Bacillus thuringiensis (Bt) is a ubiquitous soil bacterium that produces an array of insecticidal crystal proteins with varied specificities. Recently a lot of effort is being made to collect, characterize, utilize novel and more potent strains of Bt. The crystal proteins (Cry proteins) produced by Bt are important in agricultural, horticultural and public health pest management. In addition to the above recently identified non-insecticidal Cry toxins are being used in cancer research. There is continuing debate among researchers on the species status for Bt and its ecology. The various toxins particularly Cry toxins are important in ecofriendly pest management in organic farming and exported oriented crops also. Many cry genes have been engineered into wide variety of crop plants and microbes, offering efficient and effective pest management strategy. Increased efforts to isolate novel strains of Bt from wide variety habitats have yielded valuable strains with new genes. Intensive use of various Bt formulations and high level of expression of various cry genes in Bt transgenic plants have resulted in resistance development in various pest species. But with the judicious use of gene pyramiding and refugia, continued successful pest management is ensured.

Bacillus thuringiensis (Bt) is a ubiquitous, gram positive soil bacterium, which was first identified by the Japanese microbiologist, Ishiwata in 1901 from the diseased silk worm, Bombyx mori (Beegle and Yamamoto, 1992). Bt produces the insecticidally important crystalline inclusions, popularly called d-endotoxins, mainly during the late exponential phase and stationary phase of growth. The various crystal (Cry) toxins have been proved to be very specific to insects and pose no threat to the environment and non-target animals including human beings. In addition to Cry proteins Bt also produces α-exotoxin, β-exotoxin, vegetative insecticidal proteins (Vip), louse factor, mouse factor, cytolytic toxins (cct) etc, production of which is subspecies/strain specific (Konecka et al., 2007). Until late 1970s most of the subspecies of Bt isolated were lepidopteran active, later many Bt strains active on coleoptera, diptera, aracina, protozoas, hymenoptera, trematode, nematodes etc were discovered (Crichmore et al., 1998)

In majority of the cases, Bt has been isolated from the dead insects and occurrence of Bt in soil has been reported to be very less. Later researchers isolated Bt from various sources (Bernhard et al., 1997, Morris et al., 1998, Gough et al., 2002). The above efforts yielded many novel isolates of Bt with altogether new genes widening the application of Bt in various fields. Advent of recombinant DNA techniques in early 1980s has accelerated cloning and characterization of new cry genes from the already identified subspecies as well as from uncharacterized strains of Bt. At present there are about 300 different cry genes have been characterized and the sequences are available. Additionally the interest on cry genes was due to the monogenic nature of the toxin as against other complex insecticidal genes like tc genes from Photorhabdus luminescens, which are difficult to engineer into crop plants. In addition to the above, Agrobacterium mediated transformation of various crops was successful, which was instrumental in the development of Bt transgenic plants.

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In this communication the following aspects of Bt have been reviewed 1) ecology, isolation and occurrence 2) classification 3) Nature of insecticidal proteins & other toxins and their mode of action 4) field application and 5) resistance management.

1. Ecology, Isolation and Occurrence

The true ecological role of Bt has not been established completely till date since it invests >50% of its resources to produce crystal proteins (Ohba, 1986). Additionally Bt has been isolated from places where there was no apparent insect activity at all. More over Bt rarely causes epizootics in insect population, which is untypical of any obligate insect pathogens. There are many hypotheses on the possible role of the crystal proteins. These are (a) the crystalline inclusions are vestigial (b) crystal proteins have some unknown physiological role in the bacterium (c) crystalline inclusions serve as a reservoir of nutrients and (d) it is a weapon to invade insects. Since many Cry proteins have been found to be toxic to insects, it has been considered as true insect pathogen (Agaisse and Lereclus., 1995).

Usual method of isolation of Bt is from diseased or dead insects or in places with high levels of insect infestation. Saleh et al. (1969) used polymyxin B sulfate and penicillin G which reduced the number of non-Bacillus organisms while isolating Bt. Later, Travers et al. (1987) found that Bt spores did not germinate in the presence of 0.25 M sodium acetate in the medium. Employing this method they detected one spore of Bt in the background of 10^9 bacteria/gram of soil. Presently different strains of Bt has been recorded from Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Orthoptera, Mallophaga and from Nematoda, Acarina and Protozoa, Trematoda etc. (de Maagd et al., 2003). Researchers have isolated Bt from different habitats such as soil (Ohba and Aizawa, 1986, 1986a; Carozzi et al., 1991; Hastowo et al., 1992; Landen et al., 1994; Vasquez et al., 1995; Bernhard et al., 1997; Hoassain et al. 1997; Thanis et al., 1998; Kaur and Singh, 2000), fresh water (Ichimatsu et al., 1998), saw dust (Bravo et al., 1998; Helgason et al., 1998; Morris et al., 1998; De Luca et al., 1982), cured tobacco leaves (Kaelin, 2000; Kaelin and Gadeni, 2000), warehouse (Hongyu et al., 2000), dead insects (Chilcott and Wigley, 1993; Bernhard et al., 1997; Struyger et al., 1997, Schneppf et al., 1998), trematode (Horak et al., 1996), rice bran (Jung et al., 1998), stored products (Bernhard et al., 1997; Chauffaux et al., 1997), compost (Bernhard et al., 1997), phylloplane (Bernhard et al., 1997; Bora et al., 1994; Dameraud et al., 1998), feces samples of green house workers (Jensen et al., 2002), in gastroenteritis outbreak (Jackson et al., 1995), marine sediments (Maeda et al., 1998), in animal feed mill (Meadows et al., 1992), sericultural farms (Ohba, 1984; Kim et al., 1998), on livestock ecto parasites (Gough et al., 2002), periodontitis, dairy, human pus, human nose, eye, urine (Helgason et al., 2000), Antarctica (Forsyth and Logan, 2000), in ancient glacial ice (Christner et al., 2003) etc.

Many of the earlier workers have not taken sibling strains into their considerations, Bravo et al. (1998) has emphasized such grouping on siblings. Prabagaran et al. (2002) have isolated 583 strains from different agro climatic regions in India and classified the same based on crystal morphology, SDS-PAGE profile and PCR panning. Out of 583 isolates screened only 18 isolates produced crystals and were subjected to detailed investigation. Bernhard et al., (1997) and Hongyu et al. (2000 b) have grouped huge populations of Bt based on the above methods. Lopez-Meza and Ibarra (1996) characterized novel strains of Bt. It is worth to investigate in depth on limited Bt.
isolate than characterizing large population with no proven potential. Statistical analysis with thousands of Bt strains regarding potency generally does not have any practical value. On the contrary, studies with selected elite strains with novel gene/s or high potency will have commercial value.

2. Classification

Bt is evolutionarily very close to B. cereus except that the former produces crystal inclusions while the later does not. Some classical bacterial taxonomists still opine that Bt should be a subspecies of B. cereus, based on many experimental evidences.

Bt and B. cereus are one species

The various experiments that proved the similarities between Bt and B. cereus are as follows

(a) certain strains of Bt and B. cereus killed mice, when injected intra peritoneally (Lamanna and Jones, 1963) (b) the spores of both share common antigens and also exhibited cross-sensitivity to bacteriophages (c) some B. anthracis strains were sensitive to bacteriophage from Bt and the fatty acid patterns were similar (Kaneda, 1968) (d) flagellar antigens of some Bt and B. cereus isolates overlapped (e) close enzyme semological relationship, thin section spores appeared alike in fine structure, DNA homology is 80-100% (Krieg, 1975). According to Singer (1980) strains of bacteria having greater than 70% DNA homology should be considered as single species.

Bt and B. cereus are two different species

The various experimental evidences that support this argument are as follows

(a) DNA competition studies, immuno-fluorescence and phase-microscopy, pyrograms based on pyrolysis gas-liquid chromatography, biochemical characteristics (Jones, 1983) (b) Morphological and biochemical characteristics (Heimpel and Angus, 1960) (c) Later de Barjac (1990) developed identification and classification method based on serotyping and biochemical characteristics (d) Norris (1963) advanced the identification and classification of Bt by esterase patterns of vegetative cells by starch gel electrophoresis (e) various heat stable somatic O-antigens, crystal serology; phages have been examined for their usefulness in the identification and classification (de Barjac, 1990) (f) reaction to different monoclonal antibodies (Roffte and Whiteley, 1989) (g) phospholipid and fatty acid analyses (Rathout et al., 2000) (h) electrophoresis of PCR products (Perez et al., 1994) (i) 16S rRNA sequence comparison (Joung et al., 2001) (j) AFLP (Kim et al., 1997; Ticknor et al., 2001) (k) genomic restriction digestion (Schnepf et al., 1998) (l) SDS-PAGE analysis of total proteins (Helgason et al., 1998) (m) Southern blot analysis (Kronstad and Whiteley, 1986) (n) bioassay (Martin and Travers, 1989) (o) RFLP (Joung and Cote, 2001) (p) ribosomal RNA gene restriction patterns (Joung and Cote, 2002).

(q) Beattie et al. (1998) employed Fourier transformation infrared spectroscopy to distinguish B. cereus, B. mycoides and Bt. (r) Vilas-Boas et al. (2002) identified the genetic differences between sympatric populations of B. cereus and Bt by alleloyme electrophoresis and hemolytic activity (s) Rachneger et al. (2003) worked on the genomic differences that distinguished Bt from the closely related pathogens like B. cereus and B. anthracis. Serotyping based on H-flagellar antigen is regularly employed to classify Bt subspecies (Lacadet et al., 1999, Joung and Cote, 2002).

3. Nature and mode of action of insecticidal proteins and other toxins produced by Bt

The production of various insecticidal toxins is strain/stage specific i.e. all the Bt strains/subspecies may not produce all the listed toxins. The various insecticidal proteins and toxins produced by Bt are as follows
α-exotoxins

This is a heat-labile toxin; first identified by Toumanoff in 1954 from Bt aleti culture filtrates. This toxin is found to be active on Lepidoptera, Diptera, Coleoptera, Orthoptera, and Hymenoptera. The molecular weight of this toxin ranges from 45-50 kDa and is a phospholipase (Beegle and Yamamoto, 1992). The following subspecies that produce α-exotoxin are thuringiensis, finitimus, alesti, kurstaki, sotto, dendrolimus, kenyaee, aizawai, oestrinae, tolworthi, toumanoffi, kyushuensis, thompsoni, pakistani, israelensis, dakota, Indiana, kumamotoensis, tochigiensis. This toxin affects the phospholipids in the lipid bilayer and causes lytic or necrotizing action and not highly specific to insects (Beegle and Yamamoto, 1992).

β-exotoxins

This is a heat-tolerant exotoxin, first discovered by McConnell and Richards in 1959 (Beegle and Yamamoto, 1992) (Figure-1). This toxin is also known as fly-factor or thuringiensin. Vegetative cells produce this toxin, which is water-soluble, dialyzable nucleotide, composed of adenine, ribose, glucose and allaric acid with a phosphate group. Levinson et al., (1990) identified one more heat-stable toxin called type-II β-exotoxin, active against Leptinotarsa decemlineata. The Bt strains that produce β-exotoxin are, thuringiensis, galleriae, canadensis, aizawai, marrisoni, tolworthi, kumamotoensis.

The type I β-exotoxin inhibit the DNA-dependent RNA-Polymerase of wide variety of organisms (Beegle and Yamamoto, 1992).

![Figure-1: Structure of the α-exotoxin](image)

(An adenine derivative linked through a glucose moiety to the 5’ position of the phosphoallaric acid)

(Wirth et al., 2001)
Cytolytic toxins

The Cyt toxins are small proteins (20-27 kDa) and the toxin structure is a three-layered core comprising two outer layers of alpha helix hairpins wrapped around a mixed beta-sheet. The Cyt toxin alpha helices are too short to form a pore spanning the membrane. The kinetic and biochemical evidence suggests that the membrane pore is formed by aggregation of several toxin molecules (Promdonkoy and Ellar, 2000).

This toxin is particularly produced by *Bt israelensis*. Unlike the Cry toxins, Cyt toxins do not need any receptors to cause pore formation on the columnar epithelial cells of susceptible insects. Once the Cyt toxin is activated in the alkaline pH, the toxin molecules automatically insert into the phospholipid bilayer and form a pore of 1.0 nm in diameter, by aggregation of six molecules. This toxin has broad-spectrum activity (Promdonkoy and Ellar, 2000).

Vegetative insecticidal proteins (Vip)

Vegetative insecticidal protein is a novel insecticidal protein derived from *Bt*, which is highly toxic to large number of economically important pests (Estruch et al., 1996). Vip protein is secreted from the mid-log phase to sporulation phase. The molecule weight of this protein is 88.5 kDa. Vip proteins are active on black cutworm, *Agrotis ipsilon*, fall army worm, *Spodoptera frugiperda*, beet army worm, *S. exigua*, tobacco budworm, *Heliothis virescens* and corn ear worm, *Helicoverpa zea* (Estruch et al., 1996). Donovan et al. (2001) proved that the toxicity of Vip3 on *A. ipsilon* and *S. exigua* by gene knockout. The above authors found that *Bt* kurstaki HD-1 without vip3A gene was one fourth as toxic to *A. ipsilon* and one-tenth as toxic to *S. exigua*. Since the mode of action of Vip proteins are entirely different from that of Cry proteins, vip genes are very useful in *Bt* resistance management strategies, by pyramidning with cry genes. vip3A has been transformed into several crop plants including maize, rice, cotton etc and is very effective in controlling the cotton boll worm complex (Shotoshki and Chen, 2003). Yu et al. (1997) showed that Vip3A also lyses the mid gut epithelium cells of the susceptible insects as that of Cry toxins. Lee et al. (2003) reported that the mode of action of the Vip toxins is different from that of Cry1Ab toxin.

δ- endotoxins

The crystal proteins are formed mainly from late log phase to sporulation phase. Crystal formulation could be observed microscopically during the later part of stage II of sporulation. Abdel-Hameed et al. (1990) detected the presence of nascent inclusions even at the end of stage-I of sporulation. The crystal toxin is insoluble in water or inorganic solvents, but soluble in alkaline solvents. Cry1 proteins are soluble at pH 9.5, which Cry2 proteins are soluble at pH 12. Cry3A dissolves at pH below 4.0 ml above 9.5, Cry4A, Cry5B are soluble at pH 9.5, and Cry4D is soluble at pH 12. The crystals can also be soluble at neutral pH in the presence of detergents and denaturing agents like urea, 3- mercaptopropanol, DTT, and SDS (Gill et al., 1992). Separation of crystals from spores and cell debris involves isopycnic centrifugation in sucrose or cesium chloride gradients.

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Cry toxins</th>
<th>Active on</th>
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<tbody>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>Cry3, Cry7, Cry 8</td>
<td>Coleoptera</td>
</tr>
<tr>
<td>3</td>
<td>Cry 1B, Cry 11</td>
<td>Lepidoptera and Diptera</td>
</tr>
<tr>
<td>4</td>
<td>Cry 5, Cry 12, Cry 13 and cry 14</td>
<td>Nematodes</td>
</tr>
<tr>
<td>5</td>
<td>Cry2, Cry4, Cry10, Cry11, Cry16, Cry17, Cry19, Cry20, Cry 27</td>
<td>Mosquitoes</td>
</tr>
</tbody>
</table>
The mode of action of Bt Cry toxin there are at least four parameters are involved (a) Effectiveness of solubilization (b) Efficiency of protoxin to toxin conversion (c) specific membrane receptor binding and (d) membrane pore formation. All these parameters determine the specificity of a crystal protein otherwise insecticidal spectrum. When the crystals are ingested by the susceptible larvae while feeding, the crystal toxin under goes different sets of activation like (a) solubilization in higher alkaline mid gut pH and further activation of the linearised protoxins by the gut protease. Upon proteolytic activation a considerable stretch of C-terminal is removed leaving the protein resistant functional activated toxin, which is also trimmed at the N-terminal end (b) binding of the activated toxins to the receptors that are found in the mid gut columnar epithelial cells and (c) insertion of the activated toxin into the columnar epithelial cell apical membrane to create ion channels or pores (Crickmore et al., 1998).

The major proteases of the Lepidopteran larvae are trypsin (Milne and Kaplan, 1993) or chymotrypsin like (Johnston et al., 1995; Novillo et al., 1997). The activated toxin binds readily to specific receptors found on the apical brush border membrane of the mid gut microvillae of susceptible insects (Pigott and Ellar, 2007). Receptor binding is a two-stage process (1) reversible binding (ii) irreversible binding (Schneef et al., 1998, Simon et al., 2008). The later step involves tight binding of the toxin to the receptor that lead to pore formation. Insertion of the activated toxin into the lipid bilayer of the columnar epithelial cells renders the activated toxins insensitive to proteases and monoclonal antibodies (Molfersberger, 1989). The nature of the pores formed by the Cry toxins is still unclear. The pores are K+ selective (Sacchi et al., 1986), permeable to anions (Hendricks et al., 1989), permeable to solutes such as sucrose, irrespective of the charge (Schwartz et al., 1991). Cessation of the K+ pump leads to osmotic imbalance resulting swelling of the columnar epithelial cells (Knowles and Dow, 1993). Thus the disruptions of gut integrity leads to starvation and finally results in death and septicemia, which is responsible for secondary cycling of the spores in the soil. Rukmini et al. (2000) showed the proteolytic processing of various classes of Cry proteins.

Gazit et al. (1998) have proved that the Cry toxins form pore with an umbrella-like structure on the apical membrane of the columnar epithelial cells (Figure-2). The above phenomena have been verified using resonance energy transfer measurements of all possible combinatorial pairs of membrane bound helices in their membrane bound state. Aronson and Shai (2001) while reviewing the unique features on the mode of action of Bt insecticidal toxin, describe the importance of reversible, irreversible binding, aggregate formation of the Cry toxins and the final pore formation. Even though more than 300 genes have been cloned and sequenced we are not still clear about the receptors for different Cry toxins. Researchers have identified Aminopeptidase-N (APN) (120 and 106 kDa) and Cadherin like proteins (210 kDa) as the putative receptors for Cry1A toxins (Gomez et al., 2002; Banks et al., 2001 and Hare et al., 2003). Rajagopal et al. (2002) used gene-silencing technique to establish the role of aminopeptidase N (Slapn) from S. litura as receptor for Cry1C toxin. Alkaline phosphatase has also been proposed to be the Cry1Ac receptor

**Mechanism of hyper production of Cry toxins**

The stability of mRNA is an important factor for the high level of toxin production. The half-life of the cry gene mRNA is about 10 minutes, which is five fold more than the half-life on an average bacterial mRNA. In cry1Aa gene, a putative transcriptional terminator,
Lipid bilayer

(The loop connecting α helix 4 and α helix 5 may be either in an intracellular localization or may interact with the inner leaflet of the membrane because of its hydrophobicity)

Fig. 2: Schematic presentation of a proposed model for the interaction of d-endotoxin with phospholipid membrane

(Gazit et al., 1998)

which is a stem loop structure, acts as a positive retro regulator. Because of this, degradation of mRNA by 3’-5’ exoribonuclease is impeded. Between the promoter and the transcriptional start codon, from position – 560 to – 600 are involved at the posttranscriptional level for the stability of mRNA. The 5’ untranslated region from –129 to –12 is also essential for improved mRNA stability. The stability of the cry9A mRNA could also be due to interaction between the 3’ end of 16 S rRNA and the STAB-SD sequence may protect mRNA from 5’-3’ exoribonuclease activity, resulting in a stable transcript with a 5’ end at a nucleotide position – 129. Potential STAB-SD sequences are present in similar positions upstream of the cry3Ba, cry3Bb and cry3Ca genes (Agaisse and Lereclus, 1995)

Protease (Inhibitor A)

This is a zinc protease InhA2, which is capable of inactivating cecropins and attacins from insect larvae and reduce the defense response. This inhibitor recognizes an open hydrophobic region near the C terminal end of the Csecopins (Fedhila et al., 2002)

4. Field application

The functionality of any insecticidal protein is validated only by the bioassay method. All other methods like Southern blot, Western blot, Northern blot and activity staining have limited role in predicting the biological activity of an insecticidal protein. Even though PCR analysis of the Bt isolates show the presence of cry genes in various isolates, only bioassay will determine the usefulness of a particular Cry protein on a suitable target.
Martin and Travers (1989) reported that 40.3% of the Bt isolates were found to be toxic to Lepidoptera and 22.7% against mosquitoes. Chilcott and Wigley (1993) observed that 37 to 88% of the Bt collections were toxic to Diptera, 0-6% toxic to Coleoptera, 45-77% toxic to both Lepidoptera and Diptera and 20% had no insecticidal activity. Bernhard et al. (1997) tested 5303 isolates against A. ipseicon, 5136 against H. virnecmen, 3077 against Pieris brassicae and 3028 against S. litioralis. Van Frankenhuyzen et al. (1993) reported that Cry1C was toxic to S. litura, Cry1D to S. littoralis, Cry1E to S. exigua. Bai et al. (1993) observed that S. exempta was susceptible to Cry1Ab, Cry1B, Cry1D Cry1Aa and Cry1Ac.

Natarajan and Srinivasan (1999) recorded 100%, 85%, 20% mortality for 1st, 2nd, 3rd instar larvae of H. armigera at 20 mg/ml crystal protein concentration. Attathom et al. (1995) found that 15 isolates were toxic to S. exigua, H. armigera, T. incertulus, C. suppressalis and R. mari. Ichimatsu et al. (1998) reported that out of 195 isolates from water, 52 (26.7%) were toxic to Culex pipiens molestus and Clogmia albipunctata. Maeda et al. (2000) studied the toxicity of Bt isolates obtained from marine sediments in Japan and reported that 86.4% of the isolates had no toxicity against insects. Masetal activity was found in three isolates out of 1313 colonies belonging to the B. cereus/Bt group. Iriarte et al. (2000) reported that out of 122 isolates only 8 isolates were found to be toxic to Tipula clareae larvae. Kim (2000) also reported that 19 out 25 isolates from granary were toxic to P. xylostella and C. piger.

Employing the principle component analysis (PCA), Leong et al. (1980) reported that pathogenicity of Bt was influenced by the environmental factors like sunlight, leaf temperature, and vapour pressure deficit. Navon (2000) showed that effectiveness of Bt in the field was influenced by solar radiation (UV-radiation of solar spectrum), protein concentration, plant phytochemicals and allelochemicals on the leaves. Broza and Sneh (1994) reported that Bt kurstaki was an effective control agent for Chrysodeixes chalcites, H. armigera and P. opercullelata, in tomato fields in Israel. Payne and Frankenchuyzen 1995) reported the field effectiveness of Bt against the spruce budworm, Choristoneura fumiferana, in Canada. Beveridge and Klek (1999) reported that Bt terebrionis showed no toxicity to the predator, Chauliognathus lugubris. Bt was even integrated with the parasitoid C. plutellae for the control of susceptible and resistant P. xylostella (Chilcott and Tabashnik, 1999)

5. Resistance management

Until the mid 1980 there was no report on the development of resistance to Bt. It was suggested that evolutionary advantages of the pathogen might preclude or greatly reduce the likelihood of insects becoming resistant to Bt. However, as it has been shown that resistance to delta endotoxins could develop readily in many species of insect pests both in laboratory and in the field (McGaughey, 1985; McGaughey and Beeman, 1988). Tabashnik et al. (1990), showed the first documented evidence of resistance occurring against Bt kurstaki in the open field populations of P. xylostella. Kirsch and Schmutter (1988) suggested the development of resistance to Bt kurstaki in the population of P. xylostella in the Philippines. Georgiou et al. (1983) and Goldman et al. (1986) proved that C. quinquifacaitus and Aedes aegypti had developed resistance to Bt israelensis. At least one strain of Leptinotarsa decemlineata has been selected for resistance to Bt terebrionis (Miller et al. 1990). The genetic capacity of insect population to evolve resistance to Bt is now well recognized from the laboratory studies (Van Rie et al., 1990; Tabashnik and McGaughey, 1994; Van Frankenhuyzen et al. (1999)).
The summarized results on resistance development to Bt are as follows:

(1) The resistance alleles are present at varying levels in different insect species and populations.

(2) Within a single species, the genetics, mechanisms, level, and stability of resistance vary between selected populations.

(3) Selection with a blend of toxins can select for resistance to each toxin in the blend.

(4) Resistance occurs rapidly with purified toxins than with spore/crystal preparations.

(5) Cross-resistance to Bt α-endotoxins is almost ubiquitous and often unpredictable.

(6) Reselection of revertant population is rapid.

Presently, much of the effort toward resistance management or avoidance seems to focus on the presumption that there are an almost unlimited number of different Bt toxins available in nature and that resistance can be managed by using these in various mixtures, mosaic, rotational or sequential system (Tabashnik, 1994). The implementation of integrated pest management (IPM) strategies that optimize the goals of resistance management involves.

(a) Diversifying the sources of mortality to avoid selection for a single mechanism.

(b) Reducing selection pressure for a major mortality factor.

(c) Maintaining susceptible individuals by providing refuges and encouraging immigration.

(d) Monitoring for increasing resistance to any one of the mortality agents and

(e) Responding to resistance through management of strategies designed to reduce the frequency of the resistance trait (Whalon and McGaughey, 1993).

CONCLUSIONS

The true purpose of production of high quantity of crystal proteins by Bt remains a mystery. Some of the non-insecticidal isolates and isolates that occur in places where apparently no insect activity also produce high levels of the crystal proteins. Never the less, the various non-insecticidal toxins are currently being used in cancer research. Some of the isolates of Bt also exhibiting promises in the plant disease management and nematode management programmes. Even though there is a continuing debate on the species status of Bt the crystal toxins and the Vip toxins are found to be ecologically safer and effective in the management of some of the hardy pests such as different cotton boll worms, various tissue borers and leaf feeders. Many genetically transformed bacteria expressing crystal toxins are used for improved persistence in different habitats such as land and aquatic environments.

Unambiguously use of Bt formulations and Bt transgenic plants has brought down the uses of various synthetic chemical pesticides thus reduced the pesticides load in various spheres of the environment. The main concern on the use of Bt is that the crystal toxins have narrow spectrum of activity and therefore engineered toxins with improved spectrum activity are available presently. Increasing need for the isolation of novel and potent toxins from Bt have resulted in the isolation and characterization of new binary toxins like cry 34/35 and also many unassigned toxins (Ito et al., 2004; Beron et al., 2005; Schnepf et al., 2005). It has been estimated that roughly 40,000 strains of Bt are now stored, mainly in private collection worldwide. With more information on characterization of receptors for various Cry toxins and novel mechanism of resistance in insects a renewed approach is necessary to decide on the suitable gene pyramiding strategies to be adopted for the
Table 1: Diversity of Bacillus thuringiensis subspecies

<table>
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<th>Bt subspecies</th>
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(Joung and Cote, 2002)

continued success of Bt. Gene pyramiding technology also used for increased effectiveness and to slow the rate of development of resistance in the field. As more and more crop species are being genetically modified and the acreage under various Bt crops increasing year by year it is important to dove-tail the Bt technology with the integrated pest management practices.

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REFERENCES


