

# [Pest control methods for crop production](https://assignbuster.com/pest-control-methods-for-crop-production/)

LITERATURE REVIEW

Crop productivity is highly affected by the presence of pests. Insect pests are one of the major causes of low agricultural yield worldwide (Ferre et al ., 2008). In spite of the crop protection measurements, large number of crop is lost to insect pests attack (Oerke, 2006). For sustainable agricultural production to meet the needs of increased population, chemical insecticides are used against the insect pests to reduce the yield losses. Chemical insecticides are still widely used as the effective approach to control the pests. Due to the excessive and constant use of chemical insecticides, insects’ resistance development against these chemicals is the major problem. Synthetic insecticides have adverse effects towards non-target insects, human and environment. In the last forty years, increase in chemical insecticides usage is observed with no significant decrease in crop yield loss (Wilson and Tisdell, 2001; Oerke, 2006; Ferre et al ., 2008; Popp et al ., 2014).

To avoid damaging effects of synthetic insecticides, different methods of insect pests control were approached including development of insect-resistant transgenic plants. For some decades, Bt has been used as an insecticide. Bacillus thuringiensis contains crystalline toxins which are known for their insecticidal activity (Tang et al., 2006). Bt is a spore-forming soil bacterium and these spores contain crystalline Cry or Cyt proteins known as delta endotoxins which have insecticidal activity (Sanahuja et al ., 2011). Despite the registration of 182 Bt-based products by the USEPA (United States Environmental Protection Agency) till 1995, because of toxicity instability of Bt-based insecticidal spray only 2 % of them made up to the total sales of all insecticides by 1999 (http://www. epa. gov/pesticides/biopesticides/).

After extensive research efforts, genes encoding Bt toxins were isolated and used for the development of insect-resistant plants. Tobacco and tomato were used to develop first Bt-based insect resistant transgenic plants (Barton et al., 1987; Fischhoff et al., 1987; Vaeck et al., 1987). By 2013, Bt-based transgenic crops plantation reached more than 175 million hectares of global area due to their great pesticidal activity (James, 2013). Bt toxins are highly toxic to the target insects only and are harmless to non-specific insects. These toxins do not persist in the environment and are not harmful to humans (Ferre´ and Van Rie, 2002).

With the increase in use of Bt crops, there are concerns of resistance development among insect population against Bt toxins (Tabashnik et al ., 1994; Gould, 1998; Frutos et al ., 1999). However, under laboratory and greenhouse conditions, it was showed that several insects have already became resistant to Bt toxins (McGaughey, 1985; Hama et al ., 1992; Gould et al ., 1995; Sayyed et al ., 2000; Ferre´ and Van Rie, 2002; Kain et al. , 2004). To avoid or delay the occurrence of resistance, two strategies have been proposed which includes high dose/refuge and gene stacking (Frutos et al ., 1999; Ferre´ and Van Rie 2002; Shelton et al ., 2002).

Bt toxins have narrow host specificity, so Bt-crops containing only one type of Cry gene are not suitable for long term use as a wide range of insects attack plants during different developmental stages. To avoid narrow host range issues, plants were transformed with more than one type of Bt toxin all together (Sohail et al. , 2012). Bt-based transgenic plant was developed containing combination of Cry1Ac and Cry1Ab toxins, which was effective against wide range of insects. However, it failed due to the occurrence of cross-resistance as both toxins share the same receptor binding site (Sayyed and Wright, 2001; Estela et al ., 2004). Development of insect resistance can be avoided by using a combination of the toxins which share different binding sites. Combination of Cry1Ac and Cry2Ab toxins is ideal as both toxins are active against different target insects and also bind to different binding sites (Hernandez-Martinez et al ., 2008).

Broad-spectrum insect-resistant transgenic plant was developed by Sohail et al ., (2012) to combat insects attack by using previously synthetically synthesized Cry2Ab and Cry1Ac genes and cloned them simultaneously in a plant expression vector which was later transformed into tobacco. PCR-based amplification was used to confirm the transgenic plants and transformed protein expression. Insecticidal efficiency of the transgenic plants indicated significant resistance to S. exigua and H . armigera insects.

Insects develop resistance against insecticidal Cry toxins due to long term exposure. So there is a continuous need of replacing the current Cry toxins with new type of insecticidal genes. For this purpose, isolation of new Cry- type genes is needed. Cry1 and Cry2 genes were identified in different Bt isolates using PCR-RFLP and were amplified using the designed primers. RFLP analysis and PCR amplification showed the abundance of Cry1A including Cry1Ac and Cry2A containing novel Cry2Ab genes. Analysis of novel Cry2 genes sequence depicted 95 % homology to reported Cry2Ab and Cry2Ah genes (Patel and Ingle, 2012).

Occurrence of insects resistance to Cry1- type Bt toxin led to the identification and isolation of Bt genes with different receptor binding-sites and broader insecticidal activity (Lin et al ., 2008). Cry2 -type Bt genes contain around ten different sub-types, coding protoxins with smaller molecular weight ranging from 65-70 kDa (Hofte and Whiteley, 1989). Saleem and Shakoori, (2010) identified 11 Bt strains containing Cry2- type genes while genotyping local Bt isolates and subtyping of these strains revealed presence of various Cry2A -type genes. Cry2A genes were amplified with a product size of 1. 9 Kb and cloned in pTZ57R/T vector. Sequence analysis presented eight new Cry2A genes with toxicity against H. armigera and M. domestica .

Bt Cry2A toxins have structural difference to Cry1A -type toxins leading to different mechanism of action against insect pests. Thus are favorable to be used to regulate insects attack (English et al ., 1994; Morse et al ., 2001). Cry2Aa proteins, compared to other Cry toxins, differ in their mode of action and are toxic to larvae of lepidopteran and dipteran insects (Winder and whiteley, 1989). Their toxicity against lepidopteran insects is higher than Cry1 ­ -type toxins (Morse et al ., 2001). Tounsi and Jaoua, (2003) cloned a new type of Cry2Aa gene, termed as Crybn3-4 , from Bt strain BNS3 which contained an orf of 1. 9 Kb with 633 aa long encoded protein. Alignment with the known Cry2Aa genes revealed homology in the sequence along with numerous changes. Cloned Crybn3-4 was expressed in an acrystalliferous mutant of BNS3; analysis of recombinant Bt cells confirmed the presence of spore-crystal inclusions.

In another study, Bt strain Rpp39 was characterized using scanning electron microscope and the presence of Cry1Aa , Cry1Ab , Cry1Ac , Cry1Ia and Cry2Aa was identified by PCR-RFLP method. SDS-PAGE analysis of Rpp39 isolates displayed bands of 60 kDa and 130 kDa size. Cry2Aa -type gene was cloned and expressed in E. coli BL21(DE3) cells using pET-30a vector. Analysis of sequence depicted 1. 9 Kb long orf encoding 634 amino acids long protein with 99. 7 % homology to Cry2Aa1 protein, thus nominated as Cry2Aa12. SDS-PAGE analysis of induced recombinant BL21(DE3) cells showed the expression of Cry2Aa12 protein with a band at 65 kDa. Expressed protein was found to be highly toxic to larvae of P. xylostella and C. supperssalis (Tan et al ., 2008).

Cry2Ab gene was first identified by Donovan et al ., (1988) and encodes for 633 amino acids long protein with molecular weight of ~ 71 kDa (Ahmad et al ., 1989). Cry2Ab gene is considered cryptic because of the absence of functional promoter (Crickmore et al., 1994; Jain et al., 2006). Cry3A promoter has been used to express Cry2Ab gene (Dankocsik et al., 1990). Cry2Aa orf2 assists the formation of Cry2Ab inclusions in Bt strains (Crickmore et al., 1994). A novel Cry2Ab gene, entitled as Cry2Ab7 , isolated from a new native Bt strain 14-1 was cloned and its expression was examined in an acrystalliferous Bt strain (4Q7) by Jain et al., (2006). The sequencing analysis revealed an orf of 1902 bp and amino acid sequence comparison with other known Cry2Ab proteins showed a sequence variation. Cry2Aa promoter and orf1 + orf2 sequences were fused upstream of Cry2Ab gene to study the expression of Cry2Ab7 gene in Bt strain (4Q7). SDS-PAGE analysis using spore-crystal mixture from transformed Bt strain revealed the expression of Cry2Ab7 protein of around 65 kDa. Cry2Ab7 protein was dissolved in alkali to release active toxins which were found to be lethal to Helicoverpa armigera larvae.

In another study, Cry2Aa and Cry2Ab genes were cloned from the new Bacillus thuringiensis strains 22-4 and 22-11, respectively and their expression was studied in an acrystalliferous strain of Bt (4Q7) and E. coli BL21 (DE3) strain. Cry2Aa and Cry2Ab genes were fused downstream of promoter and orf1 + orf2 sequences of Cry2Aa for their expression analysis in Bt (4Q7) strain. Low expression of the Cry2Aa and Cry2Ab proteins was detected by Western blot analysis. To achieve a high level expression of Cry2Aa and Cry2Ab proteins, respective genes were cloned under the T7 promoter using pET-29a expression vector. SDS-PAGE analysis displayed a band of ~ 65 kDa size and confirmed the high level expression of Cry2Aa and Cry2Ab proteins (Kumar and Udayasuriyan, 2004).