

## Insecticidal genes in Pest Management

Lipsa Dash<sup>1</sup>, \*Sandeep Rout<sup>2</sup>, UditNandan Mishra<sup>2</sup>, Gyanaranjan Sahoo<sup>3</sup> and Ajay Kumar Prusty<sup>1</sup>

<sup>1</sup>M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Odisha, India.

<sup>2</sup>Faculty of Agriculture, Sri Sri University, Cuttack, Odisha, India

<sup>3</sup>KrishiVigyan Kendra, Odisha University of Agriculture &Technology, Angul, Odisha, India

\*Email: sandeeprou1988@gmail.com

### ABSTRACT

Sessile plants face a variety of abiotic stresses, making them vulnerable to insect pests. Successful crop establishments can only be assured with effective eco-friendly management practices. Unfortunately, most of these practices harness natural resources, but recently, genetic engineering has emerged as a viable alternative against plant insect pests. Insecticidal protein in bacteria which express constitutively once introgressed through r-DNA technology have shown efficacy against lepidopteran and coleopteran pests. Indigenous expression of plant secondary metabolites like flavonoids shows negative effects on insect pest reproduction. Genetic engineering of plants expressing insect cell wall (containing structural polysaccharides like chitin) degrading enzymes showed efficacy of insecticidal potential. The basic principle behind the digestion of ingested food is to hydrolyze it with an enzyme. Such gut enzymes of insects get attenuated when they come across the enzyme inhibitors (such as protease inhibitors, alpha-amylase inhibitors etc) present in the fed portion of the plants. All of these genes encoding insecticidal compounds are abundant in nature. This review intends to summarize the progress made in this area using genetic engineering to successfully come up with transgenes against plant insect pests.

**Keywords:** Enzyme inhibitors, genetic engineering, r-DNA technology, transgenes.

### Introduction

The most serious limiting factor in crop yield is pest infestation, which causes severe damage to crop plants. Different classes of proteins and secondary metabolites have evolved in plants as effective countermeasures against herbivorous insects over time, and the utilisation of these natural plant defence mechanisms as a tool for genetic engineering for crop pest management is currently an achievement. Hence different genes coding proteins and secondary metabolites have been isolated for developing traits showing pest resistance.

Genes of any living source when incorporated and expressed in a plant produce several products which have insecticidal property or growth and developmental impediment property against the phytophagous insects are called insecticidal genes. Such genes are available in plants and animals. After isolation, these genes are incorporated in to a crop

genetic background such that the transferred genes express themselves constitutively to produce several proteins with toxic properties [27].

### Sources of insecticidal genes

#### 1. Bacterial Sources

- ❖ Bt toxins attach to glycoprotein receptors on the cell membrane of midgut cells,
- ❖ causing pores in the membrane to open, disrupting cellular osmotic equilibrium and causing cells to expand and lyse in a process known as colloidal lysis.
- ❖ Ion leakage from the midgut to the haemolymph causes an ionic imbalance in the haemolymph, resulting in septicaemia.
- ❖ Insects stop eating ultimately leading to death.

**Table 1. Bacterial gene as insecticide**

Crop	Foreign gene	Origin of insecticidal genes	Target pests	References
Potato	<i>CryIAb</i>	<i>B. thuringiensis</i>	<i>Pthorimaeaoperculella</i>	Kunitzetal., 2007 [1]
Sugarcane	<i>CryIAb</i>	<i>B. thuringiensis</i>	<i>Diatreasaccharalis</i>	Kunitzetal., 2007 [1]
Tobacco	<i>CryIAb</i>	<i>B. thuringiensis</i>	<i>Heliothisvirescens</i>	Kunitzetal., 2007 [1]
Tomato	<i>CryIAc</i>	<i>B. thuringiensis</i>	<i>Manducasexta</i>	Kunitzetal., 2007 [1]
	<i>Bt(k)</i>	<i>B. thuringiensis</i>	<i>ManducasextaHelicoverpazea</i>	Klümper et al., 2014[2]
Brinjal	<i>CryIIIb</i>	<i>B. thuringiensis</i>	<i>Leptinotarsadecemlineata</i>	Klümper et al., 2014[2]
Cotton	<i>CryIAb, cryIAc, cryIIAb</i>	<i>B. thuringiensis</i>	<i>Helicoverpazea, Spodopteraexigua</i>	Gatehouse et al., 2013[3]
Maize	<i>CryIAb</i>	<i>B. thuringiensis</i>	<i>Ostrinianubilalis</i>	Gatehouse et al., 2013[3]
Tobacco	Sip toxin	<i>B. thuringiensis</i>	<i>Manducasexta</i>	Palma et al., 2017[4]

#### 2. Plant metabolites

##### ❖ Flavonoids

They behave like an antifeedant and also affect the insect through antibiosis, thus regulating insect growth and reproduction [5].

**Table 2. Plant secondary metabolite gene as insecticide**

Crop	Name of the compound	Insecticidal gene	Origin of insecticidal gene	Target Pest	References
------	----------------------	-------------------	-----------------------------	-------------	------------

Tobacco	Anthocyanins and O-Glycosylated flavonols	UGT gene ( <i>CsUGT72AM1</i> )	Purple-leaf tea variety, 'Moomal'	<i>Spodopteralitura</i>	Xiujuanet <i>al.</i> , 2018[6]
Banana	Phenylphenalenones		Bluggoe ( <i>Musa acuminata</i> × <i>balbiana</i> )	Banana weevil	Hölscheret <i>al.</i> , 2016[7]

### 3. Enzymes

- Another alternative to Bt genes has been suggested: transgenic expression of certain enzymes. Chitinase, a key component of insect integument, is one of the most significant enzymes.
- The resistance of transgenic tobacco plants producing chitinase against lepidopteran insects has enhanced [8].
- *Serratiamarcesens*chitinase has been discovered to work in tandem with Bt toxin against *S. littoralis* [9].
- The bacterial isopentenyltransferase (*ipt*) gene, which is necessary for cytokinin production, was found to be efficient against *Manducasexta* when combined with a promoter from the proteinase inhibitor II (PI-IIK) gene and introduced into *Nicotianaplumbaginifolia* [10].

### 4. Plant derived genes

#### ❖ Alpha-amylase inhibitors

- In plants alpha amylase inhibitors are held in seeds and tubers towards the finish of life in extensive sums.
- Alpha amylase is such a catalyst that catalyzes the endolysis of 1-4 connected glucose polymers, which leads to hydrolytic items with alpha setup.
- They have an impact in safeguard by restraining an assortment of alpha amylases from different sources by shaping irreversible edifices with them. Thus it influences absorption of sugars in bugs.

**Table 3. Plant derived gene as insecticide**

Crop	Insecticidal genes	Origin of insecticidal genes	Target pests	References
Tobacco	WAAI gene	Wheat	Lepidopteran larvae	Carboneroet <i>al.</i> , 1993[11]
Pea	BAAI gene	Bean	<i>Callosobruchus</i>	Shade <i>et al.</i> , 1994[12]
Tobacco	AlphaAI-Pc1	Bean ( <i>Phaseoluscoccineus</i> )	Lepidopteran larvae	De Azevedoet <i>al.</i> , 2006[13]

#### ❖ Proteases inhibitors

- They are found in leguminosae, gramineae and solanaceae families.
- The impacts of PIs on defenseless bugs are for the most part seen as an expansion in mortality, decline in development rate and prolongation of larval formative period [3].
- A foundational arrangement of PIs is absurd because of varieties as far as source, construction, specificities and size. Anyway they are gathered into 4 explicit gatherings Serine, Cysteine, Metallo and aspartic protease inhibitors. Among them the Potato inhibitor 1 and 11 families, the Bowman-Birk inhibitor (BBI) families and the soybean trypsin inhibitor family are generally significant.

**Table 4. Protease Inhibitor (PI) as insecticide**

Crop	Insecticidal genes	Origin of insecticidal genes	Target Pest	References
Tobacco	Tainong 57 trypsin inhibitor gene	Sweet potato	<i>Spodoptera litura</i>	Yehet <i>et al.</i> , 1997[14]
Rice	Kunitz trypsin inhibitor (SKTI) gene	Soybean	<i>Nilaparvatalugens</i>	Lee <i>et al.</i> , 1999[15]
Rice	Cowpea trypsin inhibitor (CpTi) gene	Cowpea	<i>C. suppressalis</i> and <i>Sesamia inferens</i>	Xuet <i>et al.</i> , 1996[16]
oilseed rape	Oryzacystatin I (OCI)	Rice	<i>Psylliodeschrysocephala</i>	Girard <i>et al.</i> , 1998[17]
<i>Brassica oleracea</i> var. <i>capitata</i>	Cp-Ti	Cowpea	<i>P. rapae</i>	Fang <i>et al.</i> , 1997[18]
Sugarcane	Soybean Bowman-Birk trypsin inhibitor	Soybean	<i>Diatraea saccharalis</i>	Xuet <i>et al.</i> , 1996[16]

#### ❖ Lectins

- Lectins are defined as proteins that reversibly bind to specific carbohydrates.
- They are heterogenous gathering of proteins vary from one another regarding their atomic design, carb restricting particularity and natural exercises (Esteban *et al.*, 2006).
- Lectins apply their inhibitory impact by restricting to glycoproteins implanted in peritropic matrix coating the bug midgut and in turn disturbing the absorption interaction.

- Lectins from snowdrop, pea, wheat, rice, castor, soybean, mungbean, garlic, yam, tobacco, chickpea and groundnut have been detached and described.

**Table 5. Carbohydrate binding protein (lectin) as insecticide**

Crop	Transgene	Origin of transgene	Target insect pests	References
Rice	GNA	Snowdrop	<i>Nilaparvatalugens</i> <i>Nephotettix</i> <i>virescens</i>	Yang <i>et al.</i> , 2018[19]
	GNA	Snowdrop and Spider	<i>N.lugens</i>	Yang <i>et al.</i> , 2018[19]
Wheat	GNA	Snowdrop	<i>Sitobionavenae</i>	Stogeret <i>al.</i> ,2015 [20]
Potato	GNA	Snowdrop	<i>Myzuspersicae</i>	Stogeret <i>al.</i> ,2015 [20]
Mustard	WGA	Wheat	<i>Lipaphiserysimi</i>	Stogeret <i>al.</i> ,2015 [20]
Potato	ConA	Tomato	Potato aphid	Cao <i>et al.</i> , 2015[21]

### Crops that have been genetically manipulated

GM crops, often known as biotech crops, are agricultural plants whose DNA has been altered using genetic engineering techniques. In each scenario, the goal is to introduce a novel gene into the plant that does not occur naturally. It provides resistance to specific insect pests, illnesses, environmental circumstances (e.g. herbicide resistance), or improves the nutrition profile of cultivated food crops.

GM technology has been widely adopted by farmers. From 1.7 million hectares in 1996 to 185.1 million hectares in 2016, worldwide cropland rose by 12 percent. Herbicide tolerance (95.9 million hectares), insect resistance (25.2 million hectares), or both (58.5 million hectares) are the most common features in main crops (soybean, maize, canola, and cotton) as of 2016 [22].

28 types of insect pests and parasites have been accounted for to be related with brinjal. Among these the brinjal shoot and natural product drill has been accounted for to be the genuine nuisance which lessens the harvest yield up to 60-70% and delivers the huge misfortune underway. Other than brinjal, it was likewise found to assault shoots and products of tomato, potato (*Solanum tuberosum* L.), green peas (*Pisum sativum* L.) and *Solanum torvum* Swartz. The brinjal leafy foods drill is dynamic during blustery and summer seasons and frequently causes over 90% harm [23].

Considering the significance of the brinjal and the severity of the problem with the brinjal shoot and fruit borer, management approaches are entirely reliant on chemical pesticides [24]. Continuous usage of synthetic chemicals pollutes the environment and causes hazardous residues to accumulate in the body [25]. The pesticides carbaryl, chlorpyrifos, deltamethrin, endosulfan, fenvalerate, and profenofos were discovered to have extensive resistance in the populations of brinjal shoot and fruit borer [26]. As a result, alternative pest management tactics in brinjal pest control programmes are urgently needed.

### **Brinjal that has been genetically engineered (Bt-brinjal)**

The genetically modified brinjal (also known as an eggplant or aubergine) is a group of transgenic brinjals generated by introducing the crystal protein gene (Cry1Ac) from the soil bacteria *Bacillus thuringiensis* into the genomes of various brinjal cultivars. Agrobacterium-mediated genetic transformation is used to insert the gene, as well as other genetic components such as promoters, terminators, and an antibiotic resistance marker gene, into the brinjal plant.

Btbrinjal was created to provide resistance to lepidopteran insects, specifically the Brinjal Fruit and Shoot Borer, *Leucinodes orbonalis*. In 2013, Bangladesh approved the commercial distribution of Btbrinjal.

### **Genetically modified tomato (Bt-tomato)**

A GM tomato is a tomato variety with a genetically modified gene. FlavrSavr, a tomato developed to have a longer shelf life, was the first trial genetically modified product, but it never made it to market. There are currently no GM tomatoes on the market, but scientists are working on creating tomatoes with new features such as greater insect pest resistance.

The insecticidal poison from the bacterium *Bacillus thuringiensis* has been embedded into a tomato plant. At the point when attempted under field condition, they showed protection from the tobacco hornworm (*Manduca sexta*), tomato fruitworm (*Heliothis zea*), the tomato pinworm (*Keiferia copersicella*) and the tomato natural product drill (*Helicoverpa armigera*). Root tie nematode safe tomato has been made by embedding a cysteine proteinase inhibitor quality from taro. Nuisance safe tomatoes can diminish the environmental impression of tomato creation while simultaneously increment farm income.

### **Genetically modified Maize**

A genetically modified crop is GM maize. Specific maize strains have been genetically modified to display agriculturally desired features such as insect and herbicide tolerance. Multiple countries are now using maize strains with both features.

Bt corn is a variation of maize that has been hereditarily changed to communicate at least one proteins from the bacterium *Bacillus thuringiensis* including Delta endotoxins. The protein is noxious to some bug bugs. Spores of the Bt is broadly utilized in natural cultivating, however GM maize isn't natural. The European corn drill makes colossal harm

corn crops each year. GM Corn has been effective in controlling bugs like European corn drill.

## Conclusion

Pesticides used indiscriminately have resulted in insect resistance and recurrence difficulties. Safer alternative options for effective insect pest management include the use of particular chemicals with minimal persistence and the exploitation of natural plant resistance mechanisms. Accordingly, insect resistant GM plants will lessen the utilization of those risky pesticides by consolidating qualities that encode common biodegradable proteins with no destructive impact to creatures and people. Notwithstanding, the resistance from the counter GM developments everywhere on the world, without a doubt, will track down its more and quick application in creepy crawly bug control of significant harvests. The accessibility of various insecticidal qualities with well similarity makes it conceivable to utilize those qualities in a blend for better nuisance opposition. The transgenic crops created for bother obstruction ought to be viable with different acts of IPM to be strong and practical. In the event that all around evaluated, it will immensely affect the farming usefulness in not so distant future both in creating and created nations.

## References

1. Kunitz, S.K. and Lee, S.H. (2007). Soybean trypsin inhibitor confers resistance to the brown plant hopper in transgenic rice. *Molecular breeding*, 5: 1-9.
2. Klumper, J.Z., Shi, X.P., Fan, X.L., Zhang, C.Y. and Zhao, R.M.(2014). Insecticidal activity of transgenic tobacco co-expressing Bt and CpTi genes on *Helicoverpa armigera* and its role in delaying the development of pest resistance. *Rice Biotechnology Quarterly*, 34: 9-10.
3. Gatehouse, L.N. (2013). Novel genes for insect resistance in transgenic plants. Ph.D thesis Durham University. UK. 95-97.
4. Palma, J.I. and Goldsbrough, A.P. (2017). Transformation systems for generating marker free transgenic plants. *Biotechnology*. 12: 263-267.
5. War, D.F. (2013). Biological control of insect pests: Southeast asian prospects. Australian Centre for International Agricultural Research. Canberra.
6. Xiujuan, M., Kachlicki. P., Stobiecki, M.(2018). Isolation and characterisation of key genes that promote flavonoid accumulation in purple leaf tea. *Plant Physiology and Biochemistry*. 47: 847-853.
7. Holscher, A., Yakushiji, H. and Kobayashi, S. (2016). Flavonoid Phenylphenalaceous accumulate in plant tissues of two banana cultivars in response to herbivory by banana weevil and banana stem weevil. *Planta*. 236: 1067-1080.
8. Ding, Azevedo., Li, X. and Wu, R. (2018). Transgenic rice plants harbouring an introduced potato proteinase inhibitor II gene for insect resistance. *Nature Biotechnology*. 14: 494-498.
9. Rigevev, A.K. and Menn, J.J.(1993). Pheromone Biosynthesis Activating Neuropeptide: from discovery to current status, *Archives of insect Biochemistry and Physiology*. 22: 141-151.
10. Smigoki, A.M. (2010). The long road to commercialization of Btbrinjal in India, *Crop Protection*. 29: 412-415.

11. Carbonero, P., Royo, J. and Casanera, P. (1993). Cereal inhibitors of insect hydrolases: genetic control, transgenic expression and insect pests. In: Workshop on engineering plants against pests and pathogens. 1-13, Jan 1993.
12. Shade, R.E., Pueyo, J.J., Tabe, L.M. and Murdock, L.L. (1994). Transgenic pea seeds expressing alpha amylase inhibitor of the prospects for common bean resistant to bruchid beetles.*Biotechnology*, 12: 793- 796.
13. De, Azevedo., Li, X. and LI, YH. (2006). An insect resistant transgenic cabbage plant with the cowpea trypsin inhibitor gene.*Acta Botanica Sinica*. 39: 940-945.
14. Yeh, K.W., Lin, M.L. and Kao, S.S. (1997). Sweet potato trypsin inhibitor expressed in transgenic tobacco plants confer resistance against *Spodopteralitura*, *Plant Reports*, 16: 696-699.
15. Lee, S.I., Hofte, H. and Whiteley, H.R. (1999). Insecticidal crystal proteins of *Bacillus thuringiensis*.*Microbiology Review*. 53: 242-55.
16. Xu, D.P., Xue, Q.Z. and Wu, R. (1996). Constitutive expression of a cowpea trypsin inhibitor gene in transgenic rice plants confer resistance to two major rice insect pests.*Molecular Breeding*. 2: 167- 173.
17. Girard, C., Le- Metayer, M., Ouanin, L. (1998). Growth stimulation of beetle larvae reared on a transgenic oilseed rape expressing a cysteine proteinase inhibitor.*Journal of Insect Physiology*. 44: 263-270.
18. Fang, H.J., Li, D.L. and Li, Y.H. (1997). An insect resistant transgenic cabbage plant with the cowpea trypsin inhibitor gene.*ActaBotanicaSinica*. 39:940-945.
19. Yang, M., Kachkicki, P. and Stobiecki, M. (2018). Isolation and characterization of key genes that promote flavonoid accumulation in purple leaf tea.*Plant Physiology and Biochemistry*. 47: 847-853.
20. Stoger, J., Jansens, S. and Meller, H. (2015). Specificity of *Bacillus thuringiensis*-endotoxins. Importance of specific receptors on the brush border membrane of the midgut of target insects.*European Journal of Biochemistry*. 186: 239-247.
21. Cao, A. and Leblanc, S. (2015). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Township of Quebec, *Reproductive Toxicology*. 31(4): 123-125.
22. Klumper, J.Z., Shi, X.P. and Fan, Y.L. (2014). Insecticidal activity of transgenic tobacco coexpressing Bt and CpTi genes on *Helicoverpa armigera* and its role in delaying pest resistance.*Rice Biotechnology Quarterly*. 34: 9-10.
23. Ali, M.A., Kameswara, R.P. and Krishnamurthy, R. (1980). Chemical control of brinjal shoot and fruit borer, *Leucinodes orbonalis* with newer insecticides.*Entomon*. 7: 133-135.
24. Kabir, K.H., Baksh, M.E. and Ahmed, A. (1996). Insecticides usage pattern on vegetable at farmer level in Bangladesh.*Bangladesh journal of Agricultural Research*. 21(1):251-253.
25. Dadmal, S.K. and Senapati, S.K. (2004). Estimation of loss in yield of brinjal due to pest complex under Tarai region of West Bengal.*Environmental Ecology*. 21: 764-769.
26. Shirale, D., Patil, M. and Parimi, S. (2017). Insecticide resistance in field population of *Leucinodes orbonalis* in India.*Canadian Journal of Plant Science*. 149(3): 399-407.
27. V.Ramalakshmi., Lipsa, Dash., Deepayan, Padhy., Sandeep, Rout. and Barsha, Tripathy.(2020). Role of Semiochemicals in Pest Management. Agriculture and Forestry: Current Trends, Perspectives, Issues – I. Immortal publications.209-228 pp. ISBN No: 978-93-5426-676-8.