ABSTRACT

_Bacillus thuringiensis_, commonly known as Bt, is a most promising alternative to synthetic insecticides, has been used as an effective bio-pesticide in agriculture since long time. Two insecticidal protein, Cyt (Cytolysins) and Cry (Crystal α-endotoxin) are the toxic substances responsible for the death of an insect. The durability of this novel insect control technology is questioned as many cases of insect population developing resistance to Bt cry proteins were observed and various mechanisms for resistance to Bt cry proteins were reported. Management of resistance in insect to Bt includes refugia, gene pyramiding and novel toxins.

**Key words**: _Bacillus thuringiensis_, Cry protein, Novel toxin, Refugia, Resistance.
to plant appeared in 1987 and the first transgenic plants to express Bt toxin were tobacco and tomato (Van Frankenhuyzen, 1993).

The most serious threat to the durability of this novel insect control technology is the potential of insect population to develop resistance to Bt cry proteins. McGaughey (1985) reported the first evidence of development of resistance against endotoxin in Plodia interpunctella (Hubner), the Indian meal moth. But the recognition of the potential of the Bt resistance problem became greater when the first report of high resistance to Bt toxin in the field population of diamond back moth, Plutella xylostella (L.) came in 1990 from Hawai, Florida and New York in USA (Liu and Tabashnik, 1997).

**Status of insect resistance to Bt:** McGaughey and Beeman (1988) observed that resistance in five Indian meal moth colonies increased from 2 to 29 fold within three generations and from 15 to 100 fold in 40 generations under relatively low selection pressure. The authors further noted that resistance in one colony increased more than 250 fold with higher selection pressure. The response of second instar gypsy moths to B. thuringiensis revealed significant variation in LC \(_{50}\), thus suggested the potential threat for resistance development through natural selection (Rossiter et al., 1990). A diamond back moth colony derived from a field population when exposed regularly to Dipel expressed more than 200 fold resistance to Cry 1Ab (Ferre et al., 1991).

A colony of Leptinotarsa decemlineata (Say) collected from fields sprayed with Bt formulations containing Cry 3Aa and subjected to laboratory selection up to 29 generations resulted in a 293 fold resistance (Whalon et al., 1993; Rahardja and Whalon, 1995). Selection of a colony of Heliothis virescens (F.) with Cry 1Ac resulted in 50-fold resistance to Cry 1Ac, 1-fold to Cry 1Ab and 53-fold to Cry 2Aa (Gould et al., 1992). Similarly, selection of a colonies of Spodoptera exigua (Hubner) with Cry 1Ca resulted in a high level of resistance to Cry 1Ca (850-fold) and cross-resistance to Cry 1Ab, Cry 1Aa and Cry 9Ca (Moar et al., 1995). Laboratory selection of a strain of S. littoralis Boisdouval with Bt toxin Cry 1C for 14 generations resulted in resistance ration from 10- to more than 500 fold by using two different methods of selection (Muller-Cohn et al., 1996).

Leaf residue bioassay of a field population of diamond back moth resistant to Cry 1C showed that LC \(_{50}\) for a colonies derived from the resistant population was 20 times greater than the LC \(_{50}\) for the susceptible laboratory colony (Liu et al., 1996). NO-QA strain of diamond back moth was extremely resistant to the four Bt toxins viz., Cry 1Aa, Cry 1Ab, Cry 1Ac and Cry 1F and suggested that single recessive gene conferred extremely high degree of resistance to all four Bt toxins (Tabashnik et al., 1997). The resistant strain of diamond back moth possessed an ability to survive and developed successfully on transgenic plant (Ramchandran et al., 1998).

The response of pink boll worm larvae to Cry 1Ac suggested that the strain APHIS-98-R responded quickly to selection with Cry 1Ac in only three rounds of selections (Liu et al., 2001). Tabashnik et al. (2002) observed that resistance in pink boll worm to Cry 1Ac did not confer strong cross-resistance to Cry 1Aa. Chandrashekar and Gujar (2004) concluded that American boll worm, H. armigera (Hubner) evolved 31 fold resistance to selection pressure of B. thuringiensis endotoxin Cry 1Ac within six generations. The authors further noted that Cry 1Ac selected larvae showed cross resistance to Cry 1Aa and Cry 1Ab.

In contrast to above reports, some studies have also suggested that resistance to Bt toxins supplied in artificial diets or in leaf tip bioassays does not necessarily result in development of insect populations surviving on transgenic plant expressing same Bt toxins. Cry 3A resistant Colorado potato beetle was not able to survive on Bt potato plants expressing the same toxin (Wierenga et al., 1996). Similarly, a highly resistant strain of European corn borer, Ostrinia nubilalis (Hubner) selected on a formulation of four different Bt toxins showed 70 fold increase in the levels of toxins required for mortality, but was not able to survive on transgenic corn expressing some of these proteins (Huang et al., 2002). Thus, it was suggested that in view of environmental conditions that can affect fitness of an insect population, field evaluation for resistance becomes highly important (Bolin et al., 1999).

**Mechanism of resistance to Bt:** Studies have indicated that alleles for high level of resistance to Bt protein are almost always recessive (Tabashnik,
Relatively high level of refugia was also established of resistant insects (Tang et al., 1999). Rahardja and Whalon (1995) studied the inheritance of resistance to Cry III A δ-endorotoxin in Colorado potato beetle and concluded that sex linkage of resistance did not occur and resistance to Cry III A δ-endorotoxin is autosomally inherited and there was no maternal influence. Role of toxin binding alteration study in a resistance and cross-resistance with CXC and KCBhyb strain of H. virescens suggested occurrence of at least two mechanisms of resistance in KCBhyb insects, one of them related to reduction to Cry 1Aa toxin binding (Jurat-Fuentes et al., 2003). Chandrashekhar and Gujar, 2004 studied the degradation of Cry 1Ac protein by midgut proteases as a possible mechanism of resistance and reported that degradation was higher in resistant individual compared to susceptible individual of H. armigera. The Cry 2Aa resistance in CP 73 strain of cotton pest, H. virescens was not caused by either of the two major Cry 1Aa resistance conferring genes but probably had a quantitative genetic basis (Gahan et al., 2005). Relationship of esterase activity in determining the resistance to Cry protein was studied by Gunning et al. (2005). The authors observed that total esterase activity in the resistant strain of H. armigera was significantly greater than susceptible strain proving the link between resistance and increased level of esterase. The resistance present in Hp4-P3 colonies of H. punctigera to Cry 2Ab was due to a single autosomal gene and was fully recessive (Downes et al., 2010).

Management of insect resistance to Bt

Refugia: The concept of refugia entails planting of refuges of non-Bt host plants along with Bt crops to promote survival of susceptible pests. It implies that the large number of susceptible insects produced by a refuge will dilute any rare resistant insects (Gould et al., 1997). In order to reduce the probability of insect evolving resistance to Bt, strategies currently centered on delivering effective doses of protein to the targeted insect pests and dilution of the gene pool with refuges (Carriere et al., 2001). Even a small refuge could preserve some susceptible alleles in the population. But, increasing in the refuge level of 10 or 20 per cent resulted in a low level of resistant individual and thus prevented the extensive establishment of resistant insects (Tang et al., 2001). Relatively high level of refugia was also recommended by Cerda et al. (2006) where resistance is functionally not recessive at the level of toxin expression in the B. thuringiensis crop. Based on monitoring data, Tabashnik et al. (2008) documented that refuge strategy helped to delay resistance evolution.

Gene pyramiding: Plant expressing two dissimilar Bt toxin genes have the potential to delay resistance in target insect populations more effectively than single toxin containing plant (Roush, 1998). Synergism study between two Bt δ-endotoxin on cotton boll worm, H. armigera have indicated that toxicity of the Cry 1Ac and Cry 1F mixture was about 20-times higher than the expected toxicity, thus suggested that Cry 1Ac and Cry 1F can be expressed together for effective control of H. armigera as well as for durable resistance management strategy (Chakrabarti et al., 1998). In a study with Bt transgenic broccoli plants expressing Cry 1Ac and Cry 1C indicated that after 24 generations of selection of Plutella xylostella population, resistance to pyramided two gene plants was significantly delayed compared with single gene plants (Zhao et al., 2003). However, this strategy could fail, if a single gene in a pest confers resistance to both the toxins. The CP 73 strain of the cotton pest, H. virescens was resistant to both Cry 1Ac and Cry 2Aa toxins (Gahan et al., 2005). The transgenic cotton expressing Cry 1Ac and Cry 2Ab genes may be deployed for management of Cry 1Ac resistant H. armigera (Luo et al., 2007). The alleles resistant to commercially available Cry 1Ab Bt maize in a Louisiana population of sugarcane borer, Diatraea saccharalis (F) was first time reported by Huang et al. (2007). Incorporation of Cry 1Ac and Cry 2Ab2 insecticidal proteins in cotton has an additive effect on mortality and growth inhibition of H. armigera (Brevault et al., 2009), whereas, Gao et al. (2009) suggested that cross-resistance must be considered in evaluating the utility of pyramiding Bt genes in cotton for delaying evolution of resistance.

Novel toxin: A new family of insecticidal protein produced by Bacillus during its vegetative growth stages (Vegetative insecticidal protein, Vips) bear no similarity to δ-endotoxin (Warren et al., 1996). The insecticidal activity of Vip3A against lepidopteran insects and acute bio-activity towards Agrotis ipsilon, Spodotera frugiperda and S. exigua was
documented (Estruch et al., 1996). Ingestion of Vip3A by susceptible insects like Agrotis ipsilon and Spodoptera frugiperda causes gut paralyses at concentration as low as 4 ng/cm² of diet and complete lyses of gut epithelium cells resulting in larval death at concentrations above 40 ng/cm² (Yu et al., 1997).

Incorporation of Vip3A producing lines of tobacco could delay Cry1Ac resistance evolution in Helicoverpa armigera (Jackson et al., 2007). Recently, it was suggested that introduction of Vip3Aa/Cry1Ac producing lines could delay resistance evolution in H. armigera in Bt cotton. (Jingjie et al., 2010). Thus, this may be exploited further as an alternative to ß-endotoxin.

CONCLUSION

At least one insect species, the diamondback moth, Plutella xylostella, has evolved resistance in the field, hence caution continues to be warranted. Selection pressure for resistance in other species to Bt crops may increase as adoption level rise. Therefore, the development of second generation insecticidal transgenic crops should not be seen as a panacea for the problems of pest management and Bt resistance but as a call for improved insect resistance management strategies and promote only such transgenic crops which involve expression of at least two unrelated insecticidal genes with sufficiently high level of expression.

REFERENCES


