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Role of Biotechnology in Plant Protection

Shamik Dey and Ishanu Mandal

School of Agricultural Sciences,
JIS University,
Kolkata.

Abstract:

Biotechnology is the emerging technological tool which contributes an immense role in every sector of modern Agriculture. Biotechnological approach in plant protection is highly appreciated in terms of application of Bt technology, Recombinant DNA technology, Gene Pyramiding, RNA interference, Sterile Insect Technique which manage the insect pest population in an eco-friendly way. Indiscriminate application of pesticides shows the major threat to the sustainability of environment which can be addressed through biotechnological method in a convenient way.

Keywords:

Antixenosis, Bacillus thuringiensis, DNA, RNA, Resistance.

8.1 Introduction:

Biotechnology is an innovation that uses natural frameworks, living beings, or portions of this to create or to produce new items for use in different clinical, agricultural, drug and ecological applications. Biotechnology grew quickly in view of the additional opportunity to make changes in the organic entities' hereditary material (DNA). A significant methodology in expanding crop yield is the improvement of insect the board regimens comprising of herbicide-resistance (HR) and insect resistance (IR) crops just as transgenic.

Insect biotechnological applications to bother the executives incorporate the improvement of safe production and trees that express pest explicit poisons, the plan of microbial specialists with upgraded insecticidal intensity and the designing of insect that can move deadly qualities to normal populaces following their mass delivery in the field. Insect biotechnology is otherwise called yellow biotechnology Relative genomics examinations likewise make it conceivable to recognize insect explicit qualities that can be focused on for judicious insect spray plan. Even more explicitly, insect biotechnology acquires from and is driven by logical forward leaps in sub-atomic science, especially by the improvement of instruments and procedures that permit hereditary portrayal and designing of life forms and cells likewise with recombinant DNA technology. Under the changing climate scenario, the world's population is estimated to increase by 2 billion in the next 30 years, rising from the current 7.7 billion populations to 10 billion by 2050 (Zsögön et al., 2022). In this context, there is a continuous need for an increment of food production to fulfil the need of the rising worldwide population. Additionally, it is assessed that total global food demand will increase from 35% in 2010 to 56% in 2050 (van Dijk et al., 2021). To meet these goals, it is critical to increase crop yield and reduce pre- and post-harvest losses.

Three types of resistance determine the relationship between the insect and the plant, e.g. antibiosis, antixenosis (non-preference), and tolerance (Koch et al., 2016) The development of insect-resistant plants began in 1782 when Havens published an article on a Hessian fly-resistant wheat cultivar. Since that time, several insect-resistant cultivars have been developed by the international and national research centers, the private sector using conventional or biotechnological tools. Therefore, the main objective of this review is to assess the opportunities provided by these new biotechnological tools in developing various crop plants that are resistant to a wide range of insect pests.

8.2 Biotechnology:

Biotechnology is a multidisciplinary field that involves the integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services. The term biotechnology was first used by Károly Ereky in 1919, to refer to the production of products from raw materials with the aid of living organisms.

The core principle of biotechnology involves harnessing biological systems and organisms, such as bacteria, yeast, and plants, to perform specific tasks or produce valuable substances. Biotechnology had a significant impact on many areas of society, from medicine to agriculture to environmental science. One of the key techniques used in biotechnology is genetic engineering, which allows scientists to modify the genetic makeup of organisms to achieve desired outcomes. This can involve inserting genes from one organism into another, creating new traits or modifying existing ones. Biotechnology is a rapidly evolving field with significant potential to address pressing global challenges and improve the quality of life for people around the world; however, despite its numerous benefits, it also poses ethical and societal challenges, such as questions around genetic modification and intellectual property rights. As a result, there is ongoing debate and regulation surrounding the use and application of biotechnology in various industries and fields.

8.2.1 Biotechnology in Agriculture:

The large increase in the size of a population has led to an increasing demand for resources and basic requirements such as food, shelter, clothing, etc. Another impact of the increase in population is the exploitation of the land for crops production. Thus, cultivation has been limited to a small area. In order to meet the demands with limited resources, we need to apply a great effort. Biotechnology in agriculture has changed the face of this condition. Biotechnology is the use of technology to modify or manipulate any biological system or living system for the development or improvement of products for various purposes.

It is widely employed in different fields and agriculture is one among them. Researchers have suggested different options for increasing food production. Genetically engineered crop-based agriculture is an option, others being agrochemical-based agriculture and organic agriculture. The green revolution was an initiation for increasing food production, but it couldn’t meet the growing demands. Later the idea of crop variety improvement program was put forward. However, the agrochemicals seem to be unfeasible for farmers. In addition, the environmental issues related to them also reduced their use.

8.3 Recombinant DNA (RDNA) Technology:

Hereditary designing is the most common way of utilizing recombinant DNA (rDNA) innovation to modify the hereditary cosmetics of a life form. Generally, people have controlled genomes by implication by controlling reproducing and choosing posterity with wanted characteristics.

Hereditary designing includes the immediate control of at least one quality. Frequently, a quality from one more animal category is added to a life form's genome to give it an ideal aggregate.

The utilization of hereditarily adjusted insect has the most potential for effectively uprooting certain pesticides, albeit the advancement of hereditary designing advances for farming irritation species is yet in its earliest stages. Change vectors should be created as do change philosophies Utilization of Biotechnology in entomology includes logical strategies like Hereditarily Adjusted Life forms, Recombinant DNA, particles of DNA from two unique species that are embedded into a host creature to deliver new hereditary mixes.

8.4 Transgenic Plant:

A transgenic crop is a genetic modified organism (GMO). A transgenic crop is a plant that has a clever mix of hereditary material got using current biotechnology. For instance, a GM harvest can contain a gene(s) that has been misleadingly embedded from an inconsequential plant or from various species out and out.

8.4.1 Process of Making Transgenic Crops:

To make a transgenic crop, there are four fundamental stages, namely,

- Extract DNA, cloning a quality of interest.
- Design the quality for plant invasion.
- Transformation.
- Plant reproducing.

8.5 Genetically Modified Organisms:

Biotechnology has opened new vistas for pest control. They have the ability to change the degree of articulation quality by allowing access to new particles. Capacity to change the articulation example of qualities creates transgenic with various insecticidal qualities. A most notable illustration of this genetic control in the two plants and infections are the addition into a plant or infection of the quality coding for the creation of the delta-endotoxin of *Bacillus thuringiensis* (Frisvold et al., 2010). The Bt quality Cry1Ac was utilized to foster the primary Bt-Cotton assortment. The quality was moved into the genome of cotton explants utilizing a bacterium called *Agrobacterium tumefaciens*. The changed cells were formed into full genetic modified plant currently called Bt-cotton. Bt is short form *Bacillus thuringiensis*. It is safe plants advancement. *Bacillus thuringiensis* was first found by a Japanese researcher Ishiwata in the year 1901. *Bacillus thuringiensis* (or Bt) is a Gram-positive, soil-abiding bacterium, usually utilized as an organic pesticide. Numerous Bt strains produce "Cry" during sporulation. Cry (short for gene proteins), additionally called δ-endotoxins, are proteinaceous incorporations encoded by plasmid-borne and have insecticidal activity on defenseless pest. Various forms of the Cry qualities, otherwise called "Bt qualities", have been distinguished.

Table 8.1: Various Cry Genes and their Specific Toxicity for Particular Insect Order.

S. No.	Cry gene designation	Toxic for insect orders
1.	CryIA (a), CryIA (b), CryIA (c)	Lepidoptera
2.	CryIB, CryIC, CryID	Lepidoptera
3.	Cry ll	Lepidoptera, Diptera
4.	Cry llI	Coleoptera
5.	Cry IV	Diptera
6.	Cry V	Lepidoptera, Coleoptera

In India, just GM crop endorsed for business development is cotton communicating Cry 1Ac and Cry 2Ab quality against bollworms. Different forms that were genetically altered against significant pest by presenting cry qualities in India are paddy, chickpea, mustard, and eggplant. Insecticidal yields express these Bt toxins to shield the plant from target pest.

For instance, Bt cotton shields against Lepidoptera, including pink bollworm by communicating Cry 1Ac toxins, which are explicitly deadly to Lepidoptera. Bt toxins are utilized in an assortment of transgenic crops, including cotton, for insurance against an assortment of lepidopteran pest; corn (maize), for assurance against the European corn drill, Ostrinia *nubilalis (*Lepidoptera); and potatoes, for security against the Colorado potato insect, *Leptinotarsa decemlineata.*

Table 8.2: Transgenic Plants, their Genes and Targeted Insects

S. No.	Crop	gene	Target insect
1	Brinjal	Cry IIIb	Leptinotarsa decemlineata
2	Cotton	Cry 3A 3 Cry 1A (c)	Helicoverpa zea, pink bollworm. Spodoptera exigua, Trichoplusia ni
3	Maize	Cry 1A (b)	Ostrinia nubilalis
4	Potato	Cry 3A, Cry 1A (b)	Leptinotarsa decemlineata
5	Rice	Cry IA (b), Cry 1A (c)	Rice Yellow stem borer, leaf folder, Chilo suppressalis
6	Sugarcane	Cry IA (b)	Diatraea saccharalis
7	Tomato	Cry 1A (c)	Helicoverpa virescens
8	Tobacco	Cry 1A (b)	Manduca sexta

8.6 Gene Pyramiding:

Pyramiding is the amassing of the qualities into a solitary line. Genome Altering (likewise called Quality Altering) is a gathering of advancements that enable researchers to change a creature's DNA. These advances permit hereditary material to be added, taken out, or changed at specific areas in the genome. A few ways to deal with genome altering have been created. Quality pyramiding is a helpful method in controlling diverse insect species when contrasted with transgenic assortments containing single poison characteristics. Stacked blends of various qualities in a solitary yield with their capacity to focus on a similar creepy crawly bother species is shown to be an exceptionally amazing and successful apparatus in overseeing insect opposition issues.

The key objective of the quality pyramiding approach is to create transgenic plants with additional obstruction against bothers and to improve crop yield. To transgenic crops with strong and expansive range obstruction against creepy crawly pest and sicknesses, the pyramiding of prevalent qualities (multigene technique) suggesting an exceptional method of activity is an incredible methodology. Hereditary alteration, including plant reproducing, has been broadly used to further develop crop yield and quality, just as to build infection obstruction.

Progress in site-explicit nuclease combined with increment crop genome sequencing and a more viable change framework offers extraordinary guarantee in making non transgenic crops with foreordained attribute R gene are a significant instrument for controlling nuisances and a harmless to the ecosystem option in contrast to the utilization of synthetic pesticides; albeit the particular pressing factor they apply on vermin might bring about the presence of microbes and pest that are unconcerned with explicit R qualities.

In this manner, the sturdiness of monogenic obstruction qualities is a worry. *Medicago truncatula* plants conveying two qualities that give protection from Bluegreen Aphids. They recognized potential phytohormone crosstalk set off by the joined R quality activity in light of aphid taking care of that improves opposition and limits R quality related wellness expenses for the plant.

Table 8.3: Specific Crop, their Genes Pyramiding and Targeted Insects.

S. No.	Crop	Target insect	Gene pyramiding
1.	Cotton	H. virescens	Cry1Ab +high terpenoid
2.	Rice	Brown Plant Hopper	Bph1 and Bph2
		Gall midge	Gm2 and Gm6
3.	Wheat	Russian wheat aphid	Dn2 and Dn4
4.	Soybean	Soybean looper and corn ear worm	QTLs + Cry 1Ac
5.	Broccoli	Diamond back moth	Cry1Ac and Cry1C

8.7 RNA Interference (RNAi):

RNA interference (RNAi) is an improvement of novel methodologies of gene silencing mechanism at the cellular level triggered by double-stranded RNA (dsRNA) and is likely to be the new approach underlying the next generation of insect-resistant transgenic plants. RNA interference (RNAi) is a natural interaction where RNA is associated with succession explicit concealment of quality articulation by twofold abandoned RNA, through translational or transcriptional restraint. It depicts the “turning off” of a quality by a system other than hereditary adjustment all things considered, RNAi was known by different names, including co-concealment, post transcriptional gene silencing (PTGS), and suppressing.

There are three RNAi pathways in insect: the dsRNA/siRNA-interceded siRNA pathway, miRNA-intervened miRNA pathway, and pi-connecting RNA (piRNA)-interceded piRNA pathway.

RNAi has fluctuating impacts in various types of Lepidoptera Conceivably in light of the fact that their spit and gut juice are better at separating RNA, the cotton bollworm, the beet armyworm, and the Asiatic rice drill have so far not been demonstrated defenseless to RNAi by taking care of is being worked on as an insect poison.

One first report was circulated in 2011 concerning making transgenic *Nicotiana benthamiana* and A. *thaliana* conveying dsRNA eroding in on characteristics imparted in *Myzus persicae* gut (RACK1) nd salivary organs (MpC002).

An education of the explanation level of the target characteristics and a decrease in M. *persicae* productivity was seen, anyway no destructive effects (Pitino et al., 2011).

In *Spodoptera frugiperda*, fifth instar hatchlings presented greater calming when diverged from grown-up moths (Griebler, 2008).

Table 8.4: Specific Crop and Their Targeted Gene for Insect Resistance

Crop	Species	Order	Target Gene
Nicotiana rustica	Bemisia tabaci	Hemiptera	VATPasc
Nicotiana tabacum	Helicoverpa armigera Spodoptera exigua	Lepidoptera	Nuclear receptor complex of 20-hydroxyecdysone (HaEcR)
	Helicoverpa armigera	Lepidoptera	Molt-regulating transcription factor gene (HR3)
Arabidopsis thaliana and Nicotiana benthamiana	Myzus persicae	Hemiptera	MpC001, Rack1
Arabidopsis thaliana			serine protease
Nicotiana tabacum			hunchback (hb)
Solanum tuberosum	Leptinotarsa decemlineata	Coleoptera	β-actin, Shrub
Cotton	Cotton bollworm (Helicoverpa armigera)	Lepidoptera	cytochrome P450 gene

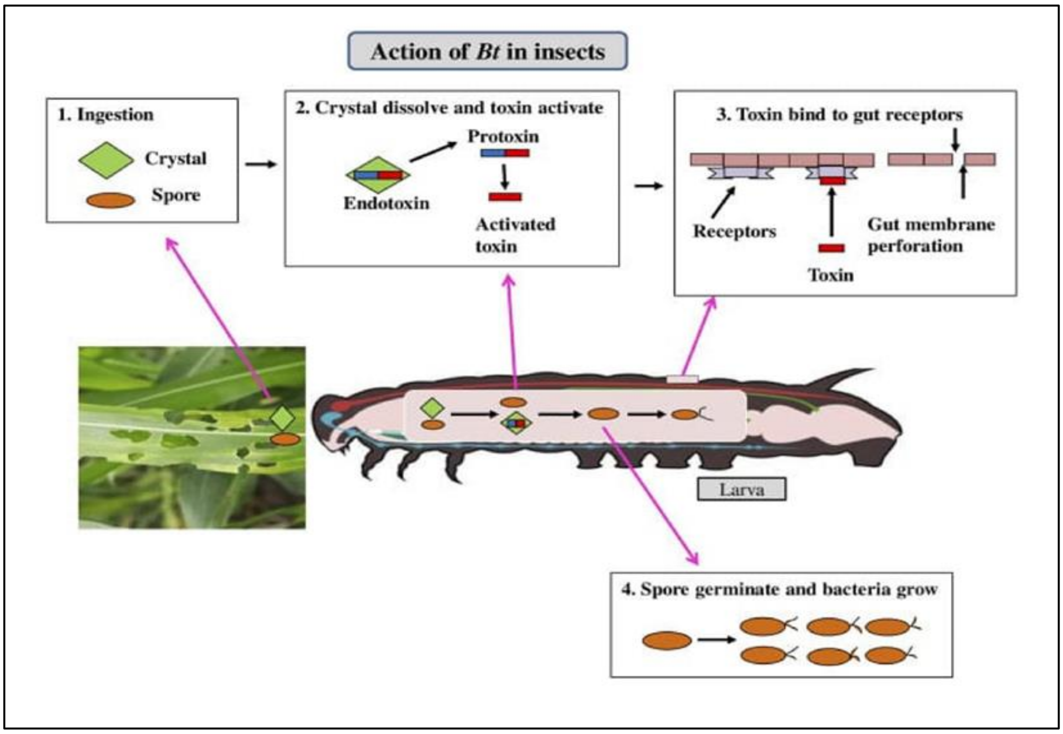

Figure 8.1: Action of B*t* in Insects

8.8 Sterile Insect Techniques:

The idea of releasing sterile insects into wild populations as a pest management intervention was independently conceived in the 1930s and 1940s by geneticist A.S. Serebrowskii in Moscow. Knipling (1937) sought after the utilization of ionizing radiation to actuate prevailing deadly transformations causing sterility. Insect are designed with a self-limiting develop giving a predominant deadly aggregate. Male insect that are homozygous for that transgene are delivered to mate with wild females, whose descendants acquire the prevailing deadly then can't get by to regenerative development. The population-level result is then indistinguishable from SIT a decrease in populace size.

In current practice, the SIT includes the mass raising of the nuisance species on a fake eating regimen, uncovering exceptionally enormous groups of people to radiation to cause chromosome harm, trailed by their delivery into an objective region. At the point when the delivered insect's mate, the subsequent eggs don't incubate because of the harm to hereditary material inside the parent's germline.

Supported inundate deliveries are required. Adequate sterile insect should be delivered for a drawn-out sufficient period to understand a major decrease in bother numbers, either concealment to a reasonable extraordinariness or nearby populace disposal. SIT programs normally discharge the two guys and females, without a useful strategy to sort the genders effectively in huge numbers.

This is wasteful on the grounds that the delivered sterile females and guys will in general court and mate with each other as opposed to searching out wild mates. Male-only deliver is generally more efficient than mixed sex releases, a large-scale investigation of irradiated Medfly quantified this as being three- to five-fold more efficient per male (Rendónet et al., 2004).

Early evacuation of females (eggs or early larval instars) inside the age bound for discharge likewise conceivably saves money on raising expenses as just the guys had the chance to be housed and taken care of. Genetics-based variations of the SIT are being created (Thomas et al., 2000; Alphey et al., 2014).

The SIT is mating-based, depending on science instead of science to handle bother populaces. It is species-specific then has no direct off-target consequences for different species inside the climate and is most appropriate to frameworks where one animal group is that the significant reason for the hurt. Area-wide SIT programs have made progress for extremely enormous scopes. Decades-long worldwide missions have stifled and killed the New World screwworm *Cochliomyia hominivorax* (Coquerel). Screwworm might be a myiasis bother whose hatchlings create in living tissue of vertebrates, strikingly dairy cattle, yet additionally other animals, natural life, and rarely people (for example in injuries).

8.9 Gene Editing:

This methodology is called switch hereditary qualities. Among the vital necessities of converse genetic material examination is that the capacity to switch the DNA arrangement of the objective life forms. These procedures can possibly drive hereditary builds through a populace, joining ‘gene drive’ systems that present greater-than-Mendelian legacy though they development has wellness costs.

Ongoing advances in hereditary alteration have focused on procedures of gene and genome altering. Gene-editing approaches could even be wont to stifle farming of insect as well as oversee opposition; for example, CRISPR gene altering has been used in a utilitarian report to spot appropriate gene focuses on diamondback moth (Huang et al., 2016).

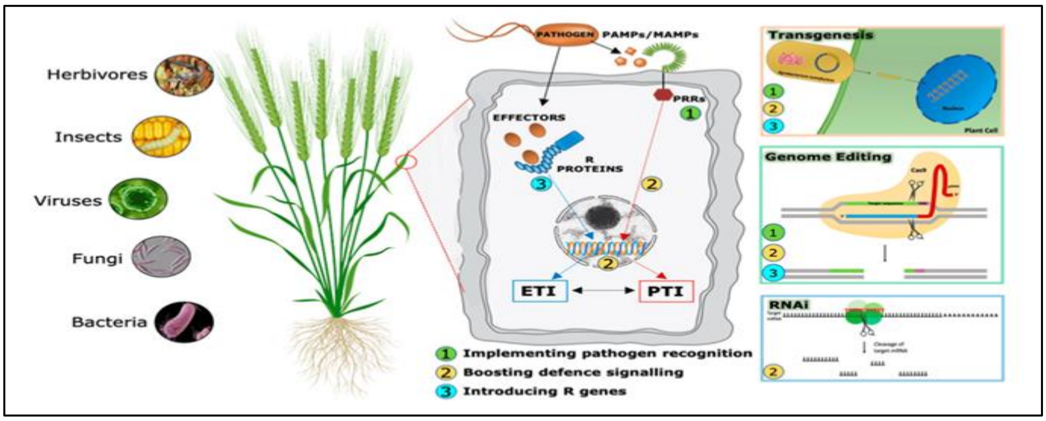

Figure 8.2: Transgenes and Gene Editing

8.10 Protease Inhibitors:

Protease inhibitors (PIs) are predominantly protein molecules that obstruct the activity of proteases, which are enzymes that facilitate the disintegration of proteins, produced by pathogens. While the function of some individual PIs and their target enzymes has been extensively studied, it is uncertain whether this defensive mechanism occurs naturally in plants.

In addition, several plants produce multiple types of PIs, and it was previously unclear whether these proteins work collaboratively to provide protection or if they serve additional purposes. The categorization of PIs into families based on the specific reactive site present in their sequences was proposed by Laskowski and Kato.

The adoption of this nomenclature simplified the categorization of PIs into four major families, namely: (I) cysteine protease inhibitors, (II) metalloprotease inhibitors, (III) aspartic protease inhibitors, and (IV) serine protease inhibitors. Plant PIs are classified according to their functional and biochemical properties, such as cysteine protease inhibitors, cereal trypsin/α-amylase inhibitors, mustard trypsin inhibitors, metallo-carboxypeptidase inhibitors, potato-type II protease inhibitors, potato type I inhibitors, serpins, soybean trypsin (Kunitz) inhibitors, Bowman-Birk serine protease inhibitors and squash inhibitors. Rawlings and Barrett presented an updated classification system for PIs, in which they are grouped into families and clans, akin to the classification system for peptidases/proteases suggested by Laskowski and Kato.

Nevertheless, this system aims to mirror the evolutionary relationships among PIs and is structured hierarchically, comprising three main levels: inhibitors, families, and clans, with the clan being the highest level of evolutionary divergence. Hartl also discovered four serine PIs from *Solanum nigrum* that provided protection against various natural herbivorous insects in both field and greenhouse experiments.

To summarize, PIs are a potential approach to attain control over plant pests, as they have been shown to safeguard specific tissues, serve as storage proteins, and regulate the activity and release of proteases.

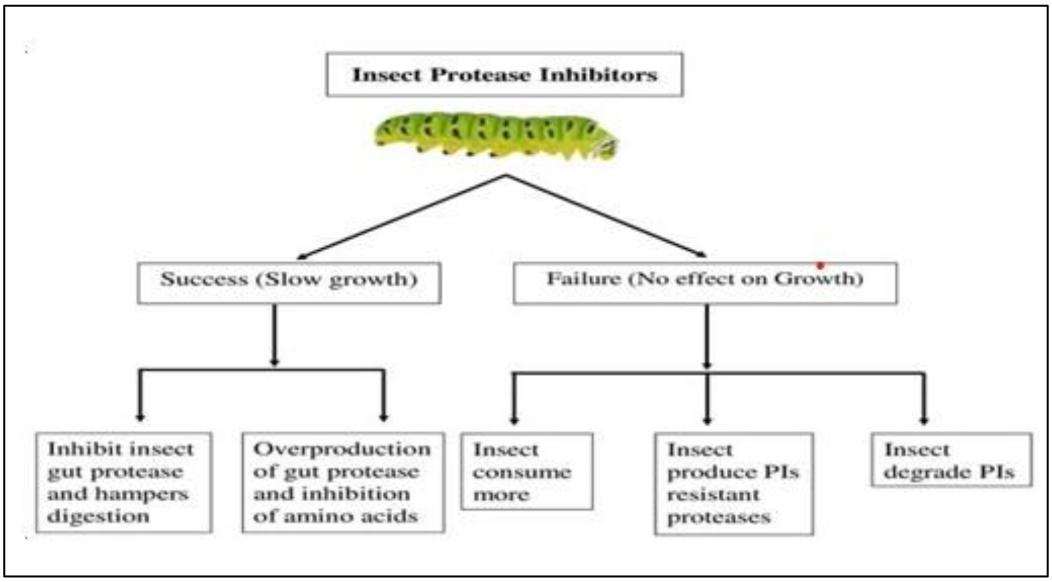

Figure 8.3: Insect Protease Inhibitors

Table 8.5: Tabular Representation of Plant Protease Inhibitors, their Origin and Applications in Plant Protection

PIs Name	Origin	Application
AtKTI4, AtKTI5	*Arabidopsis thaliana*	spider mite
AtSerpin1	*Arabidopsis thaliana*	Insect attack
AtWSCP	*Arabidopsis thaliana*	Herbivore attack
UPI	*Arabidopsis thaliana*	Fungal, insect attack
Potato type 1	*Solanum tuberosum*	Nematodes
PCI	*Solanum tuberosum*	Fungal, insect attack
mPI	*Zea mays*	Fungal, insect attack
SaPIN2b	*Solanum americanum*	Insect attack
StPin1A, NaPI	*Solanum tuberosum*	*Helicoverpa* spp.
PSPI-21, PSPI-22	*Solanum tuberosum*	Fungal attack
CDI	*Solanum tuberosum*	Recombinant proteins
PIN2	*Solanum tuberosum* L.	PPNs attack
Mi	*Solanum tuberosum* L.	
Hs1pro-1	*Beta vulgaris*	
Gpa-2	*Solanum tuberosum* L.	
Hero A	*Solanum tuberosum* L.	
PI-I, PI-II	*Solanum nigrum*	Insect attack
BBt	*Oryza sativa*	Fungal attack
CmPS-1	*Cucurbita maxima*	Insect attack
CPTI	*Vigna unguiculata*	Insect attack
SKTI	*Glycine max*	Parasitic, insect attack
SbBBI	*Glycine max*	Aphid parasitoids
Poplar Kunitz trypsin	*Populustrichocarpa* x *Populusdeltoides*	
PfKI	*Passiflora edulis* Sims	
ApKTI	*Adenantherapavonina*	Insect attack
BvSTI	*Beta vulgaris*	
BTI-CMe	*Hordeum vulgare*	
BWI-1a	*Fagopyrumsculentum*	Insect, fungal, bacterial
BBt	*Viciafaba*	Fungal attack
BBt, C/s, A/s	*Hordeum vulgare*	Fungal attack
AtKPI-1	*Arabidopsis thaliana*	Fungal attack
BBI	*Glycine max*	Therapeutic proteins
Chymotrypsin and trypsin	*Nicotiana alata*	Recombinant proteins

8.11 Biotechnology Strategy in Nematology:

An extension nematologist receives a sample containing an unknown root-knot nematode. Based on isozyme analyses of female nematodes and mitochondrial DNA (mt DNA) polymorphism in second-stage juveniles (J2), the species identities of 20 females and 100 J2 are ascertained within hours.

The extension nematologist finds that the field in question contains a mixture of 60% Meloidogyne incognita and 40% M. arenaria. This scenario, which would have been implausible 10 years ago, can occur today because of advances in biotechnology.

It is likely that within the next few years, the extension nematologist in our story will also establish the nematode race designation and determine whether the M. incognita is a pathotype capable of parasitizing root-knot resistant tomato cultivars with the Mi gene.

During the past two decades, rapid advances in molecular biology have affected understanding and treatment of human and plant diseases. The human and Caenorhabditis elegans genome sequencing projects (41,52) will yield additional useful techniques and results.

Important as the new molecular techniques and tools are to basic biological research, they have use in applied biology as well, and nematode management will definitely benefit. However, to those not directly involved in and unfamiliar with the new technologies and their terminology, the benefits of the advances are not obvious because of technical jargon.

A short glossary is included at the end of this paper to assist with terminology, and the first appearance of each glossary term in the text is emphasized with bold-face type. Despite the sometimes-confusing language, most of the principles and ideas of molecular biology are simple.

The details are often complex, but the concepts are easily understood and are becoming more accessible to the users. Many important genes for nematodes parasitism were characterized by RNAi strategies (Steeves er al.,2006).

Table 8.6: Examples of Biotechnological strategies for Nematodes control

Target for Control	Considerations	Status/Example
Major or minor natural resistance genes	The introgression and combination of natural resistance genes, for example from related or wild species, has been the mainstay of resistance breeding strategies	Marker-assisted breeding for nematode resistance has become routine in many breeding programs, although effective resistance genes are not available for all crops
Nematode migration in the rhizosphere and root entry	Disruption of sensory functions	Peptide(s) that inhibit reception of gradients by amphids RNAi disruption of amphid proteins/function
Migration in the root	Wall-degrading enzymes may be required for migration, e.g. *Endoparasites* Positional gradients in roots detected for migration to the required site in the root	RNAi downregulation of nematode expression of cell wall-degrading enzymes Inhibition of sensing gradients in roots
Avoiding host defences	Effectors that enable nematodes to evade or neutralize host defences	RNAi downregulation of expression of effectors involved in avoiding host defences
Disruption of feeding site formation or function	Effectors enable sedentary endoparasites to induce giant cells and syncytia. Disrupt feeding site formation, triggered by nematode-responsive promoter(s)	RNAi downregulation of expression of key effector(s) required for feeding site formation Nematode responsive promoter(s) linked to 'cell death' gene, e.g. barnase

8.12 Conclusion:

The advancement so far made in insect biotechnology basically gets from logical leap forwards in molecular science, particularly with the advances in methods that permit hereditary control of living beings and cells. Application of biotechnology in pest management has filled enormously. Insect biotechnology has demonstrated to be a helpful asset in assorted in diverse industries, particularly for the creation of modern catalysts, microbial insect poisons, and numerous different substances trans genesis especially by the improvement of apparatuses and strategies that permit hereditary portrayal and designing of organism and cells likewise with recombinant DNA innovation plan of microbial specialists with upgraded insecticidal intensity, and the designing of pest that can move deadly qualities to normal populaces following their mass delivery in the field. Similar genomics examinations likewise make it conceivable to distinguish insect explicit qualities that can be focused on for sane insect poison plan. Be that as it may, more secure options in contrast to synthetic irritation control have become objectives in light of the fact that the vast majority of these pesticides are conceivably unsafe to the biological system and human wellbeing. The coming of biotechnology overall and insect biotechnology specifically is a welcome advancement in the battle against pest bothers.

8.13 Acknowledgement:

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