixtures or separate.

Keywords: Bt Crops, insect Resistance, Resistance Management

1. Introduction
Bacillus thuringiensis (Bt) is a ubiquitous Gram-positive, rod-shaped and sporulating bacterium that has been isolated worldwide from a great diversity of ecosystems including soil, water, dead insects, dust from silos, leaves from deciduous trees, diverse conifers and insectivorous mammals, as well as from human tissues with severe necrosis. Bt strains produce a wide variety of insecticidal proteins active against larvae of very diverse insect orders as well as, in some cases, against species from other phyla. This has led Bt-based products to become the best-selling biological insecticides to date, since the genes encoding insecticidal proteins have been successfully used in novel insecticidal formulations and in the construction of transgenic crops. Bt strains synthesize Crystal (Cry) and cytolytic (Cyt) toxins (also known as δ-endotoxins) at the onset of sporulation but during the stationary growth phase as parasporal crystalline inclusions. Once ingested by insects, these crystals are solubilized in the midgut and then proteolytically activated by midgut proteases and bind to specific receptors located in the insect cell membrane leading to cell disruption and insect death [1]
In the past decades, more than 700 *cry* gene sequences that code for crystal (Cry) proteins have been identified and large plasmids appear to be the usual location for these genes. While many Cry proteins have useful pesticidal properties and may be exploited for the control of insect pests in agriculture, other proteins produced as parasporal crystals by Bt strains have no known invertebrate target and have been termed as parasporins [2].

Bt isolates also synthesize other insecticidal proteins during the vegetative growth phase which are subsequently secreted into the culture medium and have been designated as vegetative insecticidal proteins (Vip). Moreover, Vip proteins are classified into four families Vip1, Vip2, Vip3 and Vip4 according to their degree of amino acid similarity [3]

Bt crystal and secreted soluble toxins are highly specific for their hosts and have gained worldwide importance as an alternative to chemical insecticides. The usefulness of these insecticidal proteins has also motivated the search for new Bt isolates from the most diverse habitats in order to identify and characterize new insecticidal proteins with different specificities. Some of these isolates exhibit novel and unexpected toxic activities against organisms other than insects, suggesting a pluripotential nature of some toxins.

1.1 Structure of Bt Toxin

The three-dimensional structures of a number of Cry toxins have been published viz. Cry3Aa, Cry1Aa, Cry1Ac, Cry2Aa, Cry3Bb, Cry4Ba, Cry4Aa and Cry8Ea1. All Cry toxins contain three (Fig.1) structural domains and share a high degree of topological similarity [4, 5]. Domain I is composed of a bundle of seven α-helices connected by loops. The α-helical bundle has a central amphipathic α helix that is well conserved among all the toxins described. Various mutations in Domain I appear to abolish toxicity but not binding to cellular receptors. Whether these mutations affect overall conformation of the toxin molecule, compromising toxicity, is not known. Domain II consists of three sets of antiparallel β-sheets, each terminating with a loop. The beta sheets are packed around a central hydrophobic core forming a so-called beta-prism structure. Domain III is a sandwich of two antiparallel β-sheets that form a "jelly-roll" topology. Results of site-directed mutagenesis and truncation analysis provide strong evidence for the involvement of Domains II and III in receptor binding and insecticidal activity [5].

Domain I purportedly functions to form ion channels in the cell membrane and the hydrophobic motifs within this domain are what effect toxicity. Upon contact with the cell membrane, the domain undergoes refolding to facilitate insertion of the toxin into the membrane as with other bacterial toxins. Several articles have reported that hydrophobic α-4 and α-5 helices insert into the membrane and that this orientation is responsible for toxicity. However, there is no *in situ* or *in vivo* evidence to support these claims

Domain II is the most divergent domain among the Cry toxins and its replacement or switching with domains II and III of other toxins can affect host specificity. The loops connecting strands of the antiparallel β-sheets are exposed at the apex of the domain and represent the least conserved regions amongst the Cry toxins. Interestingly, the apical loops of Cry1A, Cry2A, Cry3A, Cry4A and Cry5A toxins are highly variable in length. Cry5Aa toxin has the longest loop whereas Cry3Aa has the shortest one. What impact loop length has on domain structure and function is not known. Certainly, the span of the loops contributes to the configuration of Domain II and, most likely, influences the interactions of all three domains as well as the binding of individual toxins to their cognate receptors. Many workers have suggested that shorter loops are more likely to disturb the structure of the core β-sheets of Domain II and, consequently, interrupt the interaction of Domains I and II [6]. Whatever their structural or functional roles, the loops appear to be key elements in receptor recognition, binding and specificity.

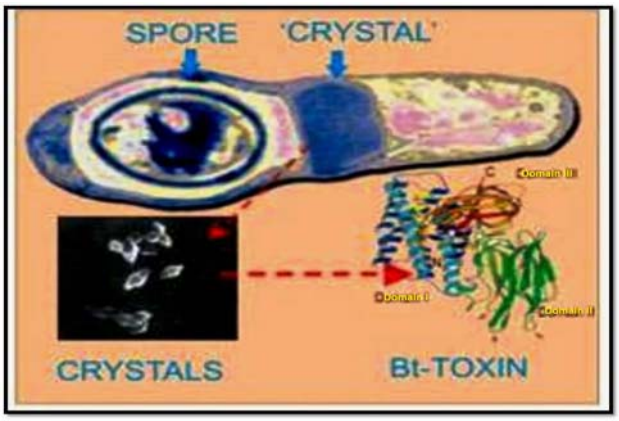

Fig 1: Three-dimensional structures of toxin [5]

Domain III has been correlated with receptor binding and channel formation in the cell membrane. *In vitro* Domain III swapping in certain Cry1 toxins, has resulted in alterations in insect specificity. Domain III swapping has been suggested as an evolutionary scheme and that such activity may be responsible for the emergence of toxins with varying specificities. Examples of toxins that may have undergone domain swapping naturally are toxins with dual specificity, especially to moths and beetles, such as Cry1I [7].

1.2 Bt Toxin Nomenclature

Since the identification and cloning of the first Bt insecticidal crystal protein gene in 1981, the number of genes coding for novel insecticidal proteins has continuously increased, generating the need for an organized nomenclature system. In the first such system, names for Cry toxins and their corresponding genes included a Roman numeral (primary rank distinction) depending on the insecticidal activity of the crystal protein, namely: CryI for proteins toxic for lepidopterans, CryII for proteins with toxicity against both lepidopterans and dipterans, CryIII for proteins toxic for coleopterans and CryIV for proteins toxic exclusively for dipterans [8]. However, this system exhibited important complications; for instance, the activity of new toxins had to be assayed against a growing list of insects before the gene and the toxin could be named, some novel homologous proteins were in fact non-toxic as expected, and others (e.g., Cry1I) exhibited dual toxicity against dipteran and lepidopteran species. To avoid these problems, the *Bacillus thuringiensis* Toxin Nomenclature Committee was created and a novel system of classification proposed [9]. In this new system, a novel toxin is given a four-rank name depending on its degree of pairwise amino acid identity to previously named toxins; additionally grouping by this criterion does not imply a similar protein structure, host range or even mode of action. Arabic numbers are used for the first and fourth ranks, and uppercase and lowercase letters are assigned for the second and third ranks, respectively (Fig. 2).

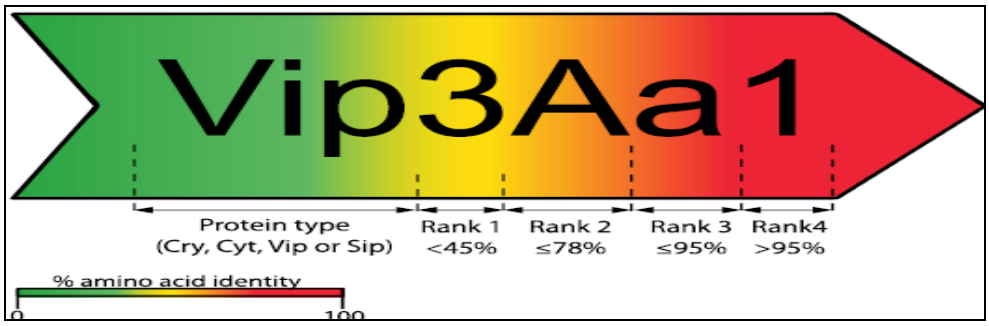

Fig 2: Schematic overview of the current nomenclature system used by the Bt Toxin nomenclature Committee for δ-endotoxins (Cry and Cyt) and secretable (Vip and Sip) toxins. In this example, numbers indicate different Vip proteins changing rank 1 depending of percentage amino acid similarity (for Vip proteins this rank may change to date among Vip1, Vip2, Vip3 and Vip4). The same rule applies for ranks 2, 3 and 4 assigning a different identification digit/letter [1]

In this way, proteins sharing less than 45% pairwise identity are assigned a different primary rank (an Arabic number e.g., Vip1 and Vip2); two proteins sharing less than 78% pairwise identity are assigned a different secondary rank (a capital letter e.g., Vip3A and Vip3C); proteins sharing less than 95% pairwise identity are assigned a different tertiary rank (a lowercase letter, e.g., Vip3Aa and Vip3Ab); and finally to differentiate between proteins sharing more than 95% pairwise identity, a quaternary rank is assigned (an Arabic number, e.g., Vip3Aa1 and Vip3Aa2) [10]. However, such quaternary ranks are assigned to each independently sequenced toxin-coding gene; therefore, although some proteins may have different quaternary ranks, they could actually share identical amino acid sequences. This nomenclature system is commonly applied to δ-endotoxins (Cry and Cyt) and secretable (Vip and Sip) Bt toxins.

1.3 Mode of Action

There are several models reviewed in the literature that seek to explain how Cry toxins exert their killing capacity, but only two are well accepted. The first one postulate that Cry toxin binds to midgut receptors, oligomerizes and inserts into the membrane to form lytic pores (Fig. 3). The notion that Cry toxins assemble lytic pores in the plasma membrane by forming oligomers is based on detection of ion fluxing in brush border membrane vesicles and synthetic lipid bilayers treated with Cry toxins but no direct evidence has been provided for such a mechanism in either living cells or an insect [11]. In fact, it has been shown that toxin oligomers incorporated into the plasma membrane of living cells do not form lytic pores and are not toxic. Furthermore, studies of mutated Cry toxins demonstrate that neither toxin oligomers nor commensurate changes in membrane vesicle permeability correlate directly with toxicity.

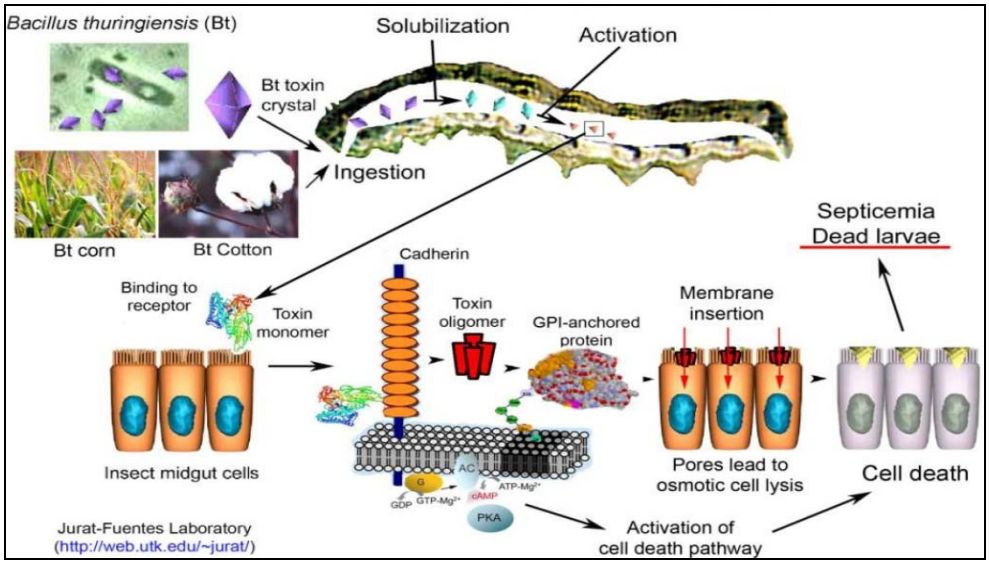

Fig 3: Showing mechanism of mode of action [6]

Advanced second model (Fig.3) challenges the notion that Cry toxin kills cells exclusively by osmotic lysis [12]. Instead, toxin monomer binds to the cadherin receptor BT-R_1 and activates Mg^{2+} dependent signal-transduction pathway leading to cell death. The model demonstrates that, in living cells Cry1Ab oligomers, integrated into the cell membrane do not correlate with cytotoxicity. Actually, toxin action is much more complicated than the proposed toxin-induced osmotic lysis. Cry toxin action is a complex, dynamic process that involves univalent binding of toxin to the highly conserved structural motif in the cadherin receptor BT-R_1. In turn, a cascade of events is triggered that leads to a form of programmed cell death referred to as oncosis. Binding of Cry1Ab toxin to the BT-R_1 receptor induces a molecular signal that stimulates heterotrimeric G protein and adenylyl cyclase with an accompanying dramatic increase in production of cAMP. The cAMP activates protein kinase A, bringing about an array of cellular alterations, which includes cytoskeletal rearrangement and ion fluxing. Acceleration of this second messenger pathway alters the chemistry of the cell

and brings about cell death. Furthermore, the killing mechanism involves promotion by the toxin of exocytotic translocation of BT-R_1 from intracellular membrane vesicles to the cell membrane. Movement of the receptor is mediated by toxin-induced signal-transduction, and amplification of this signaling is correlated directly to the execution of cell death [13].

2. 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. The global area of biotech crops has increased 100-fold from 1.7 million hectares in1996 to 181.5 million hectares in 2014 which makes biotech crops the fastest adopted crop technology in recent times. This impressive adoption rate speaks for itself, in terms of its sustainability, resilience and the significant benefits it delivers to both small and large farmers as well as consumers**.**

After twenty years of their introduction, a large majority of the area planted to GM crops still remains in just a few countries. US was the first adopter of GM crops and is still the largest cultivator, accounting for 40.3% (73.1 million hectares) of the global area under GM crops in 2014(Table 1). Brazil grows 23.3% (42.2 million hectares) and Argentina 13.4%. These three countries grow over 77% of the world's GM crops. India and Canada account for approximately 6% of the total global GM hectare each, while China and Paraguay account for 2% each. Moreover, South Africa, Pakistan and Uruguay account for less than 2% each. Together, these ten countries account for 98% of the total global GM area in 2014 while 90 percent area lies in only five countries among which India occupies fifth position with 6.4% (11.6 mha) contribution depicted in (Table 1) [14].

Table 1: Biotech crops in world [14]

Rank	Country	Area (10^6ha)	Share %	Biotech crops
1	USA	73.1	40.3	Maize, soybean, cotton, canola, sugarbeet, alfalfa, papaya, squash
2	Brazil	42.2	23.3	Maize, soybean, cotton
3	Argentina	24.3	13.4	Maize, soybean, cotton
4	Canada	11.6	6.4	Canola, Maize, soybean, sugarbeet
5	India	11.6	6.4	Cotton
6	China	3.9	2.1	Cotton, papaya
7	Paraguay	3.9	2.1	Soybean, maize, cotton
8	South Africa	2.9	1.6	Soybean, maize, cotton
9	Pakistan	2.8	1.5	Cotton
10	Uruguay	1.4	<1	Soybean, maize,
11	Bolivia	1.0	<1	soybean
12	Philippines	0.8	<1	Maize
13	Australia	0.7	<1	Cotton, canola
14	Myanmar	0.3	<1	Cotton
15	Others	<0.1	<1	Maize, cotton, soybean, canola
		181.48m ha		

2.1 Major Transgenic crops

Four GM crops account for 99% of worldwide GM crop area. These are soy, corn, cotton and canola. Half of all the GM hectares in the world are planted with GM soybeans. GM corn accounts for 30% of the total global GM area and GM cotton accounts for another 14%. GM canola accounts for 5% of the GM hectares in the world (Table 2). Advances in the biotechnological techniques resulted in development of new constructs of Bt toxin genes with promoters to be expressed in monocots or dicots in different tissues of the plant including the integration of a native *Bt* gene into the chloroplast genome of tobacco. The chloroplast genome is bacterial in nature, avoiding the need of modifying the toxin gene for higher expression. Although it is still not commercially available, this technique opens new perspectives for the Bt plant breeding in the future [15].

Table 2: Major Transgenic crops [14]

GM Crop	Area Planted (mha)	Percentage of Global Area
Soybean	73.3	50
Maize	46.8	31
Cotton	21.0	14
Rape (Canola)	7.0	5
Sugar Beet	0.5	<1
Alfalfa	0.1	<1
Papaya	<0.1	<1
Others	<0.1	<1

2.2 Transgenic Plants Expressing Bt Toxins

Bt toxins have been inserted into crop plants to provide protection against different groups of insect pests. A brief list was presented by number of workers [16] (Table 3). Many crops such as vegetables forage crops, root crops, cereals, and trees are now being transformed to be protected against insects by Bt toxins [17].

Table 3: Transgenic Plants Expressing *Bt* Toxins [18]

Crop	Gene	Target pest
Cotton	*cry1Ab/cry1Ac*	Bollworms
Corn	*cry1Ab*	European corn borer
Potato	*cry3a*	Colorado potato beetle
Rice	*cry1Ab/cry1Ac*	Stem borers and leaf folders
Tomato	Cry1 Ac	Fruit borer
Brinjal	*cry1Ab/cry1B*	Shoot and fruit borer
Canola	*cry1Ac*	Diamondback moth
Soybean	*cry1Ac*	Soybean looper
Corn	*cry1Ab/cry1A*	European corn borer
Potato	*cry1Ab*	Tuber moth

3. Resistance of Insects against Bt Crops

Resistance is a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control [19, 20]. The most pressing issue relating to the practical implementation of transgenic plants in agricultural systems is the potential for rapid development of insect resistance due to the strong temporal and spatial selection pressure of *Bt* toxins controlled by a single gene [21, 22]. Already, about 17 insect species have become resistant to Bt in the laboratory, but only one species has shown widespread resistance in the field. It is widely considered to be only a matter of time before resistance occurs in *Bt*-plants [23]. In addition to the costs associated with the loss of the product and the development of alternative control strategies (either transgenic or conventional), the development of resistance will jeopardize the use of related *Bt* bio-pesticides for all users including those not using transgenic technologies [24].

On conducting 77 studies in eight countries, many workers concluded that reduced efficacy of Bt crops was associated with field-evolved resistance in some populations of 5 of 13 species of major pests by 2010 compared with only one such species in 2005 [25] (Table 4). Factors contributing to this surge in documented cases of resistance include more extensive monitoring as well as increases in the area planted to Bt crops, the number of pest populations exposed to Bt crops, and the cumulative duration of exposure. Three of the five resistant pests are in the United States, which accounts for about half of the world's Bt crop area each year. The other two resistant pests are from India and South Africa. Four of the five resistant pests are caterpillars; the fifth is an insidious beetle called western corn rootworm (*Diabrotica virgifera virgifera*).

Table 4: Present status of resistance to Bt plants [25]

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

4. Insect Resistance Management

Transgenic crops that express Bt proteins for the control of insect pests (Bt crops) have been commercialized throughout the world. The threat insect resistance poses to the future use of Bt plant-incorporated protectants have lead into emergence of insect resistance management concept. IRM is of gained importance as it is said to be the key to sustainable use of the genetically modified Bt crops. It may be defined as a program including actions taken to delay the development of insect resistance to pest control measures in target pest populations. In other words, it involves practices aimed at reducing the potential for insect pests to become resistant to a gene. Insect resistance management (IRM) is an important part of stewarding this valuable technology. However, IRM needs for Bt crops vary among countries because of differences in pest biology, farming practices and experiences. In countries with small scale farming systems, the scale and diversity of the agricultural systems poses significant challenges for IRM but also presents opportunities. As far as possible, IRM strategies in such countries should be implemented through the technology providers rather than requiring individual farmers to carry out novel practices. Appropriate IRM strategies should consider alternative crop and non-crop hosts as sources of unstructured refuge, particularly for highly polyphagous pests such as the cotton bollworm *H. armigera*.

4.1 Refuges

Refuges are host plants that do not contain the specific insect protection trait, allowing a portion of the target pest population to escape exposure so that susceptibility to the trait can be maintained in the population. Bt resistance as recessive need to lose/mutate both copies of the receptor gene to become resistant. Refuge is more effective the less dominant that Bt resistance is because the RS genotypes don't survive well. The development of resistance is driven by the initially very rare RR genotypes, but for a long time they only have the RS types to mate with. Planting refuges minimize the differential in fitness between the more and less resistant genotypes that eventually slows evolution of resistance. Refuges may include within or between row plant mixtures but in most cases areas of non-transgenic plants external to the transgenic crop have been preferred (Table 5) [26, 27]. Determination of the size of refuges is a compromise between practical and commercial considerations which favour smaller refuges and scientific theory which favours larger refuges [28, 29].

Table 5: Size of refuges for three commercialized transgenic crops in the USA [27]

Crop	Untreated	Treated	Distance
Potato	20%	20%	< 3.2 km
Corn	20% (50%)	20% (50%)	0.8 km (0.4 km)
Cotton	4%	20-25%	0.5 km

On examining the effect of refuge size and refuge placement (mixed vs. separate refuges) on the distribution of the larvae within the plots as well as the level of resistance in diamondback moths. It was found that the cumulative number of larvae per plant on refuge plants through the season in the 20% mixed refuge was significantly lower (6.4 vs. 14.6) than the 20% separate refuge (Table 6) [30].

This finding indicates that, as in our previous greenhouse experiments, a separate refuge is more effective at conserving the number of susceptible alleles because larvae on these refuge plants will be more likely to survive to adults (either SS or RS) that can mate with RR individuals and thereby reduce the number of RR offspring. This finding provides evidence to support the use of a separate refuge for *Bt*-transgenic crops that are attacked by insects that can move between plants as larvae. On the Bt-expressing plants over the season, an average of £0.3 larva was found in any of the treatments, indicating that the diamondback moth population was being controlled by the *Bt* expressing plants (Table 6). This was also confirmed by the absence of any larvae on the Bt-expressing plants at the end of the season.

Table 6: Effect of refuge size and refuge placement (mixed vs. separate refuges) on the distribution of the larvae within the plots as well as the level of resistance in diamondback moths [29]

Average Number of larvae per plant		
Treatment	**On refuge**	**On Bt plant**
0% Refuge	–	0.1
20% Mixed refuge	6.4	0.3
20% Separate refuge	14.6	0.1
100% Refuge	21.1	–

4.2 Multigene strategy (Pyramided plants)

Pyramiding: A special case of gene stacking where at least two modes of action against the same target pest(s) are provided by two or more genes combined in a single genotype. To engineer crops that express at least two toxic compounds that act independently, so that resistance to one does not confer resistance to the other. This approach, called gene pyramiding, became a commercial reality in 2003 with the introduction of Bollgard II. A transgenic cotton plant that

expresses the original Bt protein, Cry1Ac, and a second Bt protein, Cry2Ab.The two proteins act independently in that they bind to different receptors in the insect's midgut. Insects homozygous for one resistance gene are rare, insects homozygous for multiple resistance genes are extremely rare (Table 7). A species cannot easily evolve resistance to both toxins because that would require two simultaneous, independent mutations in genes encoding the receptors [31].

Table 7: Bt toxin pyramids used proactively and separately from one-toxin plants or remedially and concurrent with one-toxin plants [32]

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

The low percentage of resistant individuals after many years of extensive exposure to Bt cotton, exemplify successful resistance management [32]. Products like Bollgard II cotton contain two Bt proteins – Cry1Ac and Cry2Ab2 - for the control of lepidopteran pests. Both proteins are highly effective against the target pests and they differ in their mode of action require less refuge than single-Bt products (Fig. 4) [33]

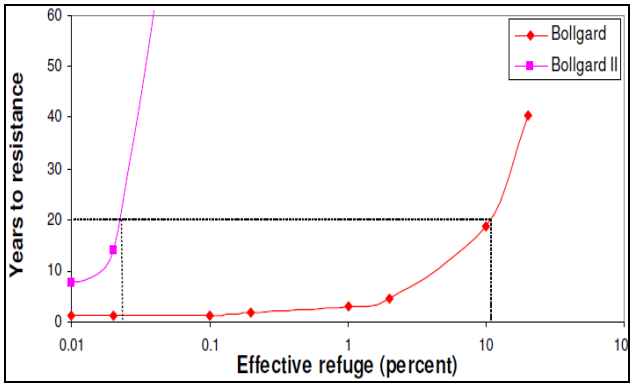

Fig 4: Effect of Products with Two Bt Proteins on rate of resistance [33]

4.3 High dose strategy

The 1998 Science Advisory Panel Subpanel agreed with EPA that an appropriate resistance management strategy is necessary to mitigate the development of insect resistance to Bt proteins expressed in transgenic crop plants. The 1998 Subpanel recognized that resistance management programs should be based on the use of both a high dose of Bt and structured refuges designed to provide sufficient numbers of susceptible adult insects. The high dose/refuge strategy assumes that resistance to Bt is recessive and is conferred by a single locus with two alleles resulting in three genotypes: susceptible homozygotes (SS), heterozygotes (RS), and resistant homozygotes (RR). It also assumes that there will be a low initial resistance allele frequency and that there will be extensive random mating between resistant and susceptible adults. Under ideal circumstances, only rare RR individuals will survive a high dose produced by the Bt crop. Both SS and RS individuals will be susceptible to the Bt toxin. A structured refuge is a non-Bt portion of a grower's field or set of fields that provides for the production of susceptible (SS) insects that may randomly mate with rare resistant (RR) insects surviving the Bt crop to produce susceptible RS heterozygotes that will be killed by the Bt crop. This will remove resistant (R) alleles from the insect populations and delay the evolution of resistance (Fig. 5) [34]. Strategy to produce transgenic plants synthesizing very high level of insecticidal proteins has been found to be highly effective in preventing or delaying development of resistant insects.

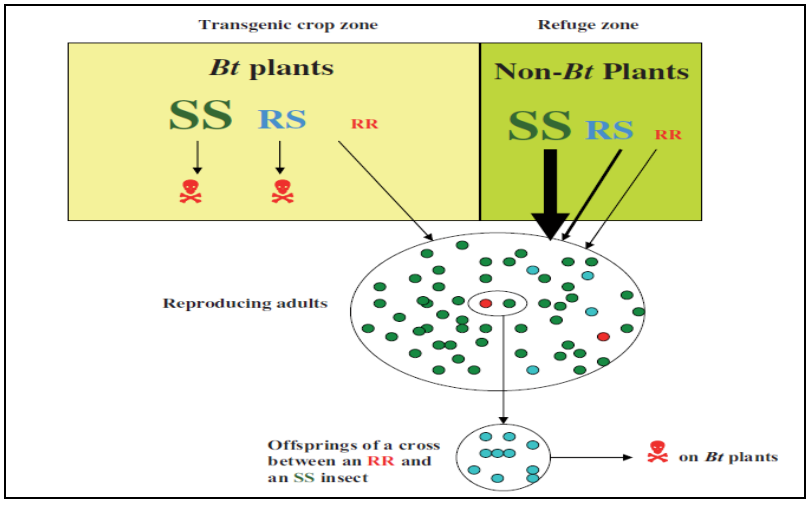

Fig 5: Schematic representation of the "high dose-refuge" (HDR) strategy. The success of the HDR strategy depends on resistance being a rare and recessive trait and the genetically modified plants producing a dose of toxin sufficient to kill all homozygous susceptible individuals (SS-green) and all heterozygous individuals with for both resistance and susceptibility alleles (RS-blue) [34]

5. Conclusion

Bt crops have brought tremendous benefit to both the environment and farmers, together with the reduction of pesticide application and cost reduction. Also, expanded use of transgenic crops for insect control will likely include more varieties with combinations of two or more Bt toxins, novel Bt toxins such as VIP. While, Modified Bt toxins that have been genetically engineered to kill insects resistant to standard Bt toxins. Increasing use of transgenic crops in developing nations is likely, with a broadening range of genetically modified crops and target insect pests. Moreover, incorporating enhanced understanding of observed patterns of field evolved resistance into future resistance management strategies can help to minimize the drawbacks and maximize the benefits of current and future generations of transgenic crops.

6. References

1. Gonclaves MF, Pereira JA. Abundance and diversity of soil arthropods in the olive grove ecosystem. Journal of Insect Science. 2012; 12:1-14.
2. Palma L, Munoz D, Berry C, Murillo J, Caballero P. *Bacillus thuringiensis* Toxins: An Overview of Their Biocidal Activity. Toxins. 2014; 6:3296-3325
3. 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
4. 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.
5. 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
6. Ibrahim MA, Griko N, Junker M, Bulla LA, *Bacillus thuringiensis* A genomics and proteomics perspective., Bioeng Bugs. 2010; 1(1):31-50.
7. 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.
8. 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.
9. Schnepf HE, Whiteley HR. Cloning and expression of the *Bacillus thuringiensis* crystal protein gene in *Escherichia coli*. Proc. Natl. Acad. Sci. 1981; 78:2893-2897.
10. Hofte H, Whiteley HR, Insecticidal Crystal Proteins of *Bacillus thuringiensis*. Microbiol. Rev. 1989; 53:242-255.
11. Crickmore N, Zeigler DR, Feitelson J, Schnepf E, van Rie J, Lereclus D *et al*. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 1998; 67:807-813.
12. Grochulski P, Masson L, Borisova S, Pusztai-Carey M, Schwartz JL, Brousseau R. *Bacillus thuringiensis* CryIA (a) insecticidal toxin: crystal structure and channel formation. J Mol Biol. 1995; 254:447-464
13. 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-R_1 expressed in insect cells. Cell Death Differ. 2005; 12:1407-1416.
14. 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
15. James C. Global status of commercialized biotech/GM crops: lessons from the laboratory and field. Journal of Economic Entomology. 2015; 96:1031-1038
16. MacBride GB, Loftis JC, Adkins NC. What do significance tests really tell us about the environment? Environ. Manage. 1995; 17:423-432
17. 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.
18. Shelton AM, Tang JD, Rousch RT, Metz TD, Earle ED. Field tests on managing resistacne to Bt-engineered plants. Nature Biotechnol. 2000; 18:339-342.
19. Fontes EMG, Carmen SS, Sujii PER, Panizzi AR. The Environmental Effects of Genetically Modified Crops Resistant to Insects. Neotropical Entomology. 2002; 31:497-513
20. Tabashnik BE, Brevault T, Carriere Y. Insect resistance to Bt crops: Lessons from the first billion acres. Nature Biotechnology. 2013; 31:510-521.
21. Groot AT, Dicke M. Transgenic crops in an agro-ecological context: Multitrophic effects of insect-resistant plants. Wageningen University Press, the Netherlands. 2001, 76.
22. Shelton AM, Zhao JZ, Roush RT. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Ann. Rev. Entomol. 2002; 47*:* 845-881.
23. Ferre J, Van RJ. Biochemistry and Genetics of Insect Resistance to *Bacillus thuringiensis*. Annual Review Entomology. 2002; 47:501-533.
24. 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.
25. McGaughey WH, Gould F, Gelernter W. Bt resistance management. Nature Biotechnology. 1998; 16:144-146
26. Tabashnik BE, Brevault T, Carriere Y. Insect resistance to Bt crops: Lessons from the first billion acres. Nature Biotechnology. 2013; 3 1: 510-521.
27. Groot AT, Dicke M. Transgenic crops in an agro-ecological context: Multitrophic effects of insect-resistant plants. Wageningen University Press, the Netherlands, 2001, 76.
28. Teulon DAJ, Losey JE. Issues relating to the practical use of Transgenic crops for insect pest management New Zealand Plant Protection. 2007; 55*:*396-404
29. Gould F. Potential problems with high-dose strategies for pesticidal engineered crops. Biocontrol Sci. Technol. 2000; 4:451-461.
30. 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.
31. 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
32. 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.
33. Tabashnik BE, Brevault T, Carriere Y. Insect resistance to Bt crops: Lessons from the first billion acres. Nature Biotechnology. 2013; 31:510-521.
34. Graham M. Insect Resistance Management Strategies For Bt Crops In Small Scale Farming Systems Head, Monsanto Company, St. Louis, USA, 2010, 1-34.
35. Sanchis V, Bourguet D. *Bacillus thuringiensis*: applications in agriculture and insect resistance management. A review Agron. Sustain. Dev. 2008; 28:11-20.