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).