Plant Health Management for Food Security
Issues and Approaches

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Dr Gururaj Katti

Dr Anitha Kodaru

Dr Nethi Somasekhar

Dr G S Laha

Dr B Sarath Babu

Dr K S Varaprasad

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Chapter 6

Application of Modern Tools of Biotechnology for Pest Management

Hari C Sharma

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru – 502 324, Telangana State

E-mail: h.sharma@cgiar.org

ABSTRACT

Recombinant DNA technology has significantly enhanced our ability for crop improvement, to meet the increased demand for food and fiber. Considerable progress has been made over the past two decades in manipulating genes from diverse sources to develop plants with resistance to insect pests, improve effectiveness of biocontrol agents, marker assisted selection for insect resistance, understand the nature of gene action and metabolic pathways, production of genetically modified sterile insects, use of molecular techniques in insect taxonomy, understand the mode of action of insecticides, and identify insecticides with newer mode of action. Despite the diverse and widespread beneficial applications of tools of biotechnology, there is a need to present these benefits to the public in a balanced manner. Testing and release of products generated through biotechnology-based processes should be continuously optimized based on experience. This will require a dynamic and streamlined regulatory structure, which is clearly supportive of the benefit of biotechnology, but highly sensitive to the well-being of humans and environment.

Keywords: *Insect pests, Recombinant DNA technology, Marker-assisted selection.*

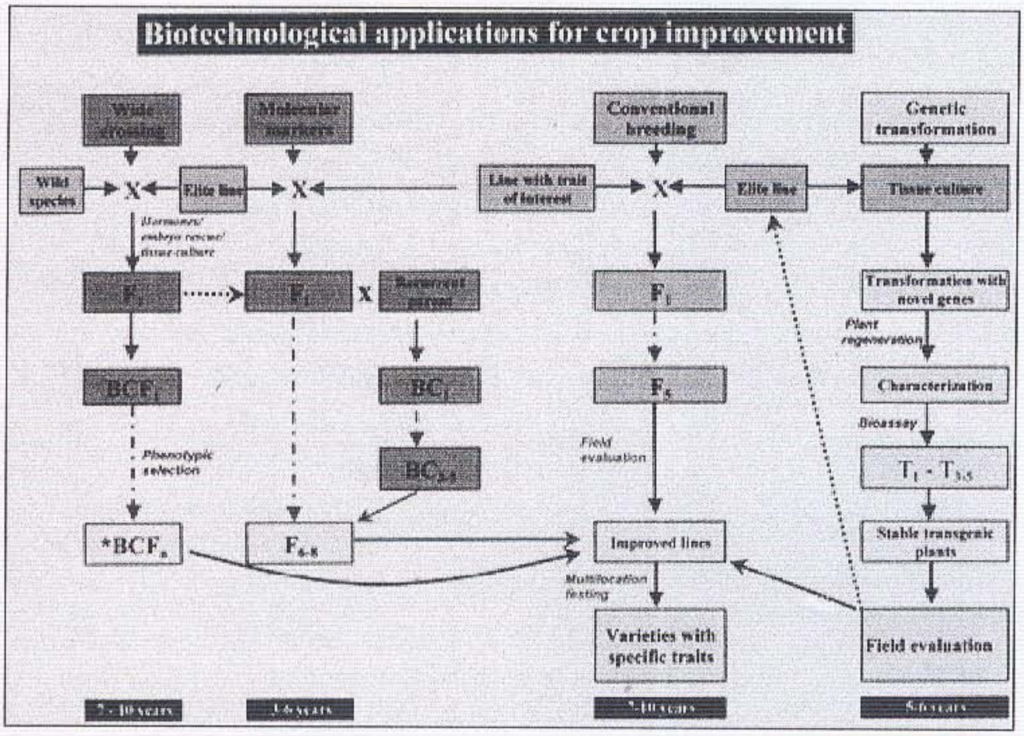
Introduction

Nearly 30 to 50 per cent of the crop yields are lost due to the ravages of insect pests, and several insect species have the potential to cause 50 to 100 per cent loss during outbreaks. Insect pests cause an estimated annual loss of 13.6 per cent globally, and the extent of losses in India has been estimated to be 17.5 per cent (Dhaliwal *et al.*, 2010). The pest associated losses likely to increase as a result of changes in crop diversity and climate change. Reduction in pest associated losses is one of the potential areas for increasing food production, and it is in this context that we can exploit the tools of biotechnology to minimize the extent of losses due to insect pests. Molecular biology has provided several unique opportunities in crop improvement that include access to molecules novel to crop species, production of transgenic crops expressing insecticidal genes, ability to change the level of gene expression, and the capability to change the spatial and temporal pattern of gene expression (Sharma *et al.*, 2002). Development of effective insect-resistant varieties and biocontrol agents will lead to a reduced reliance on synthetic pesticides, and thereby reduce farmers' crop protection costs, while benefiting both the environment and public health. The promise of biotechnology for pest management can be realized by utilizing the information and products generated through research on genomics and genetic engineering to increase crop production.

Biotech Applications in Pest Management

Genetic Engineering

- ☆ Genetic engineering of crop plants for insect resistance.
- ☆ Genetic engineering of natural enemies.
- ☆ Genetic engineering of microbial pesticides.
- ☆ Genetic engineering of metabolic pathways.
- ☆ Inducible resistance and gene switches.
- ☆ Dominant repressible lethal genetic system.



Genomics and Molecular Markers

- ☆ Marker assisted selection for insect resistance.
- ☆ Diagnosis of insect pests and their natural enemies.
- ☆ Monitoring insect resistance to insecticides.
- ☆ Development of new pesticide molecules.
- ☆ Understand plant - insect - natural enemy interactions.
- ☆ Functional genomics and metabolisms of plants and insects.

Genetic Engineering

Crop Plants for Insect Resistance

Development and deployment of transgenic plants in an effective manner is an important pre-requisite for sustainable and economic use of biotechnology for crop improvement. As a result of advances in genetic transformation and gene expression over the past three decades, there has been rapid progress in using the tools of biotechnology for developing crops for resistance to insects (Sharma *et al.*, 2004). While most of the insect-resistant transgenic plants have been developed by using *Bt* δ-endotoxin genes, many studies are underway to use non-Bt genes, which interfere with development and the nutritional requirements of insects, including:

- ☆ **Cry toxins *Bt*:** Cry1Ab, Cry1Ac, Cry IIa, Cry9c, Cry IIB, Vip I, Vip II.
- ☆ **Plant metabolites:** Flavonoids, alkaloids, terpenoids.
- ☆ **Enzyme inhibitors:** SBTI, CpTi.
- ☆ **Enzymes:** Chitinase, lipoxygenase.
- ☆ **Plant lectins:** GNA, ACAL, WAA.
- ☆ **Toxins from predators:** Scorpion, spiders.
- ☆ **Insect hormones:** Neuropeptides and peptidic hormones.

Genes conferring resistance to insects have been inserted into maize, cotton, potato, tobacco, rice, broccoli, lettuce, walnuts, apples, alfalfa, and soybean (Sharma *et al.*, 2004). A number of transgenic crops have now been released for on-farm production or cultivation by the farmers (James, 2009). The first transgenic crop with resistance to insects was grown in 1994 (Benedict *et al.*, 1996). Since then, there has been a rapid increase in the area sown under transgenic crops and transgenic crops are now grown in over 20 countries in the world. Cry type toxins from *Bt* are effective against cotton bollworm, corn earworm, the European corn borer, and rice stem borers. Successful expression of *Bt* genes against the lepidopteran pests has also been achieved in tomato, potato, brinjal, groundnut, pigeonpea, and chickpea (Sharma, 2009). Development and deployment of transgenic plants is carried out under strict biosafety regulations in each country.

Deployment of insect-resistant transgenic plants should be based on the overall philosophy of integrated pest management, taking into account alternate mortality factors, reduction of selection pressure, and monitor insect populations for resistance development to design more effective management strategies. Trasngenic crops are compatible with other methods of pest control, and yield up to 50 per cent more than the non-transgenic cultivars even under insecticide protection (Sharma and Pampapathy, 2006). Insects such as *Heliothis virescens* (F.), *Helicoverpa zea* (Boddie), *Trichoplusia ni* (Hub.), and *Spodoptera exigua* (Hub.) are many times more sensitive to insecticide sprays when they have a prior exposure to *Bacillus thuringiensis* (Harris *et al.*, 1998). Transgenic crops can be used in conjunction with other methods of pest control without any detrimental or antagonistic effects. To increase the effectiveness and usefulness of transgenic plants, it is important to develop a strategy to minimize the rate of development of resistance in insect populations through:

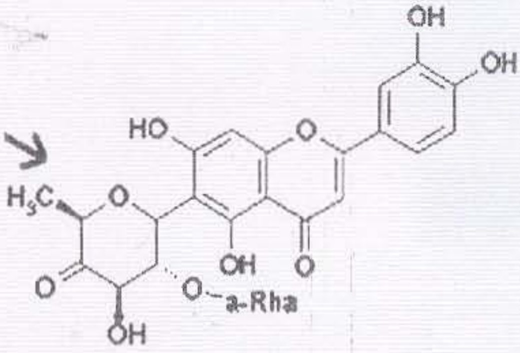
- ☆ Control of secondary pests,
- ☆ Resistance management,
- ☆ Gene pyramiding and gene deployment,
- ☆ Regulation of gene expression,
- ☆ Planting refugia and destruction of carryover population,
- ☆ Control of alternate hosts and use of planting window, and
- ☆ Follow integrated pest management from the very beginning.

Metabolic Pathways

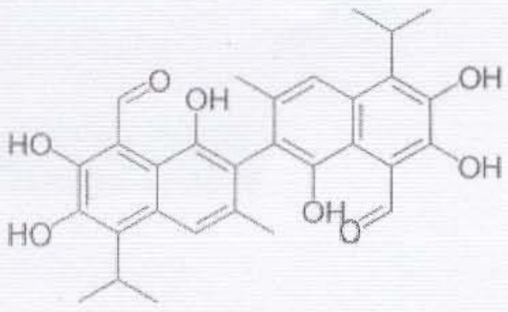
Genetic engineering can be used to change the metabolic pathways to increase the amounts of secondary metabolites, which play an important role in host-plant resistance to insect pests *e.g.*, medicarpin and sativan in alfalfa, maysin in maize, gossypol in cotton, stilbene in pigeonpea and chickpea, and deoxyanthocyanidin flavonoids (luteolinidin, apigenidin, etc.) in sorghum (Sharma *et al.*, 2002). The expression of phytoalexins in transgenic plants may be difficult due to complexities involved in their biosynthesis. Expression of a bacterial cytokinin biosynthesis gene (*PI-II-ipt*) in *Nicotiana plumbaginifolia* plants has been correlated with enhanced resistance to *M. sexta* and *M. persicae* (Smigocki *et al.*, 2000). Molecular mechanisms underlying the activation of defense genes implicated in phytoalexin biosynthesis are quite common in a large number of plant species. Biotechnology offers the promise to increase the production of secondary metabolites in plants to increase the levels of resistance to insect pests.

Inducible Resistance

Induced resistance results in changes in a plant that produce a negative effect on herbivores (Karban and Baldwin, 1997). Chemically induced expression systems or "gene switches" enable temporal, spatial, and quantitative control of genes introduced into plants or those that are already present in the plants to impart resistance to insects. A number of inducible genes have been identified in plants based on endogenous chemical signals such as phytohormones, responses to insect and pathogen attack, or wounding. Effectiveness of the chemical injury inducer, Actigard™ in providing resistance to various insect pests and pathogens in the tomato has been demonstrated by Inbar *et al.* (1998). Proteinase inhibitors and oxidative enzymes such as polyphenol oxidase, peroxidase, and lipoxygenase persist for at least 21 days after induction in damaged tomato leaflets (Stout *et al.*, 1996). Exogenous application of jasmonic and salicylic acids induces resistance to several insect pests (War *et al.*, 2012).



Maysin



Gossypol

Natural Enemies

Some of the major problems in using natural enemies in pest control are the difficulties involved in mass rearing and their inability to withstand adverse conditions in the field. Genetic improvement can be useful when the natural enemy is known to be a potentially effective biological control agent, except for one limiting factor. Some of the desirable characteristics for transgenic insects include resistance to pathogens, adaptation to different environmental conditions, high fecundity, and improved host-seeking ability (Atkinson and O'Brochta, 1999). Biotechnological interventions can also be used to broaden the host range of natural enemies or enable their production on artificial diet or non-host insect species that are easy to multiply under laboratory conditions. In addition, there is a tremendous scope for developing natural enemies with genes for resistance to pesticides (Hoy, 2000). This is of particular concern when the same vector transmits several disease causing pathogens, as it might be difficult to develop transgenic individuals incapable of transmitting different pathogens (Sharma, 2009).

Microbial Pesticides

Genetic engineering can also be used to improve the efficacy of entomopathogenic microorganisms. Efforts to improve *Bt* have largely been focused on increasing its host range and stability. Work on *baculoviruses* is largely focused on incorporation of genés that produce the proteins, which kill the insect^ at a faster rate (Bonning and Hammock, 1996), and removal of polyhedrin gene, which produces the protective viral-coat protein, and its persistence in the field (Cory, 1991). Neurotoxins produced by spiders and scorpions have also been expressed in transgenic organisms (Barton and Miller, 1991). Incorporation of benomyl resistance into *Metarhizium anisopliae* and other entomopathogenic fungi could make them more useful for use in integrated pest management (Goettel *et al.*, 1989). The role of neurotoxins from insects and spiders need to be studied in greater detail before they are deployed in other organisms because of their possible toxicity to mammals.

Dominant Repressible Lethal Genetic System

The sterile insect technique has been employed to control several insect pests. However, this system depends on large-scale production of the target insect, and use of irradiation or chemical sterilization. Release of insects carrying a dominant lethal (RIDL) gene has been proposed as an alternative to the conventional techniques used for insect sterilization (Alphey and Andreasen, 2002). This is based on the use of a dominant, repressible, female-specific gene for insect control. A sex-specific promotor or enhancer gene is used to drive the expression of a repressible transcription factor, which in turn controls the production of a toxic gene product. A non-sex specific expression of the repressible transcription factor can also be used to regulate a selectively lethal gene product. Insects produced through genetic transformation using this approach do not require sterilization through irradiation, and could be released in the eco-system to mate with the wild population to produce sterile insects, which will be self-perpetuating.

Genomics and Molecular Markers

Marker-Assisted Selection for Insect Resistance

Recombinant DNA technologies, besides generating information on gene sequences and function, allow the identification of specific chromosomal regions carrying genes contributing to traits of economic interest. Once genomic regions contributing to the trait of interest have been assigned and the alleles at each locus designated, they can be transferred into locally adapted high-yielding cultivars by making requisite crosses. The offspring with a desired combination of alleles can then be selected for further evaluation using marker assisted selection (MAS). It is important to use large mapping populations, which are precisely and accurately characterized across seasons and locations. MAS can be used to accelerate the pace and accuracy of transferring insect resistance genes into improved cultivars. Several markers have been used to identify QTLs for insect resistance in different crops (Smith, 2005; Sharma, 2009). In contrast to the markers linked to resistance genes inherited as simple dominant traits, the improvement of polygenic traits (QTLs) through MAS is difficult due to involvement of a number of genes, and their interactions (epistatic effects). Several studies on QTLs linked to stem borer resistance in maize underscore the problems involved in using QTLs in MAS. The relative efficiency of phenotypic and MAS has been found to be similar). However, phenotypic selection was more favorable due to lower costs. Maximum progress has been made in breeding for insect resistance in common bean by using a combination of phenotypic performance and QTL-based index, followed by QTL based index, and conventional selection (Tar'an *et al.*, 2003).

Understanding Gene Sequence and Function

Genes can be discovered using a variety of approaches (Shoemaker *et al.*, 2001), but a routine large-scale approach can commonly be followed by generating and sequencing a library of expressed genes. A large number of ESTs are now available in the public databases for several crops such as *Zea mays*, *Arabidopsis thaliana*, *Oryza sativa*, *Sorghum bicolor*, and *Glycine max*. A comparison of the EST databases from different plants can reveal the diversity in coding sequences between closely and distantly related species, while mapping of ESTs may elucidate the synteny between those species. For understanding gene functions of a whole organism, functional genomics technology is now focused on high throughput methods using insertion mutant isolation, gene chips or microarrays, and proteomics. These and other high throughput techniques offer powerful new uses for the genes discovered through sequencing (Hunt and Livesey, 2000).

DNA Barcoding of Insect Pests and their Natural Enemies

For developing appropriate strategies for managing insect pests, it is important to have a correct identification of the pest species. Correct taxonomic identification is also important for import and export of plant material/food grains to implement quarantine procedures. Identification of insect pests has primarily relied on morphological characters of adult life stages. However, intercepted specimens often are not in the adult stage and may be damaged, which seriously handicaps correct identification. The molecular tools now enable precise and rapid identification of insect pests, irrespective of the developmental stage and condition of the samples. The modern tools of biotechnology can be used for detection and identification of insect pests, insect biotypes, and understand genetic diversity, population structure, tri-trophic interactions, and insect plant relationships (Caterino *et al.*, 2000; Heckel, 2003). Molecular markers can also be used to gain a basic understanding of their interaction with environment, and develop sound strategies for pest management.

Development of New Insecticide Molecules

Crop protection is still dominated by chemical control, and this approach will continue to be important in crop protection in future. Traditionally, the discovery of new agrochemicals has used *in vivo* screens to identify new compounds. Functional genomics offers the opportunity to acquire in-depth knowledge of the genetic make-up and gene function of insect pests that may lead to the discovery of new processes that could be the targets for novel chemistry (Hess *et al.*, 2001). Combining genomics with high throughput biochemical screening can be used to identify a range of new chemicals for pest control. Genomic technologies are now allowing investigation of some previously intractable mechanisms involved in insect resistance to insecticides. New molecular techniques permit fundamental insights into the nature of mutations and genetic processes such as gene amplification, altered gene transcription, and amino acid substitution to underpin insecticide resistance mechanisms. This in turn will lead to high-resolution diagnostics for resistance alleles in homozygous and heterozygous form, especially for insect pests with multiple resistance mechanisms, or for resistance mechanisms not amenable to biochemical assays.

Large-scale adoption of insect-resistant transgenic crops has resulted in a significant reduction in insecticide use and increased both production and productivity (Qaim and Ziberman, 2003; James, 2009). The potential of insect-resistant transgenic crops can be enhanced through gene pyramiding by using a combination of exotic genes and insect-resistant cultivars derived through conventional breeding, and by combining resistance to insect pests and diseases of importance in a crop/region. There is a considerable debate about the environmental risks such as development of resistance, harmful effects on beneficial insects, and gene flow to the closely related wild relatives of the crops (Sharma and Ortz 2000; O'Callaghan *et al.*, 2005; Sharma, 2009; Sharma *et al.*, 2012). The evidence on these issues is still inconclusive and warrants careful monitoring before transgenic crops are deployed on a large scale. There is a need for a balanced presentation of the benefits of biotechnology to the general public for increasing crop production and improving food security. The biggest risk of modern biotechnology for developing countries is that technological developments may bypass the poor farmers because of a lack of enlightened adoption. There is a need to develop scientifically sound strategies for deploying genetically engineered insect-resistant crops for sustainable crop production. Equally important is the need to assess the bio-safety of genetically modified crops and the conventional technologies deployed for pest management.

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