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Biotechnology for insect pest control

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Abstract

Insect pests are a major constraint to increased global production of food and fiber. Biological control agents including arthropod natural enemies, entomopathogens (bacteria, nematode, virus, and fungus), plant-derived insecticides and insect hormones are receiving significant interest as alternatives to chemical pesticides and as key components of integrated pest management system. Biotechnology has a significant role in improving efficacy, cost-effectiveness and in expanding the markets for these bioinsecticides. Several molecular techniques have been employed for identifying and monitoring establishment and dispersal of specific biotypes of natural enemies. Genetic engineering and insect transformation technology provide opportunities for the development of insect natural enemies conferring beneficial traits such as pesticide resistance, cold hardiness and sex ratio alteration. Modern technologies provide an effective extraction process, formulation solvents and adjuvants, which can enhance insecticidal activity of plant-derived insecticides. Production, formulation and storage, which are extremely important for the utilization of entomopathogenic fungi and nematodes, can be dramatically improved through biotechnology and genetic engineering. The introduction of gene coding for proteinaceous insect toxins (scorpion toxin, mite toxin, trypsin inhibitor), hormones (eclosion hormone, diuretic hormone) or metabolic enzymes (juvenile hormone esterase) into nucleopolyhedroviruses genome are some approaches to increase speed of kill, enhance virulent and extend host specificity of the virus. Genetic manipulation of *Bacillus thuringiensis* (*Bt*) genes encoding for proteins toxic to insects offers an opportunity to produce genetically modified strains with more potent and transgenic plant expressing Bt toxin. In addition to the *Bt* delta-endotoxin, several proteins that are effective against certain insects such as the vegetative insecticidal proteins (VIP), alpha-endotoxin, a variety of secondary metabolites and proteins of plant origin are amenable to genetic manipulation. Biological control strategies involving beneficial insects, microorganisms that attack insect pests and plant-derived insecticide will provide sustainable control practices that work in harmony with genetically engineered plants. Biotechnology can have a positive impact on food security from insect attack and can contribute to the sustainability of modern agriculture. However, the use of biotechnology brings questions regarding the potential impact of those genetically modified organisms (GMOs) or plants to human, animal and environment. National biosafety and regulatory systems for proper management of GMOs must be in place to enable the full exploitation of biotechnology. Insect control strategies that integrate advance knowledge in biotechnology with traditional wisdom and technology will contribute to the sustainability of agriculture.

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Introduction

An estimated one third of global agricultural production, valued at several billion dollars is destroyed annually by over 20,000 species of field and storage pests. Synthetic, broad-spectrum insecticide is a satisfactory and permanent solution for pest control; however, the excessive use of chemical insecticides is a threat to human health, natural ecosystem and environment. Societal concerns over pesticide use have resulted in the development of new biologically based pest management strategies that are ecologically sound, reliable, economical and practical. These lead to the development and registration of naturally occurring and genetically altered bio-insecticides, which include arthropod natural enemies, entomopathogens (bacteria, nematode, virus, and fungus), plant-derived insecticides and insect hormones. Recent global bio-pesticide products registered include bacteria (104 products, mostly are *B. thuringiensis*), nematodes (44 products), fungi (12 products), viruses (8 products), protozoa (6 products) and arthropod natural enemies (107 products) (Waage, 1996). Bio-pesticides, unlike the chemical pesticides, can be produced at an appropriate scale with technologies that are well within reach of most developing countries. This could make possible the development of local bio-pesticide products that target local pests. However, advance knowledge in biotechnology and molecular biology can help assess the production of more potent and cost-effective bio-pesticides. Most of the modern technologies are directed at improving the performance of an engineered product relative to its wild type competition by broadening their host range, increasing the speed of action, enhancing the delivery of the product to the pest, and improving their persistence in the environment. This paper addresses the biological control agents that are receiving interest as alternatives to chemical insecticides and the recent progress that has demonstrated the potential of biotechnology in enhancing efficacy of those bio-insecticides for sustainable agricultural productivity. Development of genetic engineering bio-insecticides and community perception of these novel biological control agents are also discussed.

Insect natural enemies as bio-control agents

Biotechnology could provide solutions to a number of basic and applied problems that limit the use of insect natural enemies as biological control agents. Mass rearing of insect natural enemies for classical or augmentative release is the main task of this insect control strategy. Maintaining quality in laboratory-reared insects is difficult due to possible genetic changes caused by accidental selection, inbreeding, genetic drift and founder effects (Hopper *et al*., 1993). New DNA-based methods for monitoring genetic variations are now available such as: mitochondrial DNA analysis, DNA sequencing, restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), random amplified polymorphic DNA (RAPD)-PCR and ribosomal DNA analysis. Some of these methods are also of potential value for identifying and monitoring establishment and dispersal of specific biotypes of insect natural enemies (Edwards and Hoy, 1993). Currently maintaining insect natural enemies is only by continuous rearing or holding specimens in diapause. The development of cryobiological method for preserving embryos of insects can significantly save the rearing costs, and the valuable collection of insect natural enemies could be maintained indefinitely.

Genetic improvement is a potential approach to increase the efficacy of insect natural enemies. Transgenic techniques provide the opportunity to introduce and express foreign genes and/or disrupt existing gene functions so that the desirable characteristics may be inherited by subsequent generations, thus reducing frequent mass releases. Introduction of DNA into insect germ cells can be achieved by using physical means such as microinjection, biolistics and electroporation or by using biological means in which several transposable elements, Sindbis viruses and retrovirus are used as gene vectors (Atkinson *et al*., 2001). Microinjection is the best method for penetrating insect chorions and delivering vector DNA to the germ cells. Efforts have been most intense and successful with *Drosophila melanogaster*. Inserting cloned target DNA into the chromosome of insect cells was rarely successful until the P-transposable element was genetically manipulated to serve as a vector (Rubin and Spradling, 1982). Micro-injecting DNA carried in P-element vectors had been used for gene transfer in several insect species (McGrane *et al*., 1988, Morris *et al*., 1989).

A technique called *maternal microinjection* in which the exogenous DNA microinjected through the cuticle of gravid females without the aid of any transposable-element vector, is developed for certain species of insect (Presnail and Hoy, 1992). The four transposable-element vectors, Minos element from *D. hydei*, Hermes element from the housefly, *Musca domestica*, MosI element from *D. mauritiana* and piggyBac element from the cabbage looper, *Trichoplusia ni*, have been developed for the generation of transgenic insects and for stable genetic transformation in non-drosophilid insect species. Sindbis alphavirus and pseudotyped pantropic retroviruses have been developed as vertebrate gene expression tools, however, these two viral systems are limited to the expression of genes in individuals that have been directly infected with engineered virus (Atkinson *et al*., 2001).

Other insect transformation methods including soaking embryos in a DNA solution, injecting and transplanting nuclei and pole cells into eggs, using bacterial symbionts as vehicles for expressing foreign genes, baculovirus expression vector and yeast recombinase-mediated recombination are currently explored (Hoy, 1996). However, many of them are limited to *Drosophila* species. A universal transformation system that can be routinely applied for introducing exogenous DNA into insect species, nonetheless their genetic information are barely known, will highlight the future of transgenic insect production.

There are several potentially useful genes that can be used to improve the performance of insect natural enemies. Resistance genes for chemical insecticides will probably be the most available genes, which include a parathion hydrolase gene from *Pseudomonas diminuta* and *Flavobacterium*;, an acetylcholinesterase gene from *D. melanogaster* and *Anopheles stephensi*; the amplification core and esterase B1 gene isolated from *Culex* mosquito, which confers resistance to organophosphorous insecticide. Freeze resistance and heat tolerance genes can help the insect natural enemies to adapt to a broader range of climates and become useful and effective as biological control agents (Hoy, 1996).

The power of biotechnology on genetic manipulation of insect natural enemies is enormous, however, public concerns on the release of transgenic insect emphasized the need to assess the biological consequences of such a release; for example, the risk of any transgene being transferred to non target species. Releases of transgenic insect natural enemies into the environment should be planned and strictly following the appropriate regulatory oversight system set by responsible persons or institutes.

Table 1. Imported chemical and microbial insecticides of Thailand in 1997-2000.

Imported Product	Year 1997		Year 1998		Year 1999		Year 2000	
	Amount	Value	Amount	Value	Amount	Value	Amount	Value
Chemical Insecticide	12,543	1,645,547	12,823	2,044,493	19,525	6,589,279	12,532	2,000,546
Microbial Insecticide	73 (0.5%)	36,833 (2.2%)	78 (0.6%)	51,229 (2.5%)	43 (0.2%)	13,321 (0.2%)	20 (0.15%)	5,984 (0.30%)

Amount : x 1000 kg
Value : x 1000 Baht (1US$~40 Baht)
Source : Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand

Entomopathogens

Entomopathogen is a major segment of biological pesticides worldwide. Asian countries, where two phenomena on the widespread resistance to synthetic pesticides and the unacceptable negative consequences are so intense, have recognized the need for biological insecticide in which entomopathogen received the most attention. For instance, the imported biological insecticides of Thailand are almost exclusively entomopathogens, which include bacteria, virus, nematode and fungi. Recent figures as shown in Table 1 demonstrated the total amount of imported microbial insecticides in 1997-2000, which account for less than 1% of chemical insecticides and the total value of those microbial insecticides is less than 3% of chemical insecticides. *Bt* dominates the biopesticide market in Thailand but recently, fungus is increasing its role in integrated insect management program and is expected to increase its share in the market. Currently, efforts have been contributed to local production of *Bt*, nematode and nucleopolyhedrovirus in China, Vietnam and Thailand. It is, thus forecasted that the growth rate of microbial insecticides over the next ten years should be higher than chemical insecticides. This paper will highlight the principal factors constraining the use of those entomopathogens and to examine how far modern biotechnological processes may overcome these constraints in order to increase the market share for microbial insecticide products.

Bacillus thuringiensis and transgenic insect resistant plants

Bacillus thuringiensis (*Bt*) is a ubiquitous, spore-forming, rod-shaped, Gram-positive bacterium that produces massive amounts of one or more proteins that crystallize intracellularly during sporulation stage. These proteins (*Cry* proteins) are toxic mainly to insect larvae in order Lepidoptera, Diptera, and Coleoptera, but isolates with toxicity toward Hymenoptera, Homoptera, Orthoptera and Mallophaga and against nematode, mites, lice and protozoa have been recently discovered (Lacey and Goettel, 1995). The genes encoding the insecticidal proteins known as *cry* genes have been of particular interest. They have been classified into some 30 different groups by amino acid sequences of the proteins encoded by the genes (Yamamoto, 2001). The genetic manipulation of *cry* genes in *Bt* offers promising means of improving the efficacy and cost effectiveness of *Bt*-based bioinsecticide products. In Asian countries, *Bt* products have been used almost exclusively as direct spray for the control of foliar-feeding lepidopteran insects. Poor persistence under field condition and the dissemination of large amount of spores are two limitations of *Bt* for spray application. Cell Cap technology had been developed in which *Bt* toxin genes were cloned into a common plant-colonizing bacterium, *Pseudomanas fluorescens*. The bacteria were killed, resulting in encapsulated insecticidal proteins that had enhanced residual property in the field and had no *Bt* spores. These novel formulations provide environmentally safe and stabilized *Bt*-based bioinsecticides (Gaertner *et al.*,1993). The gene encoding Cry1Ac protein has been engineered into the endophytic, xylem-inhabiting bacterium, *Clavibacter xyli*. The engineered bacterium was then introduced into corn, and damage caused by stem borer was significantly reduced (Tomasino *et al.*, 1995). In this alternative approach, the endophytic microbe help to enhance delivery of toxin to leaf and stem-feeding lepidopteran insects. Combining genes from different strains of *Bt* to increase the activity and broaden their host range is underway using nonrecombinant and recombinant technologies. A self-transmissible *cry* gene from a *Bt aizawai* strain was transferred via conjugation-like process to a *kurstaki* recipient strain (Gawron-Burke and Baum, 1991). Recent development of *Bt*-based cloning vector system enables the construction of recombinant *Bt* strains with improved spectrum of insecticidal activity and these genetically modified *Bt* products are currently commercially available.

Advances in plant transformation, tissue culture and molecular biology offer great potential for incorporation of genes that produce the *Bt* delta-endotoxin into crops to confer resistance against insects. The two most widely used methods of transforming plant are *Agrobacterium*-mediated transfer of DNA and bombardment of cells with DNA-coated particles. *Bt* transgenic crop has received more attention when *cry* genes had been reconstructed by a combination of mutagenesis and oligonucleotide synthesis to produce synthetic genes. These genes encoded the same proteins but had codon usages typical for plant genomes and which had all aberrant processing signals removed. Expressing level of *Bt* toxin from these synthetic genes was increased by nearly two orders of magnitude when expressed in transgenic plants (Perlak *et al.*, 1991). Since then many other crops, including cereals, root crops, leafy vegetables, forage crops, and tree are/or being engineered to express *Bt* toxins. Currently *Bt* crops have been commercialized for field corn, sweet corn and cotton. Plant varieties that incorporate both *Bt* and virus resistant traits or *Bt* and herbicide resistance have been produced.

Global commercialized transgenic crops in 2001 has been reviewed by James (2001). Herbicide tolerant soybean occupied 33.3 million hectares, representing 63% of the global transgenic crop area of 52.6 million hectares. *Bt* crops occupy 7.8 million hectares, representing 15%, which include *Bt* maize, the second most dominant transgenic crop that occupied 5.9 million hectares; and *Bt* cotton, which occupied 1.9 million hectares. Stacked genes for herbicide tolerance and insect resistance deployed in both cotton and corn occupy 8% of the global area of transgenic crops (Table 2). It is interesting to note that the area of crops with stacked genes for *Bt* and herbicide tolerance increased from 3.2 million hectares in 2000 to 4.2 million hectares in 2001. It is expected that stacked genes will continue to gain an increasing share of the global transgenic crop market. Despite the ongoing debate on genetically-modified (GM) crops, millions of large and small farmers in both industrial and developing countries continue to increase their planting of GM crops. Their decision was based on evidences that clearly demonstrated the benefits of GM crops, such as more sustainable and resource-efficient crop management practices; more effective control of insect pests; and reduction of fumonisin mycotoxin level in maize that provides safer and healthier food and feed products (James, 2001). The number of farmers that benefited from GM crops increased from 3.5 million in 2000 to an estimated 5.5 million in 2001. More than three-quarters of this number were resource- poor farmers planting *Bt* cotton, mainly in China and South Africa. The experience of China with *Bt* cotton presents a remarkable case study where poor farmers are already benefiting from significant agronomic, environmental, health and economic advantages (James, 2001).

In addition to the *Bt* delta-endotoxin, a second class of protein that is effective against certain insects such as the vegetative insecticidal proteins (VIP), alpha-endotoxin, and a variety of secondary metabolites including Zwittermycin from *B. cereus* strains may be amenable to genetic manipulation (Baum *et al.*, 1999). Several single gene products of plant origin have been proven to confer resistance to insect damage and have been transferred to another plant species. Lectin and lectin-like proteins are carbohydrate binding molecules that are abundant in seeds and storage tissue of plants. The role for lectin as defensive protein in plants against insect is well documented particularly for homopteran plant pests such as aphids, leafhoppers and planthoppers, which routinely feed by phloem abstraction (Powell *et al.*, 1993). Genes encoding the pea lectin (*P-Lec*) and the snowdrop lectin (*GNA*) have been engineered into transgenic plants resulting in significant reduction of insect damage. Enzyme inhibitors that have insecticidal and/or antimetabolic activity in insects such as protease inhibitor, cysteine, alpha-amylase inhibitor and cholesterol oxidase have been proven to reduce insect damage to transgenic plants expressing these proteins (Gatehouse and Gatehouse, 1998). With these tremendous opportunities provided by modern biotechnology, new resistance genes must continue to be identified from both conventional and transgenic sources for the advent of more environment-friendly control strategies.

New genetically engineered and improved *Bt* products may provide more opportunity and choice for growers. It is expected that transgenic plants resistant to insects will be a major component of future pest-control system in agriculture. The risk of developing greater resistance of insects to *Bt* transgenic plants than to *Bt* formulations applied as sprays has been raised and will be one of the major constraints for the utilization of *Bt* crops. However, strategies have been developed to delay the evolution of resistance involving establishment of refugia for sensitive insect, high dose expression in engineered plants, pyramiding traits and agronomic practices (Gould, 1998).

Table 2. Dominant transgenic crops, 2001.

Crop	Million hectares	% Transgenic
Herbicide tolerant soybean	33.3	63
Bt maize	5.9	11
Herbicide tolerant canola	2.7	5
Herbicide tolerant cotton	2.5	5
Bt/Herbicide tolerant cotton	2.4	5
Herbicide tolerant maize	2.1	4
Bt cotton	1.9	4
Bt/Herbicide tolerant maize	1.8	3
Total	52.6	100

Source: Clive James, 2001

Entomopathogenic nematode

Insect nematodes have enormous potential for inoculative and inundative release and control of a wide range of insect pests. They are probably second only to bacteria (i.e., *Bt*) in terms of commercially important microbial insecticides. Commercially available species of nematode as bioinsecticide are in three families: Rhabditidae, Steinernematidae, and Heterorhabditidae. Nematodes parasitize their hosts by direct penetration either through the cuticle or natural opening in the host integument (i.e., spiracles, mouth, or anus). Insect death is not due to nematode itself but a symbiotic bacterium that is released upon entry into the host. The symbionts are specific with members of the genus *Xenorhabdus* associated with the steinernematids and *Photorhabdus* associated with the heterorhabditids (Lacey and Goettel, 1995). In general, both steinernematids and heterorhabditids tend to do best against soil-inhabiting insects and borers. There have been limited successes when applying to other insects. Strain selection and new formulations may be able to address this limitation. Molecular techniques such as RFLP, RAPD-PCR, AFLP, ribosomal internal transcribed spacer (ITS) analysis, satellite DNA analysis have been applied to measure genetic diversity of the nematodes and provide an initial screen to identify useful strains. The development of large- scale *in vitro* rearing systems and formulations that would allow for adequate shelf life and infectivity in the field are underway. Currently, nematodes are successfully grown in large-scale bioreactors similar to those used for the production of *Bt* or antibiotics. Formulation by chilling the produced nematodes prior to formulation and then mixing with materials that will enhance their handling, application, persistence, and storage will help to create a commercial venture. Another limitation of nematodes for insect control is their susceptibility to environmental stress, extreme temperature, solar radiation and desiccation. The potential of genetic engineering to enhance these traits is being explored. In addition, genes that confer resistance to insecticide or fungicides could also be incorporated for protective purposes (Harrison and Bonning, 1998).

Efforts to engineer entomopathogenic nematodes have been immense and relied heavily on knowledge and techniques for manipulating of the *Caenorhabditis elegans* genome (Poinar, 1991). The research has been focused mainly on enhancing the environmental stability with respect to heat tolerance. The nematode, *Heterorhabditis bacteriophora* was engineered to express C. *elegans* Hsp70A (heat-shock protein genes) to enhance tolerance of high temperatures (Hashmi *et al*., 1998). Research on genetic of heterorhabditid and steinernematid nematodes especially on pathogenicity traits are required for future genetic manipulation of more efficient strains of nematode for insect control.

Recombinant Baculoviruses for Insect Control

Entomopathogenic viruses have been employed as bioinsecticides for a wide range of situations from forest and field to food stores and greenhouses. Baculoviruses, particularly the nucleopolyhedroviruses (NPVs) are the most commonly used or considered for development as microbial insecticides mainly for the control of lepidopteran insects on field and vegetable crops. NPVs are formulated for application as sprays in the same fashion as chemical insecticide and *Bt* strains. However, only moderate success has been achieved due to several key limitations, which include a relatively slow speed of kill, a narrow spectrum of activity, less persistence in the field, and lack of a cost-effective system for mass production *in vitro*. Fermentation technology for their mass production on a large-scale commercial basis is extensively investigated to reduce the production cost.

Approaches to engineer NPVs as improved biological insecticide include deletion of genes that encode products prolonging host survival, and insertion of genes that express an insecticidal protein during viral replication. O' Reilly and Miller (1991) demonstrated that deletion of the ecdysteroid UDP-glucosyltransferase (*EGT*) gene of *Autographa californica* NPV caused infected fall armyworm, *Spodoptera frugiperda* to feed less and die about 30% sooner than larvae infected with wild-type AcNPV. Research to insert specific toxin genes or disrupters of larval development genes into baculovirus genome is progressing. The most common used strategy for engineering baculoviruses has exploited the polyhedrin or p10 promoters and the construction of recombinant baculovirus is achieved by allelic replacement of the polyhedrin gene by foreign genes. When the recombination is successful, the polyhedrin of p10 promoters drive the expression of the foreign gene to levels equivalent to those of polyhedrin or p10 in wild type virus (Miller, 1995). Hundreds of proteins from viral, bacteria, animal and plant origin have now been produced via such recombinant baculovirus expression vectors. Candidate genes for insertion into baculoviruses and potential to enhance pathogenicity and insecticidal activity are listed in Table 3 (Vlak, 1993). Introducing an insect-specific neurotoxin gene from the Algerian scorpion, *Androctonus australis* and from the straw itch mite, *Pyemotes tritici* into insect genome using the baculovirus expression system have received the greatest attention to date (Treacy, 1999). There are several insect hormones that play vital role in the control of insect morphogenesis and reproduction and are focused for engineering into baculoviruses. These include eclosion hormone that initiates ecdysis, the process leading to the shedding of old cuticle, prothoracicotropic hormone (PTTH), which is involved in triggering the molting process; allatostatins and allatotropins, which regulate the release of juvenile hormone; and diuretic hormone (DH) that regulates water balance and possibly blood pressure in insect. Another interesting gene for genetic manipulation of baculovirus is the enzyme gene, juvenile hormone esterase (JHE) that caused the reduction in JH level. A reduction in the titer of JH early in the last instar initiates metamorphosis and leads to cessation of feeding. Insertion of two or more toxin genes into baculoviruses has been studied and Hermann *et al*.(1995) found that binary mixtures of scorpion toxin, AaIT and LqhIT injected into larval of *Helicoverpa virescens* induced 5-10 fold the levels of activity. The authors suggested that simultaneous expression in baculoviruses of synergistic combinations of insecticidal proteins could lead to even more potent, insect-selective bioinsecticides.

The development of baculovirus expression system and the accomplishment of insect cell culture technology have broadened the utility of insect viruses as effective insecticides and as expression vector of foreign genes in eukaryote host for the production of useful proteins. Production, formulation and application technology in conjunction with genetic engineering for fast kill and broader host range will be necessary to enable the development of more economic and efficacious viral products for insect control.

Table 3. Candidate genes for introduction into baculoviruses and potential to enhance insecticidal activity.

Gene class	Protein	Insecticida[1] potential
Toxins	Bt-toxin	+/-
	Scorpion toxin	+++
	Mite toxin	+++
	Trypsin inhibitor	?
Hormones	Eclosion hormone	+/-
	Diuretic hormone	+
	Prothoracicotropic hormone	+
	Allatoropin	-
	Allatostain	+
	Proctolin	+
Enzymes	Juvenile	+

Source : Vlak, J. M. 1993

Entomopathogenic fungi

Although over 750 species of entomopathogenic fungi were reported to infect insects, few have received serious consideration as potential commercial candidates. The first registered mycoinsecticide was *Hirsutella thompsonii*, which has been known to cause dramatic epizootics in spider mites. The next mycoinsecticides are *Verticillium lecanii* and *Paecilomyces fumosoroseus*, which have been recently registered for control of whitefly, thrips, aphids and spider mites. Insect fungi that have much broader host range are *Beauveria bassiana* and *Metarhizium anisopliae*, which are effective against homopteran and lepidopteran greenhouse insects as well as coleopteran and lepidopteran field insects (Flexner and Belnavis, 1998). The broad host range of some insect fungi is an attractive characteristic for insect pests control. Nevertheless, there are numerous biotic and abiotic constraints on the ability of fungi to infect their hosts. These include dessication, UV light, host behavior, temperature, pathogen vigor and age. Certain aspects of the insecticidal efficacy of these fungi such as production, stability and application have been optimized by nongenetic means. For instance, advances in production and formulation technologies have contributed substantially to the cost-effectiveness and viability of mycoinsecticide as practical insect control agents.

Optimization of entomopathogenic fungi by genetic engineering is limited due to lack of knowledge of molecular and biochemical bases for fungal pathogenesis, and the unavailability of good cloning system for species other than deuteromycete fungi. The molecular and biochemical bases of pathogenicity of *M. anisopliae* which cause green muscardine diseases, have been well studied especially on host cuticle penetration by the fungus. Various genes related to formation of the appressorium (a specialized structure involved in penetration of the insect cuticle by the fungus), virulence, and nutritional stress had been cloned from *M. anisopliae*. Additional copies of the *PrI* gene, which encodes a subtilisin-like protease involved in host cuticle penetration were engineered into the genome of *M. anisopliae*. The larvae infected with recombinant strains died 25% sooner and feeding damage was reduced by 40% (St. Leger *et al*., 1996). The prospect of using recombinant fungi for insect control highlights the need for further research in identifying and manipulating genes involved in pathogenesis and monitoring of genetic exchange between strains by using isolate-specific molecular markers. (Harrison and Bonning, 1998). Despite a potentially wide array of insecticidal proteins produced by entomopathogenic fungi, fungal genes have played little part in agricultural biotechnology to date.

Botanical Insecticide

More than 2,400 plant species around the world are currently known to possess pest control properties. The promising species include the neem tree, sweet flag, onion, garlic, custard apple, pyretrum, derris, common latana, holy basil, black pepper, and common ginger (Weinzierl, 1998). Botanical insecticides may be in the form of dust or powder of crude preparations of plant parts that may be used in full-strength or diluted in a carrier. Some preparations are the water extracts or organic solvent extract of insecticidal components of plants. The most processed forms are purified insecticidal compounds that are isolated from plant materials by a series of extraction and distillations. The use of botanical insecticide is well documented in Asian countries and extracts from neem tree, *Azadirachta indica* appear to be the most widely used. In Thailand, the availability of neem-based bioinsecticide was a prerequisite to the emergence in the early 1990s of a new market of organic vegetables, fruit and rice, now widely sold through a network of “green” shops. More than 15 complex chemicals having repellent, antifeedant, insect growth regulator and insecticidal properties have been identified in aqueous and chemical extracts of neem leaves, bark, stem seed and other parts. Seeds or seed kernels provide the greatest amounts of insecticidal compounds (Schmutterer, 1990). Neem is generally considered to be most effective against the soft-bodied, immature stages of plant pests, including whiteflies, thrips, mealybugs, and various caterpillars (Weinzierl, 1998). Neem’ s broad activity against plant-eating insects, its virtual nontoxicity to mammals, beneficial insects and environment make it an extremely appealing insecticide.

Despite several appealing traits, however, botanical insecticides continue to fill only a minor role, primarily because most are very expensive in comparison with synthetic insecticides. Their availability is often limited because production levels are not sufficient to meet a highly fluctuating global market demand and bioactivity of some products often varies among seeming identical preparations (Weinzierl, 1998). Research should be focused on increasing natural production, improving extraction rates of the toxic compound and improving formulation for spray application. Gene transfer into bacteria or directly into food crops for the production of toxic compound is encouraging.

Pheromone for Insect Pest Control

Pheromones are signal compounds used by insects to communicate. The direct insect control approaches using pheromone includes mass trapping, lure and kill tactics and mating disruption tactics. The most efficient way is the lure and kill technique. Combination of pheromone and pathogen is designed not to kill the insects right away, but rather to use them as vector of the disease into the wider population. Mating disruption technology has received considerable attention, however it only works with isolated population. A large number of pheromone dispensers were deployed to interfere with orientation toward conspecifics and interrupt the life cycle of the insect by preventing mating (Suckling and Karg, 1998). All the pheromones currently marketed are made by chemical synthesis, but biotechnology is of potential interest. For instance, pheromone consists of two stereo-isomeric variants, only one of which has the desired biochemical effect. The use of enzyme technology will allow the effective form only to be produced, whereas in chemical synthesis both variants are produced. Accurate identification, increased stability and longevity, and more uniform release rate following field exposure, can enhance the performance of pheromones. These improvements can be achieved through the continued progress of biotechnology (Suckling and Karg, 1998). However, the adoption by growers of pheromone-based biological control will depend on how well the system can meet grower concerns about efficacy and cost. A better understanding of how and why the technology works will lead to the design of a more cost-effective technology.

Table 4 Awareness of cotton growers in Thailand on the impact of *Bt* cotton on the environment.

Awareness	Number of growers	%
Aware of (positive & negative)	15	24.19
Not aware of	38	61.29
Not indicated	9	14.52

Source : Kunalasiri, A. and S. Buskaew (2000)

Table 5. Acceptance of *Bt* cotton by cotton growers in Thailand.

Acceptance	Number of growers	%
Accept	48	77.42
Not accept	3	4.84
Not certain	6	9.68
Not indicated	5	8.06

Source : Kunalasiri, A. and S. Buskaew (2000)

Community Perception on Genetically Modified Organisms

Most of the agricultural countries recognize the potential of biotechnology as an important tool for agricultural productivity. In the past decades, many genetically modified (GM) microorganisms and plants have been produced and used in agriculture primarily for insect and disease control. There are growing concerns of the community over GM plants and products derived. Those who are in favor of the technology believe that GM plant could help the farmers reduce pesticide use and increase crop productivity. On the other hand, the opponents are anxious about the possible risk that may be associated with GM technology. The production of GMOs is now being considered as biosafety, trade and political issue. Community perception on GMOs will be presented here with special reference to public and farmer's perception of GM plants and products particularly the cotton engineered with *Bt* toxin genes in Thailand.

Cotton has been considered an important economic crop for Thailand since 1961 due to its high demand for the textile industry. The major constraint for cotton production in Thailand is the presence of insect pests especially the cotton bollworm, *Helicoverpa armigera*. *Bt* cotton resistant to insect attack, therefore, received much of public attention and demonstrated high potential for commercialization. The NuCOTN 33B *Bt* cotton was first introduced by Monsanto Ltd. Thailand Company in 1995 for screenhouse testing. This cotton line was described as having the *crylAc* gene from *B. thuringiensis* that is effective against the cotton bollworm, *H. armigera*. In 1996, *Bt* cotton was approved for isolated small scale field trial in Chiang Mai Province. By 2000, nine isolated large scale field trials of *Bt* Cotton were conducted in the Department of Agriculture (DOA) experiment stations and farmer's fields. During these field trials, GMO debates were so intense among the Thai community. In 1999, the National Center for Genetic Engineering and Biotechnology (BIOTEC) had conducted a survey on public awareness, understanding and opinions about genetically modified organisms (GMOs). On the understanding of GM technology, 34.15% indicated that they have no knowledge about it. Those who understand the technology at good, average and basic levels were at 15.57, 32.51 and 16.39%, respectively. Most of the Thais (90.71%) were aware of the GMOs but they have fewer concerns on GM food as compared to chemical residues being food contaminants. It is obvious that media plays an important role in public awareness (Noppakornvisate *et al*., 2000).

In the same year, the DOA made a survey on the socio-economic effect of the transgenic *Bt*-cotton to cotton growers in six provinces in Thailand. Generally, cotton growers (91.93%) knew about *Bt* cotton from the media and seed companies. They have heard of the advantages and disadvantages of the so called GM plants. Yet, only 24.19% were aware of the possible adverse effects of *Bt*-cotton on the environment (Table 4). About 77.42% of the farmers in the survey were willing to plant *Bt*-cotton as soon as it is permitted and only 4.84% denied to accept *Bt* cotton (Table 5). Most of the farmers believed that *Bt*-cotton could reduce the cost of production and lessen the use of chemical insecticides, which consequently contribute to their better living conditions (Kunalasiri and Bukaew, 2000). However, due to the strong movement of the anti-GMO and NGO groups campaigning against the technology monopoly and possible adverse effects on biodiversity, the government decision on deregulation of transgenic *Bt*-cotton for commercialization has not been made. The surveys clearly showed that the scientific community in Thailand is open-minded to the GM technology. Yet, there is a need to help the public understand the technology better, especially those who are not in the field of biological science.

Conclusion

Sustainable agriculture could be achieved not only through proper agricultural practices but also through continuous research and development of new technologies, particularly agricultural biotechnology, which is probably a very important investment to achieve greater competitiveness in the world market. Knowledge and continuous research is the key to assess the potential of biotechnology to increase agricultural productivity and to contribute to sustainability of agricultural systems. Potential improvements of bio-control agents involving beneficial insects and microorganisms that work in harmony with genetically engineered plants are examples of the utilization of biotechnology that lead to sustainable control practices.

Public concerns on extensive use of chemical insecticides, insect resistance development, and the rising cost of developing new synthetic insecticides, all suggest that integrated insect pest management utilizing biological control products will become increasingly important in the years to come. However, products of biotechnology should be handled and marketed in much the same way as chemical pesticides. It is important to provide appropriate regulatory mechanisms to ensure that products produced by using new techniques are as safe as the products of traditional biotechnology. It is wiser, especially for the Asian countries to look into bio-control products that complement synthetic pesticide instead of replaces them. Bio-insecticides should bring great benefit in reducing the use of synthetic pesticides, especially those that are toxic and persistent. An integrated insect pest management underlying less input, cost effective and friendly environment is the key for sustainable agriculture.

Despite tremendous benefits of biotechnology in insect pest management, there are still questions that required answers especially in Asian countries where biotechnology could be most profitable. Specific examples are the uncertainty of the technology in terms of successful research and adoption by the end users; high start-up investment; public awareness and acceptance; national policies on bio-safety and intellectual property issues; technology dissemination and proper implementation; human resource and institutional development; and limited funding due to long-term and continuous nature of the research. Responsible national institutes and other affiliated research centers should engage in educational and training programs aimed at the general public for better understanding of the risks and benefits of biotechnology application.

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