Chapter 9

Biotechnological Approaches for Sustainable Insect-Pest Management

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Abstract

To meet the fiber, food and other requirements of the growing world population, agricultural biotechnology is now an essential tool. Agricultural insect pests are a big threat as they damage crop yields all over the world computing to an annual loss of several million dollars. Genetically Modified (GM) Crops have been rapidly adopted worldwide to manage agricultural pests safely and effectively. Recently, crops expressing proteins from the bacterium *Bacillus thuringiensis* (*Bt*) have been developed by plant breeders that are highly resistant to many of our most serious insect pests. Transgenes from *B. thuringiensis*, are increasingly used to protect the staplecrops and vegetables from insect damage. In this chapter, we will discuss and review the role and application of biotechnology, different methods and techniques such as RNA interference technology (RNAi) and Sterile Insect Techniques (SIT) that have been proven to provide sophisticated alternative tools for targeted control

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of insect pests of different crops (cotton, maize, rice, vegetables comprising mainly potatoes and also fruits etc) for sustainable agriculture. Moreover, the chapter also provides valuable information and scientific knowledge about the methods and techniques used in the development of transgenic crops having insecticidal proteins and the importance of their use within an IPM context.

Keywords: Biotechnology, RNAi, Sterile Insect Technique, Entomopathogens, GM crops

9.1. Introduction

Insect pests have become a menace and are posing a serious threat to worldwide agriculture. Despite all the remedies and techniques deployed by farmers to protect crop plants, this issue has still not been properly solved. After the Second World War, the production of warheads decreased due to a decline in their demand which unfortunately led to the shifting of the warhead industry to production of insecticides/pesticides. In order to improve their business, the multinational companies pushed for the application of pesticides promising farmers that after some years of pesticide application, the pests would be completely eliminated. Farmers were tempted to use high inputs of pesticides in the hope of getting higher crop yields. Pesticide application gradually became more common and eventually farmers started relying entirely on pesticides to kill insects/pathogens. Even scientists shifted their attention towards production of new and powerful insecticides instead of working on development of insect resistant crop plants (Smith and Kennedy 2002). However, with the passage of time it is now being realized that this practice has gradually increased the resistance in insects (Lee 1992) as well as causing serious health hazards and adverse environmental effects all over the globe. Moreover, the massive use of broad-spectrum insecticides/pesticides has badly disrupted the ecosystem of natural predators and parasitoids that helped in controlling different pests. After the realization of this situation, there was a dire need to find a permanent and environment friendly solution to this problem. This was only possible by integrated pest management or biotechnological approaches through which insect resistant varieties of important crop plants were tailored along with maintaining a healthy ecosystem (Gelernter 2005; Boschm and Gilligan 2008).

Due to the growing dependence on insecticides, which harm the environment and human health, entomologists developed an “integrated pest management” (IPM) strategy to control insect pests (Kogan 1998; Koul et al. 2004). IPM is generally defined as “a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of; and impacts on producers, society, and the environment”. It involves the non-genetic approach which combines the use of chemical control, resistant germplasm, crop rotations to interfere with insect life cycles and modification of plant handling and harvesting methods (Carrozi and Koziel 1997).

The IPM strategy is mainly based on the prevention and management of pests by planning tactics to use before the injurious stage of the pest. These include host plant

resistance (HPR), cultural control, biological control and limited use of insecticides. However, this approach has not proven to be 100% successful.

The discovery of polymerase chain reaction (PCR) by Mullis and Faloona (1987) remarkably facilitated the DNA marker technology. Tremendous advances in forensic studies, crop breeding, human DNA fingerprinting, solutions for criminal and paternity disputes, early detection of diseases, gene cloning and engineering of organisms has been achieved through the tools and techniques of biotechnology (Kramer 2002). The field of agricultural biotechnology is progressing tremendously over the last 30 years due to the greater understanding of DNA as the blue print of genetic code. Biotechnology will serve as bridge to move our agriculture industry forward to help meet the food and fiber needs of the growing world population.

Genetic engineering is a term that is often interchangeably used with gene technology, genetic modification, or gene manipulation. Basically, it refers to the alteration of the genetic make-up of an individual using recombinant DNA tools. Through this technique specific recombinant enzymes are used to cut and insert pieces of foreign DNA into a plasmid vector which is in turn used to clone the gene of interest. This technique is similar to breeding as it also uses the principles of genetic manipulation; however in traditional plant breeding, the genes are transferred between the same species while in genetic engineering, genes may be transferred to individuals that would not interbreed in normal circumstances. Conventional breeding approaches are very laborious and time consuming. Moreover, there are chances of transfer of undesirable genes or while one desirable gene is gained, another maybe lost due to random assortment of genes in the offspring. Due to these reasons, traditional breeding approaches have not succeeded in improving agriculture to a high extent (Primrose and Twyman 2006).

Recent scientific breakthroughs in molecular biology made it possible to target pests and their insect vectors directly. Biotech crops (GM crops) offer a promising future for sustainable pest management through which pests may be controlled safely and effectively without the use of pesticides (George 2008). All genetic engineering approaches do not involve insertion of new genes. Genetic make-up of plants may also by manipulated by removing or switching off certain genes or their promoters. The increasing knowledge about insect genomes and advent of RNA interference technology may be combined to develop crops expressing double stranded RNA mediated silencing of genes in pests and insects without obstructing non-target organisms. Additionally, other approaches such as RNAi/insect transgenesis and sterile insect techniques have been proven to provide sophisticated alternative tools for targeted control of pests and their insect vectors (O' Brochta and Atkinson 2004; Robinson and Hendrichs 2005).

"Green biotechnology" may profit from the raising spectrum of insect-derived genes encoding anti-microbial peptides whose transgenic expression hve been established to confer crop resistance against economically important phytopathogens and insect pests. This chapter addresses the input of biotechnology in modern and sustainable approaches for plant protection and pest management. This chapter also explains the biotechnological approaches/methods and techniques for the development of GM crops for sustainable pest management.

9.2. Application of biotechnology in insect pest management

Biotechnology is basically the effective use of living systems and organisms to develop or make useful products for human beings, or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use". Plant diseases caused by insect pests damage crop yield all over the world computing to an annual loss of $30-50 billion (Misra and Bhargawa 2007). However, genetic engineering and biotechnology have made it possible to produce crop plants with improved resistance against such bioagressors. Molecular biology approaches have helped scientists to support and enhance worldwide plant health. Although biotechnology involves a wide range of complex and diversified tools and techniques, its basic principles are quite simple. It is necessary that the proper knowledge of the physiological and biological mechanisms of action and regulation of gene expression as well as bio-safety measures must be ensured beforehand. Some major achievements and uses of biotechnology for increasing crop production by protecting them from insect pests are discussed below:

9.2.1. Peptides

Peptides or proteins with an anti-pest infection activity have an immensely high potential for sustainable plant protection. For example microbial peptides from the bacterium *Bacillus thuringiensis* (*Bt*) show activity against various insects (James 2008). These days' insecticidal *Bt* peptides are being used in combination with other traits like herbicide tolerance; thus enhancing the potential of antimicrobial peptides (AMPs) (Marcos et al. 2008) as Cecropin was recognized as the first AMP in the 1990s. It was extracted from insects and applied ectopically on crops such as potato and rice to increase their insect resistance. The spontaneous production of insecticidal peptides/proteins is one of the responses in plants against the attack by phytophagous insects. A lot of experiments have been conducted in the last 10 years to study the activity of a few lectins that are expressed in response to herbivory by phytophagous insects (Killiny et al. 2012). They can be used in developing various insect pest management programs as they have little effect on non-target organisms. Carbohydrate-binding proteins (CBP) or lectins are a group of entomotoxic proteins present in many plant species. They may be used to control pests (Killiny et al. 2012).

The venoms of certain insects are also being used to topically kill various insect pests, e.g., spider venoms are complex mixtures of toxic chemicals (Tedford et al. 2004). The venom of the Australian funnel web spider (*Hadronyche versuta* S.) containing the x-ACTX-Hv1a toxin (Hvt) effectively killed the American bollworm (*Helicoverpa armigera* H.) and Egyptian cotton worm (*Spodoptera littoralis* B.) caterpillars when applied topically. The Hvt toxin when genetically expressed in tobacco, it protected the plants from *H. armigera* and *S. littoralis* larvae (Khan et al. 2006).

Some polypeptides from insect parasitoids and viruses are being identified and used to control insect pests. Baculoviruses or Nucleopolyhedroviruses are pathogens

having double-stranded DNA. They are usually extremely small and attack insects and other arthropods. Their genetic material is extremely sensitive. The baculovirus particle (*virion*) is protected by protein coat called a *polyhedron* which is typically fatal to the insect (Granados and Federici 1986). Nuclear polyhedrosis virus (NPV) *(S. litura)*, corn earworm polyhedrosis virus *(Helicoverpa zea B.)* American bollworm polyhedrosis virus *(H. armigera H.)*, Cabbage armyworm (*Mamestra brassicae* L.), Diamond back moth (*Plutella Xylotella*), and cabbage butterfly (*Pieris brassicae*) nuclear polyhedrosis viruses have been identified and used successfully to control lepidopterous insect pests (Ahmad et al. 2016b). Some of them are commercially available baculoviruses that are being used to control insect pests of cotton, vegetables, corn and tomatoes (Mahr et al. 2008; Ahmad et al. 2016b). The Polydnaviruses (PDVs), such as Ichnoviruses (IVs) (Hymenoptera: Ichneumonidae) and Bracoviruses (BVs) (Hymenoptera: Ichneumonidae) are a family of insect viruses that are being isolated and used to control lepidopterous insect pest.

9.2.2. RNA interference (RNAi) technology

RNA interference technique (RNAi) was discovered for the first time in the soil nematode (*Caenorhabditis elegans* M.). RNA interference is a promising technology which may enable selected measures against insect pests without impeding non-target organisms. It is a powerful technique in which gene expression in a wide range of organisms may be down regulated through double stranded RNA. In this technique, plant-delivered RNA is used to suppress the expression of a particular gene in the pest. Down regulation of gene expression in insect pests through delivery of dsRNA can cause death of the pest by interfering with developmental processes and its metabolism. For example, Citrus greening is a rapidly spreading disease causing huge losses to the citrus industry all over the world. The citrus greening disease is also called the Huanglongbing (HLB) disease. It is spread by the phloem dwelling bacterium *Candidadtus Liberibacter asiaticus* (CLas) through the Asian citrus psyllid (*Diaphorina citri* K.) insect vector. Scientists of the Citrus Research and Education Center, University of Florida have used RNAi technique to induce abnormal/deformed wing in the psyllids. The abnormal wing disc gene "awd" associated with wing development in insects interferes with the flight of psyllids and thereby reduces their survival rate which eventually leads to control of the citrus greening disease (El-Shesheny et al. 2013; Hajeri et al. 2014). The red flour beetle (*Tribolium confusum* D.) shows a very strong systemic response to RNA interference. In this regard experiments have also been conducted in which expression of dsRNAs against insect genes in transgenic plants were studied. Plants have shown resistance against insect herbivory and the termite *Reticulitremes flavipes* juveniles (Zhou et al. 2008). Plants release certain chemicals which attract insects. If we are successful in suppression of the production of such chemicals, the plant may be protected (Carrozi and Koziel 1997). The suppression of gene expression by dsRNAs in herbivorous insects such as lepidopteran lightbrown apple moth larvae, *Epiphyas postvittana* (W.) (Turner et al. 2006) larvae of American bollworm (*Helicoverpa armigera* H.) and larvae of diamondback moth *Plutella xylostella* (L.) was successful (Kumar et al. 2009). RNAi technology has indeed a

very huge potential in attaining insect resistance in plants. This is a useful approach having an immense potential for effective pest management. However, suppression of gene expression in insects has yet to be explored and more research both at basic and applied levels is required.

9.2.3. Transgene-improved sterile insect technique

Insect transgenic technology is a promising tool for insect control. Improvement in the Sterile Insect Technique (SIT) through transgenic approach might provide a new insight in to insect pest management. The SIT is an effective and ecologically safe method for controlling pests. In this system a mass of sterilized organisms or pests is reared in artificial settings and then released into nature, which leads to infertile mating in turn resulting in a reduction in the pest population (Klassen and Curtis 2005). The SIT is an environmentally friendly approach which may be used as an alternative to insecticides. Insect pests such as the pink bollworm *Pectinophora gossypiella* (S.) have been successfully eradicated through this technique in California, USA (Henneberry 2007). Moreover, the tsetse fly (*Glossina austeni* N.) in Zanzibar, the new world screwworm *Cochliomyia hominivorax* (C.) in North and Central America and various species of the tephritid fruit fly have been controlled in many parts of the world (Klassen and Curtis 2005). The Mediterranean fly (*Ceratitis Capitata* W.) has also been controlled by male-only releases (Hendrichs et al. 1995).

9.2.4. Manipulation of insect resistant molecular markers

Frego bract is a mutated type of floral bract in cotton. It is an important insect resistant trait. Due to its narrow and twisted shape, it does not allow insect eggs to stay on its surface. The eggs laid by insects on such bracts become more prone to environmental vagaries as compared to the normal broad bracts. The eggs tend to fall off from this thin and narrow bract. Although this trait seems a promising insect resistant trait, very little research work has been conducted on the trait and no commercial variety has been tailored. The frego-bract character was reported as effective in suppressing boll weevil (*Anthonomus grandis* B.) population and comparison of the fibre properties of frego lines with commercial varieties showed that it would be a useful trait for the development of insect resistant cotton cultivars (Jenkins and Parrott 1971).According to a report, cotton varieties having a combination of frego bract and red plant color could be developed which would be equal in yield and fibre quality with other commercial varieties (Weaver and Reddy 1977). However, according to some reports, frego bract genotypes are less productive and their fibre is of low quality (Thaxton et al. 1985; Singh 2004). Malik et al. (2009) found that frego bract recombinant lines are equally productive, have high photosynthetic rates and have a good quality fibre compared to the normal bract line. The variation between normal bract and frego bract recombinant lines was also determined by PCR studies and it was concluded that this useful trait can be incorporated into commercial cotton varieties for insect resistance by cloning the underlying gene for frego bract (Malik et al. 2009). In addition to frego bract, some other morphological traits have been identified which confer resistance to insect pests in cotton. Incorporation of such traits in the cotton cultivars has been advocated for stable, economic and environment friendly insect resistance in cotton by many

researchers (Maxwell and Jennings 1980, Rahman et al. 2013). These traits include trichomes, okra leaf, nectariless, gossypol glands etc. which make cotton plant unattractive to insects for feeding, oviposition, shelter etc. Moreover, plant surface waxes also help in keeping insects away in sorghum, brassica, apple and rice. Trichomeshairiness on leaves and stems is a major source of resistance to many insects especially thrips and weevil (Stephens and Lee 1961) jassid and mites (Narayanan et al. 1990). Trichomes interfere with insect locomotion, oviposition, attachment, shelter, feeding, ingestion and digestion. They have shown to decrease the infestation of aphids and leafhoppers in potatoes and alfalfa (Floyd et al. 2002).

Hairiness is a major source of resistance against sucking insects in cotton (Mursal 1994). Rahman et al. (2013) identified DNA markers for velvet hairiness in cotton. Such insect resistant trait markers may be identified in other species and may be exploited in developing insect resistant crop cultivars. Insect resistance is a complex quantitative trait. QTL (Quantitative Trait Loci) Mapping studies for tungro spherical virus and green leafhopper (*Amrasca bigutulla*) resistance have been conducted (Sebastian et al. 1996). Similarly loci for the Russian wheat aphid resistance have been mapped in barley (Nieto-Lopez and Blake 1994). Moreover, the loci for gall midge resistance in rice (Nair et al. 1995), trichome-mediated resistance in potato (Bonierbale et al. 1994) and European corn borer resistance in maize (Christensen et al. 1994) have also been identified. Identification of molecular markers/QTLs can positively assist in breeding for insect resistance. New varieties of crops may be introduced through marker assisted molecular breeding. Once the underlying genes for insect resistance are identified, they may be cloned and integrated into commercial cultivars through transformation approaches.

9.2.5. Genetically Engineered Entomopathogenic Organisms

In order to avoid pollution and contamination of the natural ecosystem, effective and safe biocontrol agents have been introduced through biotechnology and genetic engineering. These biocontrol agents including fungi, nematodes, bacteria and viruses have proven to be highly effective against common insect pests which destroy our crops. These biocontrol agents are more popularly referred to as bioinsecticides.They do not leave toxic residues and are also safe for non-target pests (Robert and Bonning 2000).

Genetic engineering of biocontrol agents includes selection of the beneficial or required strains, cloning of genes that influence the certain trait of interest and then introducing these genes into the natural population in such a way that they are successfully expressed and multiplied in the progeny. All this depends on how much we understand the biology of pathogenicity. Most progress in this field is made in case of the baculovirus insecticides. The genetic manipulation of entomopathogenic fungi and nematodes is still in the initial stages of development and testing. A review of recent progress made in case of entomopathogenic organisms is given below:

9.2.5.1. Entomopathogenic Fungi

Entomopathogenic fungi produce a wide range of insecticidal proteins; however, these have not been exploited in agricultural biotechnological approaches. Some

fungi such as *Beauveria bassiana and Metarhizium anisopliae* have a broad host range so may be used for effective biocontrol of its insect pests. The entomopathogenic fungi enter the host cuticle with the help of certain cuticle degrading enzymes. These enzymes along with serving as a means to penetration of the cuticle, also act as insecticidal proteins (St. Leger et al. 1998). The complex interactions between entomopathogenic fungi and its host insects have now been studied and researchers have come up with a successfully engineered entomopathogenic fungus *M. anisopliae*, which is highly effective against lepidopteran larvae (St. Leger et al. 1998; Robert and Bonning 2000).

Despite the 700 species of entomopathogenic fungi known, only one has been genetically engineered up till now. The main reason for little or slow progress in the development of entomopathogenic fungi is the little or no knowledge about the molecular biology of its pathogenicity. Moreover, good cloning systems are available for only a few species of deuteromycetes (Goettel et al. 1989). More fungal genes involved in pathogenicity need to be identified. It is said that various genes are expressed at various stages of fungal-host relationship (St. Leger 1993). *M. anisopliae* takes around 5 to 10 days to kill a host insect so scientists have genetically manipulated its genome in such a way that additional copies of the *Pr1* gene have been introduced which facilitate its penetration into the cuticle (St. Leger et al. 1996). Larvae infected with recombinant strains of fungus died earlier than those infected with the wild type *M. anisopliae*. *Pr1* also activates the trypsins which start melanization of the larvae. This reduces contamination of the environment as the melanization of larvae restricts the fungus sporulation and in turn dissemination of spores from one place to another (St. Leger et al. 1998).

9.2.5.2. Entomopathogenic Bacteria

Relatively more amount of work has been conducted on entomopathogenic bacteria as they are easy to culture and genetically manipulate via their plasmids. The most emphasis has been put on *B. thuringiensis* (Bt). Bt toxins are known for their specificity and safe nature. *Bt* produces several insecticidal crystal proteins (ICPs). These ICPs accumulate in the cytoplasm in crystalline form and are highly toxic against lepidoptera, diptera and coleopteran larvae. Next to *Bt*, a lot of work has been conducted on genetical improvement of Enterobacteriaceae which are kept in the guts of nematodes and then released into the hemocoel of *B. sphaericus* for mosquito control. The mosquito larvicidal genes have been cloned from *B. sphaericus* and are being genetically pyramided for infection of dipteral insects (Robert and Bonning 2000). Genetically engineered endophytic microbes are highly valuable in the sense that they may be used to kill stem and leaf feeding lepidopteran insects. The gene encoding Cr1Ac has been manipulated into the endophtic bacterium *Clavibacter xyli* (Tomasino et al. 1995). This bacterium was introduced into corn seedlings through seed inoculation or wounding. It was found that the damage caused by the European corn borer *Ostrinia nubilalis* was reduced from 55% to 65%.

9.2.5.3. Entomopathogenic Viruses

A variety of existing peptides or toxins are ineffective against insect pests when ingested or topically applied. Certain insect viruses such as baculoviruses help these toxins reach the site of action in the hemocoel of the insect. The viruses expressing

neurotoxins are the most effective biocontrol agents and may be used as bioinsecticides. Baculoviruses are the only viruses genetically altered to act as bio-insectcides (Black et al. 1997). Around 20 recombinant baculoviruses have been successfully engineered up till now. The development of recombinant insect virus requires a plasmid transfer vector consisting of a viral genomic restriction fragment containing the desired sequence/alteration. The alteration is incorporated into the viral genome by homologous recombination between the parent genome and transfer vector. In order to produce a recombinant virus, it is necessary for the insecticidal protein to have no effect on the replication of the virus in host insect until the insect dies. Moreover the protein must be highly effective in small doses and must have a speedy reaction (Possee et al. 1997). *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV) is the most studied virus in this context. The gene coding for polyhedral envelope protein was deleted from the AcMNPV genome which resulted in a 6 times greater and faster infection of the virus against the insect 'first star' *Trichoplusia ni* compared to the wild type AcMNPV which had that gene intact. This is due to the fact that rapid release of the virus into the gut of the insect leads to faster death. The promoters driving certain insecticidal genes are also activated early on in the life cycle of a virus to increase its efficiency. American Cyanamid and DuPont Company tested the first ever genetically engineered baculoviruses as bioinsecticides (Black et al. 1997). Small scale field trials of these bio-insecticides are being conducted and soon baculovirus insecticides will be on the market.

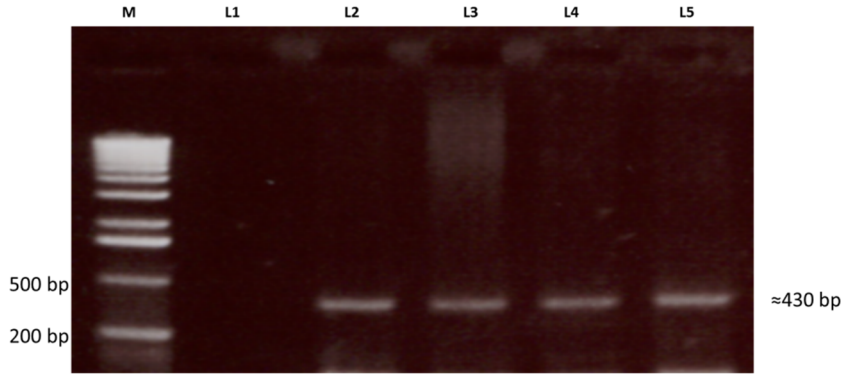

Fig.1A PCR for the detection of NPV from infected samples from different Geographical regions of Pakistan by using NPV specific primers: L1 Non infected *Spodoptera litura* Larva, L2- NPV infected *Spodoptera litura* larva (RY Khan strain), L3-NPV infected *Spodoptera litura* larva (Multan Strain), L4- NPV infected *Spodoptera litura l*arva (Faisalabad strain), L5- +NPV *Spodoptera litura* DNA, M- 1 kb DNA Marker. (Ahmad et al. 2016b)

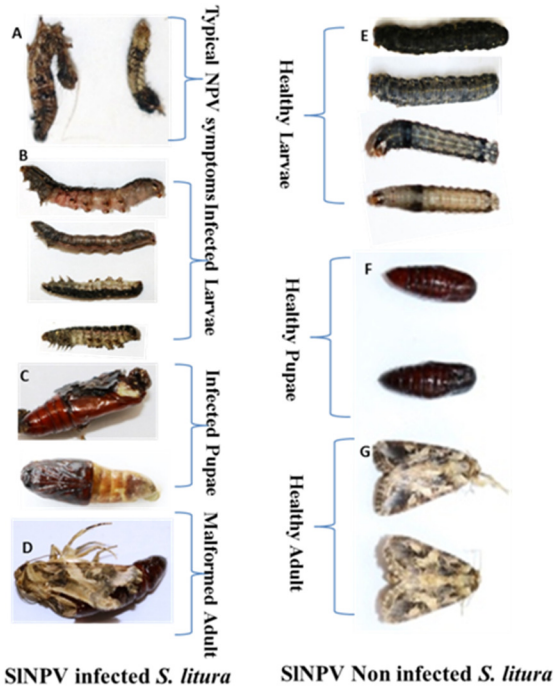

Fig.1B. Different stages of Healthy and NPV infected *Spodoptera litura* . ***A-*** typical NPV symptom as attached with walls of vials downward. **B-** Different NPV infected dead instars. **C-** NPV infected pupae. **D-** Malformed NPV infected Adults. **E.** Healthy larvae. **F-** Healthy pupae. **G-** Healthy Adults (Ahmad et al. 2016b)

Despite the fact that entomopathogenic fungi, bacteria and virus have been genetically altered and are being used to some extent as biocontrol agents, there are some disadvantages. Some problems and limitations we are facing in this strategy are limited host range, slow mode of action and restricted persistence in the field (Robert and Bonning 2000). But all this can be dealt with by further genetic manipulation and in depth understanding of the molecular basis of pathogenicity.

9.2.6. Development of transgenic crops and insects

The time required for developing a transgenic plant varies in different crop species. It also depends on the gene and available resources as well as regulatory approval. The main technique involves a series of steps:

The first step is the isolation of nucleic acids (DNA or RNA). This is done by following a proper extraction protocol. The extracted nucleic acid (DNA/RNA) is precipitated in the form of thread-like pellets. The second step is cloning of the gene of interest. This is done by generating DNA fragments (cut by a restriction enzyme) and then joining them to a vector which is then allowed to multiply in a host cell. Vector having the desired sequences is selected, isolated and clones are produced.

To determine whether the desired gene was cloned completely, the restriction enzymes are again used. Gene manipulation is further done by replacing an existing promoter sequence with a new one, adding a selectable marker and promoter gene and incorporating gene enhancer fragments and introns (Lemaux 2008).

While developing GM plants, promoters are used for differential expression of genes. Selectable marker genes are generally linked to the gene of interest to help in its detection inside the plant tissues. For this purpose antibiotic resistance and herbicide resistance marker genes are used so that the cells containing the inserted gene may be detected. Reporter genes are also cloned into the vector in close proximity to the gene of interest so that after transformation, the transformed cells may be easily identified. The reporter genes are also used to determine the correct expression of the inserted gene. Instead of inserting a whole new gene into an organism, gene expression can also be promoted by cloning genetic sequences in front of the promoter sequences or within a certain genetic sequence itself (Lemaux 2008).

Transformation is the most common method used to introduce a gene into the plant/animal cell. This is done through various techniques such as partial bombardment, gene gun method or through the use of the *Agrobacterium* bacteria. The transformed cells are grown *in vitro* and then cultured to form small plants expressing the inserted gene. The aim of transformation is to introduce the gene of interest into the nucleus of the cell without killing the cell and allowing it to continue its normal activities. Once a plant stably inherits and expresses the gene of interest in subsequent generations, it is called "transgenic". PCR (Polymerase chain reaction) is one of the quickest and most effective methods used to determine the integrity of the transgene in the plant cell (Lemaux 2009). The detection of transgenic cells is done by analysis of PCR products in agarose gel to see whether the DNA fragment equivalent in size to the inserted gene has been amplified or not.

Another method used to expose the transgenic status of the plant is called Southern Blot analysis in which autoradiography is used. Northern blot analysis determines whether the transcript or the messenger RNA (mRNA) of the introduced DNA is present and is correctly transcribed in the transgenic plant. This is also done through autoradiography. Western blot analysis is an analytical technique used to detect whether the transgenic plants are producing the specific protein product of the inserted gene. Protein samples are extracted from the transgenic plant cells, denatured and then transferred to a nitrocellulose membrane. The protein is then detected by using probes or antibodies specific to the target protein (Towbin et al. 1979).

9.2.7. GM crops

Pest management technologies and practices in agriculture are cutting down the use of insecticides, the most prominent being the use of biotechnology. With the discovery of restriction enzymes in the 1980s, the field of molecular biology has progressed tremendously. After the first transformation experiments conducted on tobacco in 1983, *Bt* and some other insecticidal genes/proteins have been transferred to some crop plants to confer protection against insect pests. Scientific evidence strongly suggests that the GM crops grown so far are very safe and non-hazardous to

the environment. Most of the GM (genetically modified) crops are known as *Bt* crops (due to the insertion of *Bt* gene). The benefits of *Bt* crops over the conventional varieties have been recognized all over the world and these insect resistant crops have been successfully grown on millions of hectares (James 2008). Crops such as maize, cotton, potato and rice, egg plant, and some cruciferous vegetables have been transformed successfully with *cry* genes coding for proteins that are highly active against the most important pests (Ferre et al., 2008). *Bt* crops, especially cotton contains *cry1Ac* or a fusion of *cry1Ac* and *cry1Ab* genes that are highly active against the lepidopteran (chewing pests) that feed on the cotton bolls. The *Bt* genes express a protein product which makes the cotton bolls toxic; so that whenever a pest tries to chew on the boll, the poison enters its gut eventually killing the pest (Ferre et al. 2008).

Recently we have developed a new plant system in oil seed rape (*Brassica napus* L.) lacking toxic mines and improving the quality of oil and fodder. We are investigating the defense response against sucking and chewing insect pests to control them in a proper way (Ahmad et al., 2016a).

The Colorado potato beetle poses a serious threat to the potato crop. Genetically modified *Bt* potatoes containing the *cry3Aa* gene against the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae,) have been planted commercially in North America and Europe. The development of a *Bt* rice product containing both *cry1Ac* and *cry1Ab* to offer complete protection against the rice stem borers is under pavement. *Bt* vegetable crops such as eggplant and cruciferous vegetables are also under development. In *Bt* eggplant, the eggplant fruit and shoot borer *Leucinodes orbonalis* (Lepidoptera: Pyralidae) is targeted while the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) is trying to be controlled by *Bt* crucifer vegetables (Shelton et al. 2008).

Some crops such as corn and soybeans have been genetically modified to resist herbicides. These crops now help farmers to eliminate the milkweed growing in between the rows of these plants without affecting the corn or soybean plants. These crops have been modified to be tolerant against the chemical glyphosate which is found in most herbicides (Losey et al. 1999).

9.2.7.1. GM cotton

Cotton is one of the world's leading fiber crops being grown in more than 75 countries with a total production of more than 26.6 billion kilograms. It fulfils 40% of the total worldwide fiber demand. China is the highest cotton producer in the world followed by India, USA, Pakistan and Brazil (Cotton production Worldwide Statistics 2014). Four species of cotton *Gossypium herbaceum, G. arboretum, G. barbadense* and *G. hirsutum*, are mainly grown in Asia, Egypt, parts of western and South America as well as the West Indies (Rahman et al., 2014). The most grown species is *G. hirsutum*. Cotton seed is used in animal feed and also produces oil. A large diversity of arthropods (more than 1300 herbivorous insects) inhabits cotton all over the world (Naranjo 2011). A number of insect vectors are being reported with corresponding plant pathogens producing havoc in respective arenas (Ahmad et al. 2016b).

Transgenic *Bt* cotton (producing the proteins *Bt*; Cry1 and Cry2) was firstly grown on a commercial scale in Australia, Mexico and the USA in 1996 but has now spread to millions of hectares all over the globe. It has effectively reduced the use of insecticides by 94.5 million kilograms and improved cotton yield by US$7.5 billion. *Bt* cotton controls boll worms which are a serious and highly damaging pest for cotton worldwide. The USA, Australia and Mexico were the first countries to allow commercial cultivation of *Bt* cotton, followed by China and South Africa in 1997, Argentina in 1998, Colombia and India in 2002, and Brazil in 2005 (James 2008). In 2009 *Bt* cotton was planted on 15 million hectares in more than 11 countries reducing 140 million kg use of insecticides (Steven 2011). The hybrid cultivation approval for planting in India was increased from 4 in 2002 to 131 in 2007. Now India is the highest *Bt* cotton producer in the world followed by China, and then the USA. Pakistan being one of the top 5 cotton producing countries of the world has introduced several *Bt* cotton varieties indigenously. In fact *Bt* is the only transgenic crop being commercially cultivated in Pakistan. The *Bt* cotton varieties approved for cultivation this year include Tarzan-1, VH-259, MNH-886, BH-178, NS-141, CIM-599, FH-114, CIM-602, IR-NIBGE-3, FH-118, CIM-598, FH-142, Sitara 009, IR-NIBGE-824, A-One IUB-222, Sayaban-201, Sitara-11M, A-555, KZ-181, Tarzan-2 and CA-12 (Rahman et al. 2014).

The Cry1 or Cry2 carrying cottons are resistant to nearly all sorts of bollworms as well as a variety of other pests such as leaf worms, leaf perforators and semiloopers. However these single genes expressing cottons are not so effective against pests such as *Spodoptera* spp., *Trichoplusia ni*, *Pseudoplusia includes* and cutworms (James 2008). In 2015, *Helicoverpa armigera* H. and *Pectinophora gossypiella* (S) infestation and resistance has been reported on Bt cotton containing Cry 1Ac in Pakistan (Ahmad et al. 2016c). Now double gene expressing cottons (Bollgard II, WideStrike) have been produced and are being grown in Australia. These dual gene constructs have a broad spectrum of resistance against the Lepidoptera so they can control many pests that were previously left unharmed by the single gene constructs (Adamczyk and Gore 2004).

It has been 18 years now ever since *Bt* cotton was first cultivated. Despite its advantages, some negative impacts have been seen. Among the disadvantages of *Bt* cotton, one of the most disastrous effects is that the low or no use of insect repellent sprays has increased the population growths of non-target pests in many *Bt* cotton growing countries. Some pests such as green mirid (*Creontiades dilutus*), green vegetable bug (*Nezara viridula*), leaf hoppers (*Austroasca viridigrisea* and *Amrasca terraereginae*), and thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*) (Lei et al. 2003, Wilson et al. 2006) have become more prominent in Australia. Wu et al. (2002) reported that a complex of mirid plant bugs such as *Adelphocoris suturalis*, *A. lineolatus*, *Lygus lucorum*, and *L. pratensis* have become problematic in China. In Henan Province (USA), it was observed that populations of some sucking pests such as leafhoppers (*Empoasca biguttula*), cotton aphids (*Aphis gossypii*) and spider mites (*Tetranychus cinnabarinus*) increased in *Bt* cotton fields (Williams 2006). According to a report by Sharma et al. (2005) an outburst of pests such as tobacco caterpillar (*Spodoptera litura*), mealy bugs (*Pseudococcus corymbatus*, *Pulvinaria maxima*, and *Saissetia nigra*), thrips (*T. tabaci*) and okra

leafhoppers (*Amrasca biguttula biguttula*) occurred in different regions of India and Pakistan.

9.2.7.2. GM rice

Rice (*Oryza sativa L.*) being one of the most widely eaten cereals is grown on an area of 152 million hectares of the world (FAO 2007). About 90% of the total rice produced in the world comes from Asia (Maclean et al. 2002). Rice is grown on approximately 5,559,750 acres of Pakistan with a total production of 4,500,000.00 tones (FAO 2013). Punjab province is the major contributor as 59 % of the rice growing area of the country is located there. Quite a few varieties of rice are grown in Pakistan, however the main ones are Basmati Rice (long grain rice, comprising of more than 50% of the total cultivation) and IRR6/9 (short grain rice, comprising of 40 % of the total cultivation) (FAO 2007).

A wide variety of insect pests such as Hemiptera, Diptera, Lepidoptera, and Coleoptera damage this crop all over the world (Dale 1994). Lepidopteran stem borers are chronic pests in all rice ecosystems. They account for a yield loss of 2.3% in Asia (Savary et al. 2000) with 3.1% loss in China (Sheng et al. 2003). However, the most important tropical and temperate species are the yellow stem borer, *Scirpophaga incertulas* (Pyralidae) and the striped stem borer, *Chilo suppressalis* (Crambidae) respectively. Stem borer damages the rice tillers during vegetative stage called dead hearts whereas at the reproductive stage, it causes the production of panicles containing unfilled grains called whiteheads. Some foliage feeders from the Lepidopera group also infest rice species. Among these leaf folders, *Cnaphalocrocis medinalis* and *Marasmia* spp. (Pyralidae) are the most common. Leaf folders being very visible to farmers stimulate them to use insecticides (Matteson 2000). However, they can be controlled without the application of insecticides as leaf folders cause the least amount of damage to rice because they only affect at the vegetative growth stage which can compensate for damage to foliage.

Scientists have identified several genes in rice germplasm having resistance to plant hoppers, leafhoppers and the Asian rice gall midge (*Orseolia oryzae*). They have been crossed through breeding to tailor modern rice varieties. Although thousands of different rice accessions have been studied and evaluated, genes resistant to lepidopteran rice pests have not been identified up till now (Heinrichs 1994). After the huge success of transgenic *Bt* technology in cotton, extensive research and experiments to form *Bt* rice have been conducted over the years. The *Bt* gene transformation in rice was reported for the first time by Fujimoto et al. (1993). Rice lines expressing *cry1Aa*, *cry1Ab*, *cry1Ac*, a *cry1Ab/cry1Ac* fusion, *cry1B*, *cry1C*, and *cry2A*, and a fusion of *cry1Ac* with *cry2A* genes, have been shown to be resistant to stem borers, leaf folders and other foliage-feeding Lepidoptera. In addition to cry toxins through *Bt* gene, some protease inhibitors have also been expressed in rice to enhance resistance against the chewing pests. Plant lectin genes have also been used to control plant hopper and leafhopper pests of rice through genetic engineering. Lectin gene from garlic (*Allium sativa* leaf agglutinin gene, *ASAL*) and snowdrop lectin gene, *Galanthus nivalus* agglutinin (*gna*), has been used to induce resistance in rice against plant hoppers and leafhoppers in laboratory and field tests (Saha et al. 2006). Field trials were also conducted in Pakistan, Spain, Iran and India (Mahmood-

ur-Rahman et al. 2007). *Bt* Rice will bring prosperity for Pakistani farmers as well as industries and business activities as Pakistan exports about 2 million tons or about 10 percent of world trade annually. However, *Bt* rice is currently not being grown commercially in any country of the world. China has banned the commercial cultivation of *Bt* rice due to environmental and health concerns. Iran grew 4,000 ha of *Bt* rice for seed multiplication in 2005 (James 2008) but commercial production in Iran is not currently allowed.

9.2.7.3. GM Maize

Maize, *Zea mays* L. (corn), grown on 365 million hectares of the world producing about 750 million metric tons of grain per annum. It is the second most cultivated crop after rice. The largest producer of maize is the United States, followed by China, Brazil, Mexico, Argentina and lastly India being the least contributor (FAOSTAT 2007). Asia accounts for 30 % of the global maize growing area. In Pakistan maize is grown on an area of 2.4 million acres with an annual production of 3.25 million metric tons. Punjab and NWFP (North West Frontier Province/Khyber Pakhtunkhwa) are the largest producers contributing to about 30 and 60 % of the total production respectively. About 0.25 million acres of maize are grown in AJK (Azad Jamu Kashmir) (Business Recorder 2014).

Genetically-modified (GM) maize was first planted commercially in the United States in 1996 and in Canada in 1997 but now more than 24% of the worlds area is under *Bt* maize cultivation(James 2007). A single Cry protein was introduced into the first GM maize plants produced. This Cry toxin is resistant to European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae) and other lepidopteran maize pests. *Bt* maize containing endotoxin proteins such as *cry1Ab*, *cry1Ac* or *cry9C* shows resistance against the European corn borer and the Mediterranean corn borer *Sesamia nonagriodes* (Lepidoptera: Noctuidae). *Bt* maize containing the transgenes *cry3Bb*, *cry34Ab* and *cry35Ab* are effective against the rootworms of the genus *Diabrotica* (Coleoptera: Chrysomelidae) (Richard et al. 2008). Recently, herbicide resistant maize containing genes/proteins introduced by Monsanto is being cultivated In Pakistan.

9.2.7.4. GM fruits and vegetables

Fruits and vegetables are vital for good health. However, they are subjected to severe pest infection. About 30 % of the insecticides/pesticides applied all over the world are used to control insect pests of vegetables. The problem of fruit and vegetable pest infestation is more acute in the developing countries which are inhabited by 83% of the world's population (Shelton et al. 2008). Most vegetables are consumed in India and China. Transgenic or genetically modified vegetables and fruits are providing new dimensions for pest control in these countries. Production of transgenic vegetables resistant to insects and insect-transmitted pathogens or viruses has been successful to some extent. It may be possible to confer resistance against multiple viruses if genes from different viruses are collected and pyramided within a single T-DNA region of a binary plasmid. Generally the coat protein (CP) is genetically engineered to confer virus resistance (Fuchs and Gonsalves 2007). Some virus resistant vegetable and fruit species have been engineered that express the CP genes. For example, papaya ring spot virus (PRSV) has been controlled by genetically

modified papaya expressing the CP gene (*Carica papaya* L.). It was commercially released in Hawaii in 1998 (Gonsalves 1998). After its success in Hawaii, China also recommended the cultivation of this genetically modified papaya (Hautea et al. 1999).

Citrus is a popular fruit among young and old alike. A total of 108 million tones of citrus are produced in about 52 countries of the world. Brazil is the largest producer of citrus whereas Pakistan ranks at 13th position in the world (FAOSTAT 2007). However its production is badly affected by certain insect pests such as whitefly, citrus psylla, leaf miner and citrus caterpillar. Huanglongbing (HLB) disease is seriously threatening and causing considerable economic fatalities to the citrus industry. This disease is globally widespread. The bacterial pathogen responsible for this disease is transmitted by the Asian citrus psllyid (ACP) *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). HLB can only be managed if its vector, ACP is controlled. The Psyllid nymphs and adults feed on the phloem sap of infected trees acquiring the *C*Las bacteria and transmit to healthy trees. Many attempts have been made to control the vectors through various biological/biochemical techniques. The parasite *Tamarixia radiata* was found to be effective in stopping the spread of these diseases as it is a natural enemy/predator of psylids (Baniqued 1998). Genetically modified citrus expressing the *Bt* endotoxin and a group of lectins (WGA, CoA, LL and PL) has been produced by a group of scientists working in the Department of Entomology and Nematology, University of Florida, USA observing increased ACP mortality in those GM citrus plants (Killiny et al. 2012). However these GM citrus lines have not been commercially released and more work is currently going on.

Potato (*Solanum tuberosum* L.) is a staple food in many parts of the globe. It is one of the most important food crops. It is ranked on fourth position after maize, rice and wheat. Potatoes are rich in antioxidants and have a high nutritious value. Unfortunately potatoes are attacked by a variety of insect pests, including insects that attack foliage and tubers e.g potato leafhopper (*L. decemlineata)*, potato tuber moth, *Phthorimaea operculella* and black cutworm, *Agrotis ipsilon* L.. The insertion of desired genes by genetic manipulation for developing new potato cultivars seems promising. Potatoes are not produced by seeds; they are propagated through tuber cuttings. They serve as a great challenge to plant breeders because *S. tuberosum* is tetraploid, making it difficult to manipulate the genes of choice between cultivars and have them expressed in progeny. So the insertion of genes of interest by genetic engineering and tailoring new potato cultivars seems promising. Potatoes were one of the first successful GM crop plants (An et al. 1986). However, the commercialization of genetically modified (GM) potato cultivars has not been so successful because Japanese and European markets are unwilling to accept them (Shelton et al. 2008). GM potato cultivars expressing the Cry3A *Bt* toxin are very effective against coleopteran pests such as *L. decemlineata* and were commercially available in the USA from 1996–2000. *Bt cry1Ia1* is effective against lepidopteran pests such as *P. operculella* (Douches et al. 2004). The *cry1Ac* and *cry1Ac9* gene has been shown to be effective against the tuber moth control (Davidson et al. 2005).

Potato cultivars carrying the *cry 3A* toxin for resistance against the Colorado potato beetle *Leptinotarsa* L. *decemlineata* were the first GM food crop approved for human use. They were commercially produced in the USA in 1995. Due to consumer

concern, the *Bt* potato was banned from the market in 2000. GM potato cultivars resistant to aphids have not been produced yet. However, genetically modified varieties resistant to potato virus Y and potato leaf roll virus along with resistance to Colorado potato beetle were produced commercially for a short time and then they were also banned in 2000 along with the *Bt* potatoes. After this ban, potato virus Y is continuously spreading and no effective management has been devised (Davis et al. 2007).

Transgenic tomato and pepper resistant to CMV (Cucumber Mosaic Virus) through expression of the viral CP have been released in China. Further research on CMV resistant tomato is recently being conducted in Indonesia and the Philippines. It is predicted that more virus resistant vegetables and fruits will be released in future (Fuchs and Gonsalves 2007). We have depicted and displayed a novel research, first time in Pakistan, to control the important tomato virus vector silver leaf whitefly (*Bemisia tabaci* G.) via employment of *PR1a* and *PIN2* gene expressions via plant hormonal mediated defense systems (Ahmad et al. 2016a)

Bt tomato line 5345, resistant to lepidopteran insects was genetically engineered. The genetically modified tomato contains two novel proteins; Cry1Ac and neomycin phosphotransferase (NPTII). The Cry1Ac protein is effective against the lepidopteran insects, including the Colorado potato beetle. In mentiond vegetable crops,e.g., tomato, newly emerging pathogens such as phytoplasama can be detected and hence successively managed by advance molecular and genomic appraisals (Ahmad et al. 2016b).

Excitation and elicitation of plant defense and signalization seems to be vital and modern insect management suitability especially against American bollworm (*Helicoverpa armigera* H.), occuring on tomato, based on modern insect integrated genomics relied mechanics. Conclusive remarks, of our molecular study, revealed activation of signalizing genes of *PIN2* by phytohormones methyl jasmonate and suppression or impairment of benzothiadiazole responsive genes *PR1a,* favoring the better *H. armigera* percent reduction and decrement with simultaneous provisions of plant growth promoting rhizobacteria (PGPRs) mainly *Pseudomonas spp.* of bacteria.

Sweet corn is the only GM vegetable grown on commercial scale in USA these days. It expresses the *cry1Ab* endotoxin which is highly effective against Lepidoptera, European corn borer, *O. nubilalis* (Speese et al. 2005). The main advantage of this *Bt* sweet corn is its non-harmful behavior towards the insect predators of *O. nubilalis* (Hoheisel and Fleischer 2007) as well as a complex of epigeal coleopterans (Leslie et al. 2007).

Eggplant (*S. melongena* L.) is an annual plant and a very popular food crop grown in the tropical and sub-tropical regions of the world. However, it is affected by several destructive diseases such as *Phomopsis* blight, *Verticillium* wilt, and several viral diseases (Chen et al. 2001). Insects such as thrips, cotton leafhopper, jassids and aphids seriously damage this vegetable, the most damaging is the eggplant fruit and shoot borer (FSB), *Leucinodes orbonalis* Guenée (Lepidoptera: Crambidae). Farmers tend to use insecticides to get rid of these pests. However chemical control is not a sustainable solution. Maharashtra Hybrid Seeds Company Limited (Mahyco)

transformed *cry1Ac* gene in the eggplant. The first transgenic eggplant resistant to FSB (FSBR egg plant) was developed in 2000.

Brassica vegetables including cabbage, cauliflower, broccoli, turnip, Chinese cabbage and mustards are grown in most parts of the world. They are mostly attacked by the Lepidopteran larvae especially the diamondback moth, *P. xylostella* (L.) (Lepidoptera: Plutellidae). It is responsible for up to 90% worldwide losses to cabbage and cauliflower accounting to yield losses up to 1 billion US dollars every year (Talekar and Shelton 1993). Cry1 *Bt* genes have been successfully expressed in several *Brassica* species, making them resistant to *P. xylostella* and other Lepidoptera (Paul et al. 2005).

9.2.7.5. GM Insects

A genetically modified insect is an insect that has been genetically modified for various reasons such as agricultural production, oil production and pest control. Insect transgenic technology will provide new dimensions for effective control of insect pests. Transgenic fruit flies (*Drosophila melanogaster*) also known as the vinegar flies are being used as a model plant in biological research to study genetics, physiology, microbial pathogenesis and life history evolution. Fruit fly is preferred over other animals due to its short life cycle, simple genome (only 4 pairs of chromosomes) and low maintenance requirements. Genetically modified mosquitoes were produced and released in the 1970s. The particular specie of mosquitoes held responsible for the transmission of the dangerous dengue virus were also sterilized by irradiation through the sterile insect technique (SIT). The British company Oxitec used a technique called RIDL that produces fertile adults but induces a high death rate of the descendants. The adults generated with this technique released in the environment are not sterile but their descendants have a survival rate of only about 5% (or much higher in presence of tetracycline) (D'Andrea 2013).

Although the SIT is already successfully applied for some species, however its various steps such as large scale rearing; sex separation for male-only releases and sterilization methods may be improved through the use of biotechnology to increase the efficiency of this program. Genetic control based on the SIT uses the approach of releasing a mass of artificially reared sterile insects so that they may cause infertile matings eventually reducing the level of pest population (Klassen and Curtis 2005). The SIT is considered an environmentally safe alternative to insecticides for insect species that can be artificially reared on a large scale. The SIT has been successfully employed in to suppress or get rid of pests such as the pink bollworm (*Pectinophora gossypiella* in California, USA (Henneberry 2007).

As stated earlier, the use of RNAi technology for controlling disease causing pests has shown a great potential. RNAi technology has been used in insects such as flour beetle (*Tribolium castaneum*) (Tomoyasu et al. 2008), light brown apple moth (Turner et al. 2006) and grasshopper (Dong and Friedrich 2005) as well as in adult mosquitoes through topical application method. Expression of dsRNA in transgenic plants also provides a continuous source of dsRNA for the feeding insects. When the insects feed on that certain plant, RNAi responses reach the midgut of insects, eventually leading to plant resistance against that particular pest. The dsRNAs can also be applied in the form of molecular pesticides after producing large amounts of

dsRNA through the established bacterial expression system. The successful development and use of insect transgenic systems will help us understand the diverse aspects of biology answering many questions so far not addressable, eventually leading to sustainable agriculture.

9.3. Conclusion and future prospective

The challenge for any pest control measure or technique is to minimize negative effects on non-target insects as far as possible while allowing farmers to produce profitable crops. Massive use of pesticides/insecticides has led to destruction of our natural ecosystem and is posing serious problems for human health. Tailoring plants with built-in resistance to various insect pests serves as a remarkable strategy to control even the worst insect pests without having any hazardous effects to the environment. This is accomplished by engineering plants resistant to insects through genetic modification. Production of genetically modified crops is a safe and environmentally friendly approach for sustainable pest management. A considerable amount of work has been done on various crops and insects using the diversified tools of biotechnology. Scientists have been successful in development of *Bt* crops using the *Bt* endotoxin gene derived from *B. thuringiensis*. With the advent of these crops, a considerable decline in chemical control has been observed. For example, the pest pressure and damage potential in cotton crop is very high. It is seriously damaged by the Lepidopteran insects. Large amounts of broad-spectrum insecticides are used routinely on most conventionally grown cotton, resulting in damage to non-target insects and widespread insect pesticide resistance problems. In contrast, the *Bt* insect-resistant cotton seems to provide environmental benefits in many areas where it is now grown. The insecticide reduction is apparently clear in *Bt* cotton. Since there is an increasingly urgent need to balance food production and biodiversity conservation in our overpopulated world. GM crops, if developed carefully, tested rigorously and applied wisely, have the potential to provide at least some of the solutions to sustainable and environmentally benign world agriculture. This does not mean complacency and the uncritical acceptance of all crops developed using this technology, but it does require a thoughtful case-by-case rational approach.

China, India and Pakistan account for nearly 40% of the world's population. Insect pest problems are prevailing seriously in these countries so they have readily accepted the GM technology. All three of the countries have already adopted *Bt* cotton and it is likely that *Bt* rice will be commercialized in China in the near future. The adoption of GM crops in these countries leads to the probability of also accepting GM vegetables and fruits. Although GM vegetables seem promising for the management of insect pests and the diseases transmitted by them, however, they have not been so eagerly cultivated as other crops such as cotton and maize. Rice is a staple food in Asia. After the huge success of *Bt* cotton and *Bt* maize, *Bt* rice is gaining importance. It has the potential to eliminate pests from the rice fields in an eco-friendly way. However, *Bt* rice has still not been commercialized. Farmers are still reluctant to grow *Bt* rice may be due to the reason that it is not popular in foreign markets. More studies on the bio-safety and experimentation on wider scale under varying environments are required.

While the field of insect biotechnology is still at an infant stage, it has a tremendous potential to revolutionize agriculture and lead to a sustainable management of all the plant pathogenic problems faced by mankind. The use of genetic engineering to combat the problem of insect pests by increasing host plant resistance requires a comprehensive understanding of the crop plants as well as the complex physiology and biology of insects. There are quite a few mysteries about insects that are yet to unfold. Scientists are working on new insecticidal proteins such as cholesterol oxidase which is effective against boll weevil. Similarly vegetative insecticidal proteins for corn rootworm and black cutworm are on the verge of development. The SIT is an environmentally friendly approach which may be used as an alternative to insecticides. RNAi technology also has a huge potential in controlling insect pests through the use of biotechnological approaches. Moreover, identification of insect resistant molecular markers, insertion of novel insecticidal genes/proteins and multigene pathways as well as manipulation/replacement of existing genes through gene therapy may lead to development of highly insect resistant cultivars resulting in a sustainable solution of insect pests.

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