

## RESEARCH ARTICLE

## MODERN BIOTECHNOLOGICAL APPROACHES IN INSECT RESEARCH

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## ABSTRACT

To meet the growing demand for food it is essential to increase the production of food. Insect pests are major constraints to global production for food and fibre that can be reduced utilizing modern biotechnological tools. In insect research field, the biotechnological tools have been applied to study various issues such as insect identification, insect control and insect genetic relationships. It has a significant role in improving efficacy, cost-effectiveness and in expanding the markets for the bio insecticides. Molecular techniques employed for identifying and monitoring establishment and dispersal of specific biotypes of natural enemies. Production, formulation and storage of entomopathogenic fungi can be dramatically improved through biotechnology and genetic engineering. Proteinaceous insect toxins (scorpion toxin, mite toxin, trypsin inhibitor), hormones (eclosion hormone, diuretic hormone) and metabolic enzymes (juvenile hormone esterase) introduced into NPV and GV genome virus to increase its efficacy to kill insect. 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 2011, planting of Bt cotton in India surpassed the historical milestone of 10 million hectare for the first time and occupied 88% of the recorded 12.1 million hectare cotton crops. However, field resistance of Bt crops to various insects have been noticed and to combat this problem two approaches namely refuge and pyramiding were recently introduced. 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. RNAi technology enables engineering of a new generation of pest-resistant GM crops. Insect control strategies that integrate advance knowledge in biotechnology with traditional wisdom and technology will contribute to the sustainability of agriculture.

## KEYWORDS

Biotechnology,  
*Bacillus thuringiensis*,  
Cryobiology, GM  
crops, Insect,  
RNAi

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## INTRODUCTION

Each year, in agriculture, billions of dollars are spent worldwide in controlling insect (Krattiger, 1996). But inspite of this expenditure, up to 40% of a crop is lost due to insect damage, particularly in developing countries (Oerke, 2006). Over the years the widespread use of pesticides has led to pesticide resistant insects, a reduction in beneficial insect populations and many harmful effects to humans and the environment (Fitt, 1994; Gatehouse *et al.*, 1994; Gunning *et al.*, 1991; and Haq *et al.*, 2004). These problems have compelled the researchers to think for a solution in a different way so as to develop different insect control strategies using both synthetic and natural molecules that are more

environmentally friendly. One such approach has been the use of transgenic plants expressing plant defence molecules. Genetic modification through biotechnology can potentially provide a much larger array of novel insecticidal genes that are otherwise beyond the scope of conventional breeding. In the year 1987 first transgenic plant was developed that expressed an insecticidal gene produced in it. This transgenic tobacco plant produced cowpea trypsin inhibitor at levels of up to 1% of the soluble protein and had enhanced protection against the lepidopteran pest *Heliothis virescens* (Hilder, 1987; and Harsulkar, 1999). The development of DNA-based techniques is generally known as biotechnology. Modern agricultural biotechnology or genetic engineering includes manipulation of the genetic make-up of

organisms for use in the production or processing of agricultural products. Genetic engineering is the formation of new combination of heritable material by insertion of nucleic acid by whatever means outside the cells, into virus, bacterial plasmid or other vector systems so as to allow their incorporation into the host in which they do not naturally occur but capable of continued propagation. (Smith,1996).

Since the commercialisation of biotech crops in 1996, farmers have adopted the technology at such a dramatic rate, that in 2011, 16.7 million farmers in 29 counties planted 160 million hectares of the biotech crops. In India alone, Bt-cotton has increased cotton yields by up to 60%, and has reduced insecticide sprays by around half. This in turn has led to an income increase of up to US \$11.9 billion per annum (James, 2011).

### 1. Role of biotechnology in Insect world

In insect research field, the biotechnological tools have been applied to study various issues such as insect identification, insect control and insect genetic relationships. It has a significant role in improving efficacy, cost-effectiveness and in expanding the markets for the bio insecticides. (Tipvadee, 2002). Molecular techniques employed for identifying and monitoring establishment and dispersal of specific biotypes of natural enemies. (Tipvadee, 2002). It provides opportunities for the development of insect natural enemies conferring beneficial traits such as pesticide resistance, cold hardiness and sex ratio alteration. A number of insect-specific baculoviruses (NPVs) have been modified to contain genes which, when expressed in the host insect, produce insecticidal effects (Bishop, 1989). The most well known examples of these technologies in both plants and viruses is the insertion into a plant or virus of the gene coding for the production of the delta endotoxin of *Bacillus thuringiensis* (Merryweather *et al.*, 1990). It is seen that experiments have been conducted with transformed baculoviruses containing genes coding for insect hormones and, in some instances, other manipulations that interrupt on the insect endocrine system (O'Reilly and Miller, 1989; and Hammock *et al.*, 1990). Biotechnology also provides an effective extraction process, formulation solvents and adjuvant, which can enhance insecticidal activity of plant-derived insecticides. (Tipvadee, 2002). It 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, in breeding, genetic drift and founder effects (Hopper *et al.*, 1993). These had led to the development of cryobiological method for preserving embryos of insects that can significantly save the rearing costs, and the

valuable collection of insect natural enemies could be maintained indefinitely (Denlinger and Lee, 2010). The gene encoding the cowpea trypsin inhibitor was subsequently transferred into rice and potato but as it did not provide any sustainable insect protection so it was not commercialised. Mitochondrial DNA has been employed as a marker to differentiate between endemic and released populations of *Trichogramma* and also to measure their dispersal distance and their intensity in the field. Production, formulation and storage of entomopathogenic fungi 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) metabolic enzymes (juvenile hormone esterase) into nucleopolyhedroviruses NPV and granulosis virus GV genome are some approaches to increase speed to kill, virulence and host specificity. (Tipvadee, 2002). 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. (Tipvadee, 2002). The concept of DNA fingerprinting has been recently used in insect field. The chemical structure of everyone's DNA is the same. The only difference between organisms (or any insects) is the order of the base pairs. Using these sequences, every person could be identified solely by the sequence of their base pairs. Able to determine whether two DNA samples are from the same insect, related insects, or non-related insects. (Tipvadee, 2002). To express transgenes in plants cells, appropriate promoter sequences have been introduced alongside the gene to ensure efficient transcription of mRNA. Cauliflower Mosaic Virus (CaMV 35 S) promoter has been used in majority of insect-resistant transgenic plants. Pi gene was transferred to tobacco plants and such plants afforded resistance against *Heliothis zea*, *Spodoptera litura* and *Manduca sexta* (Srinivasan, 2006). RNA interference (RNAi) caused by exogenous injection of double-stranded RNA (dsRNA) has emerged as a powerful technique for down-regulating gene expression in insects. This method was used to explore the functions of proteins, such as metalloproteinases, metalloproteinase inhibitors and heat shock proteins, in development and immunity of the model beetle *Tribolium castaneum*. This technology enables engineering of a new generation of pest-resistant GM crops (Knorr and Vilcinskis, 2011). 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*. (Gould, 1998). Fermentation technology for their mass production on a large-scale commercial basis is extensively investigated to reduce the production cost (O'Reilly and Miller, 1991)

## 2. *Bt (Bacillus thuringiensis)*

*Bacillus thuringiensis*, a natural soil bacteria that secretes a deadly endotoxin. Bt toxins are highly effective for many pest organisms, like Lepidopterans, coleopterans, Dipterans and other related species, but not toxic to mammals and most other non-target organisms. The use of genes encoding endotoxins from *Bacillus thuringiensis* is now a well-established technology for producing transgenic plants with enhanced resistance to the larvae of lepidopteran insect pests (Duke, 2011). Regarding mechanism of bacterial toxin action, when the insect larvae feed on transgenic plant, crystals and spores are ingested into the midgut of the insect. Since the pH is alkaline in nature, so the the crystals become toxic to insect midgut leading to septicaemia.

Bt cotton was first released for commercial production in the USA in 1996 and subsequently grown in several countries including Argentina, Australia, China,<sup>1</sup> Colombia, Indonesia, Mexico, South Africa, and India (James, 2011). Since then other transgenic crop species producing Bt toxins have been commercialized including maize, tomato and potato. The adoption of Bt crop varieties by farmers has been rapid reflecting the benefits of these crops such as reduced insecticide use, lower production costs and higher yields (Brookes and Barfoot 2005). Only two Bt crops are grown in Australia (Table 1.). *B. thuringiensis*, a Gram-positive soil bacterium, produces a proteinaceous parasporal crystalline inclusion during sporulation (Schnepf, 1998). There are two main categories of Bt toxins: Cry and Cyt. These two groups are classified further by a detailed nomenclature system that describes groups Cry1 to Cry55 and Cyt1 to Cyt2 (Höfte and Whiteley, 1989; Crickmore *et al*, 1998; and Van Frankenhuyzen, 2009). The Cry toxins are divided into three larger families that are not related phylogenetically. The largest Cry family is the three domain family, and genes from this family are present in the majority of commercialised Bt crops (Tabashnik *et al.*, 2009). The larvae of insect orders primarily affected by Bt toxins are Lepidoptera (butterflies and moths), Diptera (mosquitoes) and Coleoptera (larval and adult beetles) (Knowles and Dow, 1993). However, Bt toxins are not toxic to people, wildlife, or most beneficial insects

(Marvier *et al.*, 2007; Romeis, 2006) and therefore the opportunities for biological control are great. The effect of Bt toxins on a range of lepidopteran insects has been studied including: *Bombyx mori* (Endo and Nishiitsutsuji 1980), *Helicoverpa armigera* (Estela *et al.*, 2004), *Heliothis virescens* (Ryerse 1990; and MacIntosh 1991), *Manduca sexta* (Lane *et al.*, 1989; and Knight *et al.*, 1994) *Ostrinia nubilalis* (Hua *et al.*, 2001; Li *et al.*, 2004; Siqueira *et al.*, 2004; and Tang 1996), *Plutella xylostella* (Wright *et al.*, 1997), *Sesamia nonagrioides* (Moar *et al.*, 1995), *Spodoptera exigua* (Moar *et al.*, 1995), *Spodoptera frugiperda* (Adamczyk *et al.*, 1998) and *Spodoptera littoralis* (Avisar *et al.*, 2004). The Cry toxins produced in Bt crops generally target lepidopteran pests, although some also target coleopteran pests (Tabashnik *et al.*, 2009). The first commercialised Bt crops contained only one Cry toxin, but second generation Bt crops have between two to six different toxins (Tabashnik *et al.*, 2009). In 2011, planting of Bt cotton in India surpassed the historical milestone of 10 million hectare for the first time and occupied 88% of the recorded 12.1 million hectare cotton crops (Gautam *et al* 2013, Agrobios).

**Table 1:** Bt crops grown commercially

Trade name	Crop	Bt protein
Ingard ®	cotton	Cry1Ac
Bollgard II ®	cotton	Cry1Ac , Cry2Ab

Stevens J *et al.*, 2012

## 3. Resistance in Bt crop

Most recently there have been reports of field resistance to Bt crops in pink bollworm (*Pectinophora gossypiella*), cotton bollworm (*Helicoverpa* spp. armyworm (*Spodoptera frugiperda*) and western corn rootworm (*Diabrotica virgifera virgifera*) (Tabashnik *et al.* 2008). Some insects collected from the field have Bt resistance that has been characterized in the laboratory. A decrease in field performance of Bt corn against *S. frugiperda* was observed in Puerto Rico and against *Busseola fusca* in South Africa. In south-eastern US, problems with control of *H. zea* on Bt cotton have also been reported.

### 3.1 Management of resistance to Bt crops

There are two main strategies for management of insect resistance to Bt crops: Refuge and pyramiding (Tabashnik *et al.*, 2008; and Gould, 1998).

**Refuge:** The main approach for delaying evolution of resistance to Bt crops is the refuge strategy. Farmers are mandated to maintain an abundance of host non-Bt crops as a refuge surrounding their Bt crops. The theory behind this strategy is that any Bt resistant larvae that arise on the Bt crops will mate with susceptible individuals from neighbouring non-Bt crops.

**Pyramiding:** Major strategy to combat the evolution of Bt resistance is gene pyramiding. For eg. the development of second generation Bt cotton that has at least two Bt toxins such as the Monsanto Bollgard II cotton variety. Another resistance management strategy which is still in the research phase of development is the use of insecticidal genes with completely different modes of action such as proteinase inhibitors. The success of combining multiple Bt genes for resistance management is contingent on the individual toxins having different targets to prevent cross resistance.

#### 4. Use of transgene and their mode of action

The reliance of a worldwide industry on one insect resistance trait has led to the development of Bt-resistant insects (Heckel *et al.*, 2007), especially since at least four cases of field based resistance have already been documented (Tabashnik *et al.*, 2008; Storer *et al.*, 2010;

and Van Rensburg, 2007). This in turn has led to a search for new insecticidal proteins and their encoding genes that have commercial potential for plant protection (Haq *et al.*, 2004 ; and Lynch *et al.*, 2003). They include alpha amylase inhibitors (Carlini *et al.*, 2002; and Franco *et al.*, 2002), vegetative insecticidal protein (Bhalla *et al.*, 2005, and Fang *et al.*, 2008), chitinases (Kabir *et al.*, 2006) and protease inhibitors (Ferry *et al.*, 2005; and Maheswaran *et al.*, 2007) , as well as several other proteins directed to targets in the insect gut (Table 2).

#### 5. Tools used in Genetic engineering (Moussa *et al.*, 2005)

- Polymerase Chain Reaction (P.C.R.)
- Restriction Fragment Length Polymorphism (R.F.L.P.)
- Random Amplified Polymorphic Technique (R.A.P.D.)
- Amplified Fragment Length Polymorphism (A.F.L.P.)
- Microsatellite Loci (M.S.L.)

**Table 2: Use of transgene and their mode of action**

TRANSGENE	SOURCE AND MODE OF ACTION	EXAMPLE OF USE
<b><i>Bacillus thuringiensis</i> (Bt) endotoxin</b>	The <i>Bacillus thuringiensis</i> endotoxin	The <i>Bacillus thuringiensis</i> endotoxin"
<b>Vegetative insecticidal protein (VIP)</b>	VIPs are produced by <i>Bacillus cereus</i> and <i>Bacillus thuringiensis</i> . They have similar activity to endotoxins from Bt. Vip1/Vip2 are toxic to coleopteran insects and Vip3 is toxic to lepidopteran insects	Highly toxic to <i>Agrotis</i> and <i>Spodoptera</i> species. VIP induced gut paralysis, complete lysis of the gut epithelial cells and resulted in larval mortality.
		VIP3Ac1 had insecticidal activity against larvae of <i>S. frugiperda</i> , <i>Helicoverpa zea</i> and <i>Trichoplusia ni</i>
<b>Chitinase (enzyme)</b>	Chitinase catalyses the hydrolysis of chitin, which is one of the vital components of the lining of the digestive tract in insects and is not present in plant and higher animals.	Transgenic rapeseed ( <i>Brassica napus</i> ) expressing <i>M. sexta</i> chitinase and scorpion insect toxin increased mortality and reduced growth of <i>Plutella maculipennis</i>
<b>Cholesterol oxidase (enzyme)</b>	Cholesterol oxidase is a bacterial enzyme that catalyzes the oxidation of cholesterol and other 3-hydroxysterols, resulting in production of the corresponding 3hydroxysterols and hydrogen peroxide. Functions by damaging midgut membranes	Cholesterol oxidase from <i>Streptomyces</i> caused stunting of <i>H. virescens</i> , <i>H. zea</i> and <i>Pectinophora gossypiella</i> when incorporated into an artificial diet
<b>Lipoxygenases (enzyme)</b>	Dioxygenase enzymes are widely distributed in plants and catalyse the hydroperoxidation of cis-cis-pentadiene moieties in unsaturated fatty acids. Functions by damaging midgut membrans	Lipoxygenase from soybean retards the growth of <i>Manduca sexta</i> when incorporated into artificial diet
<b>Alpha-amylase inhibitors</b>	Alpha-amylase inhibitors block starch digestion.	Development of pea weevil larvae ( <i>Bruchus pisorum</i> ; Coleoptera) was blocked at an early stage after ingestion of transgenic peas expressing an alpha amylase Inhibitor from the common bean ( <i>Phaseolus vulgaris</i> )
<b>Trypsin modulating Ostatic factor (TMOF)</b>	A peptide that blocks trypsin biosynthesis in mosquitoes ( <i>Aedes aegypti</i> ; Diptera [AeaTMOF]) and fleshflies ( <i>Sarcophaga</i> ; Diptera)	Injection or oral ingestion of Aea-TMOF caused inhibition of trypsin biosynthesis and larval growth in <i>H. virescens</i> . Mortality of <i>H. virescens</i> increased when fed transgenic tobacco plants expressing Aea-TMOF (Stevens J <i>et al.</i> , 2012)



**Table 3. Properties of Genetically modified Crops**

Crop	Properties of the genetically modified variety	Modification
<b>Corn</b>	Insect resistance via producing Bt proteins. Added enzyme, alpha amylase, that converts starch into sugar to facilitate ethanol production	New genes, some from the bacterium <i>Bacillus thuringiensis</i> added/transferred into plant genome
<b>Cotton</b>	Kills susceptible insect pests	Gene for one or more Bt crystal proteins transferred into plant genome
<b>Potato</b>	Bt resistance against Colorado beetle and resistance against 2 viruses	New Leaf: gene for one or more Bt crystal proteins transferred into plant genome
<b>Soybeans</b>	Kills susceptible insect pests	Gene for one or more Bt crystal proteins transferred into plant genome
<b>Tomato</b>	Showed resistance to the tobacco hornworm, tomato fruit worm, the tomato pinkworm and the tomato fruit borer	The insecticidal toxin from the bacterium <i>Bacillus thuringiensis</i> has been inserted into a tomato plant
<b>Chickpea</b>	Showed resistance to <i>Helicoverpa armigera</i> army worm	The insecticidal toxin from the bacterium <i>Bacillus thuringiensis</i> have been inserted

## 6. Role of molecular marker

DNA markers tightly linked to the gene of interest can be used at any crop stage for testing the presence of the gene rather waiting to observe its phenotypic manifestations. Simple Sequence Repeats (SSRs) markers are one of the most fundamental applications of the biotic tools. It was found to play a significant role in studying the mode of inheritance of a gene (i.e. whether the gene is homozygous or heretozygous). The microsatellite marker linked to BtCry1Ac resistance trait in *Helicoverpa armigera* pest was identified. Recently, the microsatellite marker linked to BtCry1Ac resistance trait in *Helicoverpa armigera* pest was identified by (Moussa *et al.*, 2005). Also, Identification of mealybug pest species in Egypt and France has been investigated using a DNA barcoding approach (Abd-Rabou *et al.*, 2012) DNA based markers have led to tagging of several plant resistance genes and also mapping of virulence genes and their subsequent cloning for Insect Biotypes.

## 7. Basic Research done in India

- National Botanical Research Institute (NBRI), Lucknow
- National Research Centre on Plant Biotechnology (NRCPB), New Delhi.
- International Centre for Genetic Engineering & Biotechnology (ICGEB, New Delhi).
- Central Institute for Cotton Research, Nagpur.
- National Chemical Laboratory (NCL), Pune.
- Bhabha Atomic Research Centre (BARC), Mumbai.
- University of Agricultural Sciences, Dharwad.
- Many other State Agricultural Universities.

## 8. Biotechnology in Integrated Pest Management

IPM is today a widely accepted strategy to reduce overdependence on chemical insecticides and their potentially negative impact on environment and socio-economic conditions. Biotechnology has considerable potential to contribute towards sustainable biological elements of IPM. Biotechnology development to date has

been directed at more conventional models for pest control technologies. It has enormous potential to improve pest management. (Osir and Gould, 1994; DeVault *et al.* 1996; and Waage, 1996).

Biotechnological research has been now focussed on improving natural enemies of pests as pest control agents. Natural enemies includes bacteria, viruses, fungi, nematodes, predators, etc. We already know about *Bacillus thuringiensis* (Bt), which is widely used as a biopesticidal formulation to control caterpillar and beetle pests of crops, and flies which are disease vectors. Research has focused on increasing the host range and virulence of Bt by combining genes with different host specificities and properties. Emphasis has been done on stabilizing and improving the virulence of these bacteria. Insect viruses also have a market in their natural form as biopesticides, mostly against caterpillar pests of forestry and field crops. Biotechnological research has focused on engineering of certain viruses to express genes whose toxins kill faster than the wild type viruses.

The second principle area of biotechnology for pest control has been the development of crop varieties resistant to pests and diseases. This has concentrated on incorporating insect and virus resistance into the plant genome. In addition, modification of the genome of plant associated microorganisms has been followed as a strategy to confer insect resistance to plants.

### 8.1. Biotechnology in biopesticide development

Today there are over a hundred commercial biological control products on the market, and many more are locally produced and supplied for particular productions systems. However, most commercial biological control have focused on insect pathogens, because of their relative ease of mass production and their capacity to be used in the same manner as formulated chemical insecticides. Bt has been the principle target of product development, and accounts for most sales in the US\$ 75 million global market for biological control products. However, this is only less than one per cent of global

pesticide sales (Waage,1997). As a product, Bt is valuable in IPM systems because it is much less harmful to predators and parasites than broad spectrum chemical insecticides. Therefore, it can be substituted for chemical products in "insecticide treadmill" situations and will allow the recovery of natural enemy populations. A key advantage of biological agents relative to chemical pesticides is their capacity to both kill pests (functional response) and reproduce at the expense of pest (numerical response) thereby giving some control in the future pest generations. *Bt* focused more on maximizing the effect of its insect killing toxin. In other words, its commercial development has focused on using it like a chemical insecticide and not as a living biological control agent.

### 8.2. Biotechnology and crop resistance

Engineering genes for Bt toxins into plants is an ingenious method of delivering these toxins to pests which might naturally avoid them, such as insects which feed inside plants. From an IPM perspective, this technology has more similarities to plant resistance breeding than biopesticide development (Thomas and Waage, 1996). Most resistance breeding to date has focused on methods that result in vertical resistance wherein resistance is based on a single gene. It has gene-for-gene relationship whereby each gene of resistance in the host has a matching gene of parasitic ability in the parasite. Qualitatively, the resistance is either present or absent. This is contrary to horizontal resistance breeding, whereby resistance is based on many genes. Quantitatively, horizontal resistance is exhibited in varying degrees, from minimum to maximum. Vertical resistance is convenient because high levels of resistance can be achieved and the method is compatible with breeding schemes used for enhancing crop performance through control of major genes. However, its gene-for-gene nature, can sometimes lead to its breakdown through the evolution of resistance breaking pest genotypes, as in the case of brown plant hopper on rice. In an IPM context, the single technology solution promised by a high level of vertical resistance is not necessarily desirable if this brings the risk of resistance by the pest. The action of other IPM components like natural enemies can reduce pest populations and hence the rate of evolution of pest resistance. This means that partial resistance, or other forms of resistance like horizontal resistance which is built on the quantitative effect of many genes, can be effective and sustainable. Unfortunately, the tradition of plant breeding and now biotechnology for resistance to pests favours vertical resistance, with its inherent risks. Suggested solutions to resistance problems involve more complex strategies of gene deployment. This includes mixed or intercropped populations of resistant and susceptible plants, or genetic methods to restrict expression of genes to certain parts of plant or certain times. Resistance management is

therefore a strong possibility, but the track record of chemical pesticides is not encouraging.

## 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. Genetic engineering for transferring agronomically useful traits across plant species that cannot be achieved by conventional means in order to reduce insect invasion and increase plant tolerance. 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. 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. 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. Sustainable agriculture could be achieved not only through proper agricultural practices but also through continuous research and development of new technologies, particularly agricultural biotechnology. Responsible national institutes and other affiliated research centres 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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**Cite this article as:** Talukdar Digangana. Modern biotechnological approaches in insect research, *Int. Res. J. of Sci. & Engg.*, 2013; 1(3): 71-78.

**Source of Support:** Nil,

**Conflict of Interest:** None declared