International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 6, Issue1, 2015, pp120-125
http://www.bipublication.com

Review Article

Entomic Resistance Genes for Genetic Engineering in Agricultural Furtherance

Pankaj Kumar[1*] and Meenakshi Sindhu[2]

[1]Department of Biotechnology, Dr YS Parmar University of Horticulture and Forestry,
Nauni Solan, Himachal Pradesh. India.

[2]Centre of Biotechnology, Maharshi Dayanand University Rohtak –124001(Haryana). India

Email: - meenakshisindhu23@gmail.com

*Corresponding author, Email: - pksharmabiotech@gmail. Phone No. - +91-9459786594.

[Received-11/02/2015, Published- 23/02/2015]

ABSTRACT

Genetic engineering for insect pest's management in crop plants offers the potential of a user-friendly, environment-friendly and consumer-friendly method of crop protection to meet the demands of sustainable agriculture. Food and energy insecurities are currently two foremost problems being faced worldwide. Losses due to pests and diseases have been estimated to be around 37% of the agricultural production worldwide, with 13% due to insects. Engineering insect resistance in transgenic plants has been achieved through the use of insect control protein genes of Bacillus thuringiensis. Till now, researchers have focused on the introduction of genes for expression of modified Bacillus thuringiensis (Bt) toxins. Successful results on the control of Bt-susceptible pests have been achieved in the laboratory and finally in the field and now commercialized Bt transgenic crops are used worldwide. Other alternative methods exploit plant-derived insect control genes with promising results. Today insect-resistance transgenes, whether of plant, bacterial or other origin, can be introduced in to plants to increase the level of insect resistance so as to contribute to sustainable agricultural practices.

Keywords— Genetic Engineering, Bacillus thuringiensis, Insect resistance genes, Crop Improvement

[1] INTRODUCTION

The term genetic engineering is used to describe the process by which the genetic makeup of an organism can be altered using "recombinant DNA technology." This involves the use of laboratory tools to insert, alter, or cut pieces of DNA that contain one or more genes of interest. Food and energy insecurities are major threat being faced worldwide. Losses due to pests and diseases have been estimated around 37% of the agricultural production worldwide, with 13% due to insects in economically important crop plants [1]. Genetic engineering technology for insect pest management is an effective approach for crop improvement using insect resistance genes. The

world population is likely to reach by 9 billion by 2050 [2]. In Indian scenario, it is estimated that by 2030, India would be most populated country in the world with a population of 1. 5 billion to be fed [3]. With rapid changes in land use from wild to agricultural to urban and increasing degradation of cultivable land, there is an urgent need to revolutionize the way we do farming and food production. With the increasing demands, longer and healthier life spans, reducing agricultural land, loss of biodiversity and germ plasm and changes in global climate patterns, there is an urgent need and increasing pressure to produce more and there are two ways to fulfill and those are either by increasing the yield and productivity of crops or by reducing losses due to abiotic and biotic reasons. Joining hands with the modern biotechnological tools or plant genetic engineering and development of GM crops is a preliminary and partial solution to the crisis. Today insect-resistance transgenes whether of plant, bacterial or other origin, can be introduced in to plants to increase the level of insect resistance. Insect resistance was first reported in tobacco[4] and tomato[5]. Approximately 40 different genes conferring insect resistance have been incorporated into different crops.

[11]SOURCES OF INSECT RESISTANCE GENES

Various sources of insect resistance genes are: microbial, plant, or animal origin, RNAi approach etc. Insect resistance genes of microbial origin include Insecticidal Crystal Protein- *cry* gene(s) and Vegetative Insecticidal Protein- *vip* gene(s). *Bacillus thuringiensis* (Bt) a common soil bacterium was first isolated in Thuringia region of Germany. Bt produces insecticidal crystal protein-*cry gene(s)* (Table 1) that paralyze the larvae of some harmful insects, including the cotton bollworm and the Asian and European corn borers, all of which are common plant pests whose infestations produce devastating effects on important crops. Mode of action of Bt toxin is that when it is ingested by the larvae of target insect, Bt protein is activated in the gut's alkaline condition and punctures the mid-gut leaving the insect which is not able to feed. The insect dies within a few days (Fig. 1). It is because of its ability to produce the insecticidal protein that much research is being done to exploit the organism's agronomic value. To date, there are more than 200 types of Bt proteins identified with varying degrees of toxicity to some insects. In earlier Bt technology, Bt can be easily cultured by fermentation. Thus, over the last 40 years, Bt has been used as an insecticide by farmers worldwide. Organic farming in particular has been benefited itself from Bt insecticide, as it is one of the very few pesticides permitted by organic standards. The insecticide is applied either as a spray, or as ground applications. It comes in both granules and liquefied form. The efficiency of both applications is quite limited, as target organisms often do not come in contact with the insecticide as they are found on the underside of leaves or have already penetrated into the plant. Scientists are working to overcome this problem through the use of modern biotechnology. In modern Bt technology, Scientists have isolated the Bt gene responsible for the production of the insecticidal protein from the bacterium and incorporated it into the genome of plants. Thus, these plants have a built-in mechanism of protection against targeted pests. The protein produced by the plants does not get washed away, nor is it destroyed by sunlight. The plant is thus protected from the bollworm or the corn borer round the clock regardless of the situation. Sources of *vip* gene(s) are *Bacillus thuringiensis* and *Bacillus ceresus.* More than 50 Vip proteins have been identified. Ingestion of vip proteins cause swelling and disruption of midgut epithelial cells by osmotic lysis in the target insect. There are different types of vip proteins: vip1and vip2 active against coleopteran insects, vip3A, and vip3Aa1and vip3Bb1 active against lepidopteran inscets.

Insect resistance genes of plant origin include *Protease Inhibitor* (PI) gene and *Lectin* gene *etc.* Protease inhibitors act as antimetabolic proteins, which interfere with the digestive process of insects. They inhibit the activity of the gut protease of the insects and reduce the quantity of the proteins that can be digested and also cause hyper production of the digestive enzymes which enhances the loss of sulfur amino acids, as a result of which the insects become weak, with stunted growth and ultimately die (Table 2). The first time use of a plant-derived PI gene was described and transformed tobacco plants with trypsin inhibitor gene (CpTI) from *Vigna unguiculata* were obtained [6]. Regenerated plants expressing CpTI under the control of cauliflower mosaic virus 35S promoter had significantly enhanced resistance to *Heliothis virescens.* *α-Amylase* gene and protein α-amylase inhibitors have been isolated from a variety of plant species and microorganisms. The physiological role of α- amylase inhibitors in plants is uncertain, but there is some evidence that they may act as protein reserve in seeds. α-amylase inhibitors function in a similar manner as proteinase inhibitors, interfering with insect nutrient utilization. When tested in artificial diet, purified α-amylase inhibitors from wheat showed insecticidal effect to coleopteran storage pests *Collosobruchus maculatus* and *Tribolium confusum* [7] (Table 3). Lectins are carbohydrate-binding proteins found in many plant tissues, but are often present in relatively large amounts (usually approximately 1% of total protein, but in some species, e.g. *Phaseolus vulgaris*, up to 30%) in seeds and other storage tissues. Lectins are carbohydrate binding proteins that bind glycans of glycoproteins and glycolipids with high affinity. The toxic effects of lectin are mediated through its binding to the midgut epithilial cell with consequent disruption of the cell function. The bound lectins may inhibit nutrient absorption or disrupt midgut cells by stimulating endocytosis of the lectin and possibly other toxic metabolites present in the midgut (Table 4).

Gene Designation	Molecular weight(KD)	Toxicity
cry IA	131-133	Lepidoptera
(a)(b)(c)		Lepidoptera
IB	137	Lepidoptera
IC	134	Lepidoptera
ID	133	Lepidoptera
IE	137	Lepidoptera
IF	134	Lepidoptera
IG	130	Lepidoptera, Diptera
cry IIA	71	Lepidoptera, Diptera
IIB	71	Lepidoptera, Diptera
IIC	71	Coleoptera
cry IIIA	73	Coleoptera
IIIB	73	Coleoptera
IIIC(a)(b)	73	Diptera
cry IVA	134	Diptera
IVB	128	Diptera
IVC	77	Diptera
IVD	72	Lepidoptera,
cry V	80	Coleoptera

Table 1: *Bacillus thuringinesis* Crystal Protein Genes classification (Source: Hofte and Whiteley, 1989)

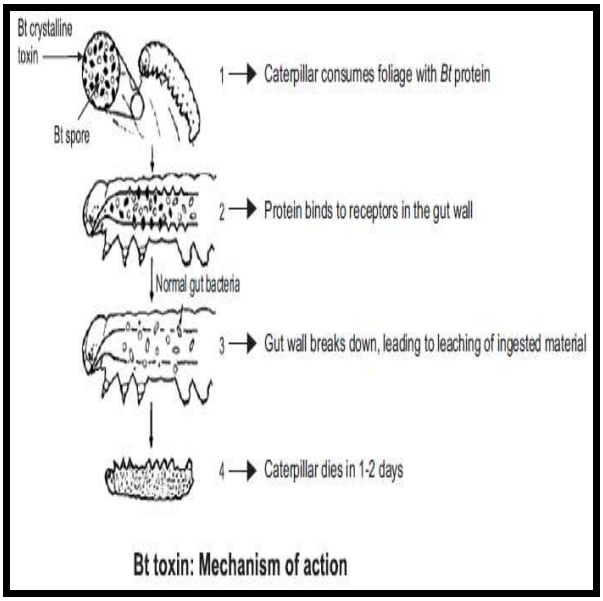

Fig1. Mechanism of action of Bt (*Bacillus thuringinesis*) toxin

Gene Source/ Gene	Effect Evaluated on Predators
Cowpea trypsin inhibitor (CpTI)	Coleoptera,
Tomato proteinase inhibitor-I	Lepidoptera
Tomato proteinase inhibitor-I I	Lepidoptera
Potato proteinase inhibitor-I (Pot-PT-I)	Lepidoptera
Potato proteinase inhibitor –II (Pot-PT-II)	Lepidoptera, Orthoptera
Rice cysteine proteinase inhibitor (OC-1)	Lepidoptera, Orthoptera
Soyabean Kunitz Trypsin , inhibitor (SKT-1)	Coleoptera , Homoptera
Barley Trypsin inhibitor (CMe)	Lepidoptera
Mustard serine proteinase inhibitor(MTI-2)	Lepidoptera
Soybean serine proteinase inhibitor (PI-IV)	Lepidoptera

Table 2: A few Protease Inhibitor Genes

Plant Gene	Encoded Protein	Plant of Origin
α-AI-Pv	α-amylase	Common
WMAI-1	α-amylase	bean
14K-CI	Serine and α-amylase inhibitor	Cereals Cereals

Table 3: A few α-Amylase Genes

Various lectins have been proved toxic towards members of Coleoptera, Lepidoptera [8] and Diptera [9]. Most importantly, lectins can be used to control sap-sucking insects belonging to the order Homoptera, which includes some of the most devastating pests spread worldwide. Gene *ASAC – Allium cepa* agglutinin gene from garlic was transferred to rice by *Agrobacterium* mediated gene transfer. Gene transfer and integration was confirmed by PCR, Southern, Northern, Western Blot & ELISA analysis. In plant bioassay, significant resistance was observed against Sap Sucking Plant Looper as compared to Control.

Plant Gene	Encoded Protein	Plant of Origin
GNA (Galanthus nivalis agglutinin)	Lectin	Snowdrop
p-lec	Lectin	Pea
WGA Agglutinin (Wheat germ agglutinin)	Lectin	Wheat germ
Jacalin	Lectin	Jack fruit
Rice lectin	Lectin	Rice

Table 4: Examples of Lectin Genes

Insect resistance gene(s) of animal origin include, *Proteinase Inhibitors* (*Anti-chymotrypsin* from *Manduca sexta*, *Anti-elastase* from *Manduca sexta*), *α-Antitrypsin* (α_1*AT*)(*Antitrypsin* from *Manduca sexta*, *Bovine pancreatic trypsin inhibitor (BPTI)/pancreatic, Spleen inhibitor (SI)*), *Chitinase* (*Chitinase* from *Manduca sexta*). It is known that over expression of protease inhibitors (PIs) can protect plants against some insect species [10]. Thomas *et al*., (1995) expressed insect encoded *anti-trypsin, anti-chymotrypsin and anti-elastase* protease inhibitor genes from *Manduca sexta* in transgenic tobacco [11]. When transgenic plants were tested against sweet potato whitefly type B, *Bemisia tabaci*, insect reproduction was reduced by as much as 98% compared to controls. This result suggests that *M. sexta-derived PI* may be useful in protecting crop plants against insects. Chitinase expression normally occurs in insects during molting when insects shed their old exoskeleton and peritrophic membrane (both contain chitin as major component) and re synthesize new ones. Thus, insect feedings on plants that constitutively express an insect chitinase gene might be adversely affected. Ding *et al*., (1998) showed that transgenic tobacco plants expressing insect (*Manduca sexta*) chitinase transgene have enhanced resistance to budworm (*Heliothis virescens*) larvae, even if the mechanism of *Manduca chitinase*-mediated resistance is unknown. Avidin is a glycoprotein from chicken (*Gallus gallus*) egg white that binds its ligand, biotin, with very high affinity. Biotin is a coenzyme required for all forms of life, so

feeding avidin to many insects causes a biotin deficiency that leads to a stunted growth and mortality [12]. Morgan *et al.,* (1993) reported that avidin is toxic to seven species of stored product beetles (Coleoptera) and moths (Lepidoptera). It was shown that avidin is also toxic to housefly (*Musa domestica*) [13]. Kramer *et al*., (2000) reported that when expressed in transgenic maize at levels of ≥100 ppm, avidin is toxic to and prevents development of insects that damage grains during storage (*Sitophilus zeamais*, *Sitotroga cerealella*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *etc* [14]. Avidin expressed in transgenic tobacco and apple conferred a high level of insect resistance to potato tuber moth, *Phthorimaea operculella* and lightbrown apple moth, *Epiphyas postvittana*, respectively.

[III] BT TECHNOLOGY: CURRENT STATUS

According to James Clive (2013), an estimated 28.8 million hectares of land were planted with crops containing the Bt gene by the end of 2013. For the first 17 years of commercialization (1996-2012), benefits from insect resistant crops are valued at US$68.9 billion, 60% of the global value of biotech crops of US$116.9 billion; and for 2012 alone at US$12 billion, 64% of the global value of biotech crops of US$18.7 (Table 5) [15].

Bt crop	Country
Cotton	Argentina, Australia, Brazil, Burkina Faso, Canada, China, Colombia, Costa Rica, European Union (EU), India, Japan, Mexico, Myanmar, New Zealand, Pakistan, Paraguay, Philippines, Singapore, South Africa, South Korea, United States of America (USA)
Eggplant	Bangladesh
Maize	Argentina, Australia, Brazil, Canada, Chile, China, Colombia, Egypt, El Salvador, EU, Honduras, Indonesia, Japan, Malaysia, Mexico, New Zealand, Panama, Paraguay, Philippines, Russian Federation, Singapore, South Africa, South Korea, Switzerland, Taiwan, Thailand, Turkey, USA, Uruguay
Poplar	China
Potato	Australia, Canada, Japan, Mexico, New Zealand, Philippines, Russian Federation, South Korea, USA
Rice	China, Iran
Soybean	Argentina, Australia, Brazil, Canada, China, Colombia, EU, Japan, Mexico, New Zealand, Paraguay, South Korea, Taiwan, Thailand, USA, Uruguay
Tomato	Canada, Chile, USA

Table 5: Countries that commercialized Bt crops and its products, from 1996 to 2013
(Source: ISAAA GM Approval Database. http://www.isaaa.org/gmapprovaldatabase)

[IV]CONCLUSION

With the advent of modern biotechnology, newer tools permitting gene transfer across the species opened an avenue for solving the age old problem. There is an urgent need of joining hands with the modern biotechnological tools and development of GM crops is a preliminary and partial solution to increase the yield and productivity of crops, reducing losses due to abiotic and biotic reasons. Genetic engineering opened the possibility to use insecticidal genes from different origin for breeding resistant plants. So far, only cry genes from *Bacillus thuringiensis* have been commercially exploited for this purpose, however many other genes of bacterial, plant, or other origin are successfully being used to confer insect resistance. In the future, special attention should be paid to produce insect resistant transgenic plants that will hinder the development of insect resistance to the recombinantly expressed antimetabolites or toxins. A possible way to achieve this is the stacking of two or more insecticidal genes with different mode of action into the same plant. Although a great amount of work has been done in the field of transgenic, yet much more is yet to be explored when it comes to studying the effect of transgenic products on human health and biodiversity. More detailed field studies should be planned and executed with the

help of allied sciences. A collaborative work with positive approach is required in order to harness the true potential of transgenic for the benefit of mankind.

[V]REFERENCES

1. Gatehouse, A. M. R., Hilder, V. A., Boulter, D. (1992): Potential of plant-derived genes in the genetic manipulation of the crops for insect resistance, in: Biotechnology in Agriculture N° 7: Plant genetic manipulation for crop protection, CAB International, pp.155-181.
2. Varshney RK, Bansal KC, Aggarwal PK, Datta SK and Craufurd PQ (2011). Agricultural biotechnology for crop improvement in a variable climate: hope or hype? *Trends in Plant Science* 16 363-71.
3. Jagadish MN (2012). Indian farmers need help to feed over 1.5 billion people in 2030. *GM Crops and Food* 3 89-92.
4. Vaeck, M. *et al*., (1987).Transgenic plants protected from insect attack. Nature 328, 33-37
5. Fischoff, *D. A. et al.,* (1987), Insect Tolerant Transgenic Tomato Plants. *Nature Biotechnology* 5, 807 – 813
6. Hilder, V. A., Gatehouse, A. M. R., Sheerman, S. E., Barker, R. F., Boulter, D. (1987): A novel mechanism of insect resistance engineered into tobacco. *Nature* 330: 160-163.
7. Gatehouse, A. M. R., Fenton, K. A., Jepson, I., Pavey, D. J. (1986): The effects of α-amylase inhibitors on insect storage pests: inhibition of α-amylase *in vitro effects on development in vitro. J. Sci. Food Agric. 35: 373-380.*
8. Czalpa, T. H., Lang, B. A. (1990): Effect of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and Southern corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol*. 83: 2480-2485.
9. Eisemann, C. H., Donaldson, R. A., Pearson, R. D., Cadagon, L. C., Vuocolo, T., Tellam, R. L. (1994): Larvicidal activity of lectins on Lucilia cuprina: Mechanism of action. *Entomol. Exp. Appl*. 72: 1-11.
10. Wolfson, J. L., Murdock, L. L. (1987): Suppression of larval Colorado potato beetle growth and development by digestive proteinase inhibitors. *Entomol. Exp. Appl*. 44: 235-240.
11. Thomas, J. C., Adams, D. G., Keppenne, V. D., Wasmann, C. C., Brown, J. K., Kanost, M. R., Bohnert, H. J. (1995): Miuca sexta encoded protease inhibitors expressed in Nicotiana tabacum provide protection against insects. *Plant Physiol. Bioch.* 33: 611-614.
12. Ding, X., Gopalakrishnan, B., Johnson, L. B., White, F. F., Wang, X., Morgan, T. D., Kramer, K. J., Muthukrishnan, S. (1998): Insect resistance of transgenic tobacco expressing an insect chitinase gene. Transgenic Res. 7: 77-84.
13. Kramer, K. J., Morgan, T. D., Throne, J. E., Dowell, F. E., Bailey, M., Howard, J. A. (2000): Transgenetic avidin maize is resistant to storage insect pests. *Nat. Biotechnol.* 18: 670-674.
14. James, Clive. 2013. Global Status of Commercialized Biotech/GM Crops: 2013. *ISAAA Brief* No. 46. ISAAA: Ithaca, NY.
15. *http://www.isaaa.org/gmapprovaldatabase/*
16. Höfte H, Whiteley HR. 1989. Insecticidal crystal proteins of Bacillus thuringiensis. *Microbiol Rev.* 53(2):242-55.