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MANAGEMENT OF INSECT PEST RESISTANCE WHILE USING TRANSGENIC PLANTS

K.V. RAMAN
International Service for the Acquisition of Agri-Biotech Applications (ISAAA),
260 Emerson Hall, Department of Plant Breeding and Biometry,
Cornell University, Ithaca, NY 14853, USA

ABSTRACT

Pest resistance is a serious global problem. Over 600 pests (insects, weeds, pathogens) are reported to have developed resistance to chemical pesticides. Several important pests have overcome, or have the potential to develop resistance to, plant defensive mechanisms through conventional plant breeding and biotechnology. Durability of plant defense mechanisms is especially critical in the rapidly advancing area of plant genetic transformation which is primarily focusing on the use of *Bacillus thuringiensis* (*Bt*) genes to impart pest resistance in several important crops. Several major private and public sector organisations are now focusing on creating transgenic plants with the engineered d-endotoxin from *Bt*. There are now reports of resistance to *Bt* in both laboratory and field strains of various insect pests. The broad application of *Bt* technology has a very high potential to accelerate the instances of resistance to *Bt* and radically reduce its utility. Strategies to delay the development of resistance while using *Bt* engineered plants are many and would need to be experimented under the different agro-ecosystems of developing countries. A brief description of the various strategies available for experimentation is discussed.

Key Words: ***Bacillus thuringiensis,*** **transgenic, insecticidal crystal proteins, D-endotoxins**

RÉSUMÉ

La résistance aux pestes est un problème mondial important. Plus de 600 pestes (insectes, mauvaises herbes, pathogènes) ont développé une résistance aux pesticides chimiques. Plusieurs pestes importantes ont développé (ou ont le potentiel de développer) une résistance aux mécanismes de défense des plantes acquis par l'amélioration conventionnelle et la biotechnologie. La viabilité des mécanismes de défense de plantes est important dans le domaine de transformation génétique des plantes qui focalise premièrement sur l'utilisation de gènes de *Bacillus thuringiensis* (*Bt*) pour imprimer la résistance aux pestes à plusieurs cultures importantes. Plusieurs organisations importantes des secteurs public et privé se focalisent sur la mise au point des plantes transgéniques à l'aide de d-endotoxine provenant de *Bt*. Il y a maintenant des écrits sur la résistance au Bt de diverses races de pestes d'insectes aussi bien au laboratoire qu'en champ. L'application à large échelle de la technologie de *Bt* a un haut potentiel d'augmentation de chance de résistance au Bt et de réduction radicale de son utilité. Les stratégies pour retarder le développement de résistance pendant qu'on utilise les plantes manipulées avec le *Bt* sont nombreuses et devraient être expérimentés dans les différents agroécosystèmes des pays en développement. Ce papier donne une brève description de différentes strategies disponibles à expérimenter.

Mots Clés: ***Bacillus thuringiensis,*** **transgénique, proteines crystal d'insecticide, D-endotoxine**

INTRODUCTION

Developing crop varieties resistant to pests has been an important research agenda of many crop improvement programmes in the private and public sectors worldwide. The advent of molecular-based technologies, including gene cloning and foreign gene transfer to plants, has enabled plant breeders to utilise traits that were not available with conventional breeding methods. One such trait is insect resistance conferred proteins produced by the soil-borne bacterium, *Bacillus thuringiensis* (*Bt*). Genes encoding these proteins have been cloned and transferred to several crop plants to improve insect pest control. While some researchers feel that this is a step in the right direction to reduce the use of chemical pesticides, others are concerned that the development of insects resistant to *Bt* sprays may render these transgenic plants useless and create insect pests that are even more difficult to control.

It is estimated that over 300 laboratories are generating *Bt* genes and conjugates for use in transgenic plants. These include small and large public and private companies, universities, and government or national research centres. The first plants to be commercialised will probably be from the private sector currently involved with field trials of transgenic cotton, maize, rice and potatoes containing the chimeric *Bt* gene constituitively expressed at a high dose (Table 1). This means that the toxins will be continuously expressed throughout the growing season, and perhaps beyond it (in plant residue). If the current regulatory guidelines persist, broad-scale introduction of transgenic technology could take place with little effort to develop a deployment strategy which will slow the rate of resistance development. Insects will respond to this new selection pressure in the same way they have to other selection pressures: by evolving resistance. Most plant protection scientists agree that resistance evolution is the most important issue facing the development and deployment of transgenic plants in agriculture. This paper summarises information on *Bt* insecticidal proteins and some of the important strategies which might be helpful in delaying or preventing the breakdown of resistance of plants engineered with the *Bt* gene.

TABLE 1. Private companies involved with *B. thuringiensis* in plants

Company	Commercial Status	Crops
Agracetus/ W.R. Grace	field trials	Cotton
Agrigenetics	field trials	Maize
Calgene	field trials	Cotton
Ciba-Geigy	field trials	Maize
ICI Zenena	field trials	Maize
Mitsubishi/Plantech	field trials	Rice
Monsanto	field trials	Cotton, Potatoes
Plant Genetic Systems	field trials	Potatoes

Bt INSECTIDAL CRYSTAL PROTEINS

The gram-positive, soil-borne bacterium *Bt* produces several proteins with insecticidal properties called insecticidal crystal proteins or insect control proteins (ICPs). Seven classes of proteins that are toxic to insects in the orders Lepidoptera, Coleoptera and Diptera, and to nematodes have been identified (Feitelson *et al.*, 1992). Each class has a unique specificity as outlined in Table 2. *Bt* ICPs are advantageous insecticides since they have a very specific activity, are not persistent in the environment and are not toxic to most beneficial insects or to mammals (Fuchs *et al.*, 1993).

D-endotoxin is the main toxin found in the ICPs. Because this toxin is found in bacteria, fermentation technology allows environmentally sound plant protection through use of *Bt* formulated as conventional sprays. The environmental benefits include protection of groundwater, decreased impact on human health, and lower insecticide residues on crops. While the mode of action of *Bt* ICPs is not yet fully elucidated, it is known that the ICPs bind to specific receptor site(s) on the brush border membrane of the insecticide midgut epithelium. The identification of the receptor sites indicate that they are glycosylated proteins (Hofmann *et al.*, 1988). The binding of *Bt* ICPs to these receptors causes pores to form in the membrane, resulting in osmotic imbalance from an influx of water, ions and other small molecules. Cellular swelling and lysis occurs, which results in midgut paralysis and cessation of feeding by the insect. The insect eventually (2-7 days) starves to death. The six common steps involved in the mode of action of ICPs include; (1) ingestion, (2) binding to midgut "receptors", (3) disruption of midgut epithelium (pore formation), (4) chemi-osmotic disequilibrium, (5) cessation of feeding, and (6) death due to starvation.

Bt ICP genes have been cloned and characterised as the preliminary steps towards producing *Bt* expressing transgenic plants. Many plants have already been transformed with *Bt* genes (Table 3). In order to improve the expression of *Bt* gene products in transgenic plants, Perlak *et al.* (1991) modified the DNA sequence of CryIA (b) and CryIA (c) *Bt* genes to make them resemble more closely a plant gene. Sequence modifications included codon usage preferred by plants and the elimination of potential polydenylation sites, ATTTA sequences and A/T rich regions . Both partially and fully modified *Bt* genes were developed. These genes retained their wild type amino acid sequence but were significantly different in the nucleotide sequence (up to 22% modified). The synthetic genes expressed *Bt* protein at levels 10-100 fold higher than achieved with endogenous *Bt* genes. Several groups have since made similar modifications in other *Bt* genes and have reported similar increases in gene expression (Perlak *et al.*, 1993). Expression levels in the plants were often over one hundred fold higher than the threshold level needed to kill the particular target insect pest.

TABLE 2. Overview of *B. thuringiensis cry* genes

Host range	Gene
Lepidoptera	*cryIA* (a)
Lepidoptera	*cryIA* (b)
Lepidoptera	*cryIA* (c)
Lepidoptera	*cryIB*
Lepidoptera	*cryIC* (a)
Lepidoptera	*cryIC* (b)
Lepidoptera	*cryID*
Lepidoptera	*cryIE*
Lepidoptera	*cryIF*
Lepidoptera/Diptera	*cryIIA*
Lepidoptera	*cryIIB*
Lepidoptera	*cryIIC*
Coleoptera	*cryIIIA*
Coleoptera	*cryIIIB*
Coleoptera	*cryIIIC*
Coleoptera	*cryIIID*
Diptera	*cryIVA*
Diptera	*cryIVB*
Diptera	*cryIVC*
Diptera	*cryIVD*
Lepidoptera/Coleoptera	*cryV*

Current work focuses on transformation vectors containing more than one *Bt* gene and on the design of synthetic *Bt* genes that are translational fusions of two or more *Bt* genes with different insect specificities (Honée *et al.*, 1990). Control strategies utilising more than one control mechanism may prove to be more durable, especially given the recent reports of insect resistance to *Bt* ICPs. Among the gene strategies recommended for the use of single gene, multiple genes (pyramid or stacked), chemiric genes and gene promoters which are constitutive, tissue specific or inducible (wound, phenology, elicitor) which may be able to control gene expression at high or low dose, may all help in delaying the development of resistant insects.

Bt RESISTANCE MANAGEMENT

The are currently 16 insect species that have demonstrated resistance to *Bt*. The most common ones include Indian meal moth, Almond moth, Tobacco budworm, Sunflower moth, Colorado

TABLE 3. Plants containing *Bacillus thirungiensis cry* genes

Plant	*Bt* class
Tobacco	CryIA(b)
Tobacco	CryIA(a)
Tomato	CryIA(b)
Tomato	CryIA(c)
Cotton	CryIA(b)
Cotton	CryIA(c)
Rice	CryI
Rice	CryIA(b)
Poplar	CryIA(a)
Potato	CryIA(c)
Potato	CryIIIA
Cranberry	CryIA(a)
Maize	CryIA(b)
Sweetgum	CryIA(b)
Walnut	CryIA(c)
Brocolli	CryIA(c)

potato beetle and Diamondback moth. Of these, only Diamondback moth is reported to have field-derived resistance to *Bt* spray applications (Table 4). Resistance of up to 1641-fold has been observed in field selected Diamonback colonies (Shelton *et al.*, 1993) and up to 820-fold in laboratory selection experiments (Tabashnik *et al.*, 1991). There is, therefore, a major concern amongst researchers in the private and public sector to develop resistance management strategies. Five major principles are recommended to delay resistance development. These are: (1) reduction of selection pressure from each mortality mechanism in the target pests; (2) diversification of mortality sources so that a pest is not selected by a single mortality mechanism; (3) maintenance of susceptible pest individuals by providing refugees or promoting immigration of susceptible pest individuals; (4) development of resistance progress estimation and/or prediction through development of diagnostic tools and monitoring; and (5) making pest resistance management part of a national biosafety policy.

These strategies play an important role in a larger pest management programme. There is now a general consensus amongst scientists in the USA that *Bt* resistance management research is a high priority and needs to focus on the following nine recommendations to: (1) establish baselines and monitor shifts in *Bt* susceptibility; (2) conduct research on ecological and genetic factors of *Bt* resistance; (3) experimentally validate resistance management strategies; (4) integrate *Bt* with other pest control tactics; (5) assure an appropriate regulatory environment for *Bt*; (6) characterise *Bt* cross resistance; (7) estimate *Bt* resistance gene frequencies; (8) map and clone *Bt* resistance genes; and (9) establish a national scientific advisory group for *Bt* resistance.

These strategies have been reviewed by McGaughey and Whalon (1992). The most widely considered strategies are those that utilise a "refuge"; a portion of the crop that is not transgenic and serves as a host for all insects, both susceptible and resistant. In a refuge strategy, only *Bt* resistant insects are able to survive the dose of *Bt* in the transgenic plants. Initially, very few resistant insects should be present, given that the initial resistance allele frequency is very low. The non-transgenic refuge plants should host large numbers of insects, the vast majority susceptible to *Bt*. Assuming that insects move from plant to plant and there is random mating, the few *Bt* resistant insects in the field should mate with susceptible insects producing progeny that are heterozygous for the resistance allele. Since *Bt* resistance is assumed to be recessive, these heterozygous offsprings should be *Bt* susceptible and killed by transgenic plants. Experiments to prove such theories have now been conducted in the laboratory and by way of computer simulations (Roush, 1994). Such studies indicate that the seed mixture of transgenic and non-transgenic plants is an attractive alternative. The best strategies can only be recommended after conducting carefully designed field experiments in different agro-ecosystems of both developed and developing countries. There is now an increased awareness amongst several eminent scientists that *Bt* resistance will be a major problem and that its occurrences will increase in future, and that this in turn will have an impact on the utility of *Bt* for pest control.

It is, therefore, important to address this problem from both a fundamental and applied standpoint. Except for a few well designed field experiments most work in the USA and elsewhere has concentrated on resistance management modelling. Such work is speculative and cannot decisively indicate a deployment strategy in the absence of continued monitoring and experimentation (Raman and Altman, 1994). It is, therefore, important that as *Bt* transgenic plants are introduced into developing countries, efforts be placed on devising suitable resistance management strategies under the diverse agroecosystems under which this technology is

TABLE 4. Insect species with developed resistance to *Bacillus thurigiensis* insect control proteins

Nature of resistance	Insect species
Stored Grain Pest	Indian Mealmoth
Laboratory-selected Resistance	Almond moth Tobacco budworm Sunflower moth Colorado potato beetle
Field-derived resistance	Diamondback moth

likely to be commerialised. This is the only way that the durability of such new plant materials will be maintained.

APPROACH

For *Bt* transgenic plants to be successfully used, emphasis should be on adopting an integrated system of pest management. Integrated Pest Management (IPM), is a comprehensive approach to pest management that uses multiple tactics to avert or reduce pest problems in agroecosystems. Conventionally and biotechnologically derived host plant resistance must be used along with other means of pest management (cultural, biological, mechanical, chemical, etc.). Deployment strategies must be designed from the on-set of host plant resistance programmes to delay or prevent the problem of pest resistance. The use of multiple genes, combining the host plant resistance derived through conventional and biotechnological means to pyramid or tack resistance genes, rotation or alteration of genes, use of gene promoters, manipulation in the levels of expression (spatial and temporal) of genes, preservation of susceptible pest genes through refugia, and integration of host plant resistance strategies into an overall integrated pest management programme should be considered in deployment strategies. Transgenic plants must be integrated and utilised within the context of IPM. This will reduce the selection pressure on the pest and, hence, help increase the life span of the biotechnology innovations. Integration of conventionally and biotechnologically derived host plant resistance pest management strategies, into an IPM will not only help in the management of resistance to these strategies but also resistance management to other tactics of pest management by diversifying the pest mortality mechanisms. If IPM is successfully adapted and implemented, the objective of resistance management will automatically be achieved. Hence, IPM should become part of a national agricultural policy and national programmes should revise their national biosafety framework to include pest resistance management issues. These must become an integral component of national biosafety framework.

The International Service for the Acqusition of Agri-biotech Applications (ISAAA) fully endorses and has agreed to cooperate with the proposed approaches of Michigan State University (MSU) in the USA to test tactics for deployment of transgenic plants so that they can be integrated into suitable delivery systems. The MSU approach has five research and training components: biochemical evaluation, variety evaluation, modeling, tactics evaluation and decision support systems. The main objectives of such a study are to: (1) evaluate transgenic crop varietics for risk of resistance development; (2) develop and validate principles, strategies and tactics for environmentally sustainable deployment of transgenic plants; and (3) to enter a cooperative network that will provide for trained scientists from developing countries who will share in the research technology, human and technical networking, and strategies and tactics for managing resistance to transgenic plants.

This project, if funded, will have strong ties with several international development programmes such as ISAAA, USAID, international centres, national programmes, private industry, IPM-CRISP and others. Training will be a key component. Already, some work has been initiated in this area by the Global Pest Resistance Management Programme located at MSU. Through effective resistance management training, pesticide use patterns change, and the effective life span of pesticides and host plant resistance technology increases. Effective resistance management can mean reduced pesticide use without loss of productivity for subsistence and commercial farmers

REFERENCES

Feitelson, J.S., Payne. J, and Kim, L. 1992. *Bacillus thuringiensis*:Insects and beyond. *Biotechnology* 10: 271-275.

Fuchs, R.L., Berberich, S.A.and Serdy, F.S. 1993. Safety evaluation of genetically engineered plants and plant products: Insect-resistant cotton. In: *Biotechnology and Safety Assessment*. Thomas, J.A. and Myers, L.A. (Eds.), pp. 199-212. Raven Press, New York.

Hofmann, C., Liithy, P., Hutter, R. and Pliska, V. 1988. Binding of the delta-endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly *(Pieris brassicas). European Journal of Biochemistry* 173: 85-91.

Honée, G., Vriezen, W. and Visser, B. 1990. A translational fusion product of two different insecticidal crystal protein genes of *Bacillus thuringiensis* exhibits an enlarged insecticidal specturm. *Applied Environmental Microbiology* 6: 823-825.

McGaughey, W.M. and Whalon, M.E. I992. Managing insect resistance to *Bacillus thuringiensis* toxins. *Science* 258: 1451-1455.

Perlak, F.J., Fuchs, R.L., Dean, D.A., McPherson, S.L., and Fischhoff, D.A. 1991. Modification of the coding sequence enchances plant expression of insect control protein genes. *Proceedings of Natural Academy of Sciences, USA* 88: 3324-3328.

Perlak, F.J., Stone, T.B., Muskopf, Y.M., Petersen, L.J., Parker, G.B., McPherson, S.A., Wyman, J., Love, S., Reed, G., Biever, D.and Fischhoff, D.A. 1993. Genetically improved potatoes: protection from damage by Colorado potato beetles. *Plant Molecular Biology* 22: 313-321.

Raman, K. V. and Altman, D.A. 1994. Biotechnology initiatives to achieve plant pest and disease resistance. *Crop Protection* 13 : 591-596.

Roush, R.T. I994. Can we slow adaptation by pests to insect-resistant transgenic crops? In: *Biotechnology for Integrated Pest Management.* Persley, G. and MacIntyre, R. (Eds). CABI, In press.

Shelton, A.M.,Robertson, J.L.,Tang, J.D., Perez, C., Eigenbrode, S.D., Preisler, H.K., Wilsey, W.K. and Cooley, R.J. I993. Resistance of diamond back moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. *Journal of Economic Entomology* 86: 697-705.

Tabashnik, B.E., Finson, N. and Johnson, N.M. 1991. Managing resistance to *Bacillus thuringiensis*: Lessons from the diamond back moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* 84: 49-55.