IMPLICATIONS OF BIOTECHNOLOGY IN INTEGRATED PEST MANAGEMENT

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I. INTRODUCTION

There are many factors that affect agricultural production and productivity globally which include increasing population, diminishing of natural resources, climate change and emerging insect pests. Among all these, insect pests are of great concern and are reported to cause about 50 to 70 percent loss of plant produce (Melaku, 2022). These insect pests have been one of the leading constraints to global food production. It has been worked out that around 70,000 species of insect pests cause damage to agricultural produce with a loss of several billion dollars particularly in developing countries (Tipvadee, A 2002). To overcome this problem, use of synthetic broad-spectrum insecticides is easy and a permanent solution giving enormous success. The indiscriminate use of these broad-spectrum insecticides has led to various problems like health hazards, development of resistance in insects, their resurgence, toxicity to natural enemies and various environmental issues (Singh *et.al*, 2018). This necessitated the shift from chemical control to biotechnological mode of pest control. Nowadays scientists are exploring various biotechnological methods for taking care of every agricultural problem that was not earlier managed by conventional ways. Biotechnology in terms of pest management is a controlled manipulation of biological systems to achieve effective insect pest control. Other than increased productivity, biotechnology has helped in cost effectiveness, quality food production and safeguard of the environment. Integrated pest management is an effective and applied ecology-based approach to the management of insect pests that relies on various combinations of management practices like habitat manipulation, biological control, modifications of cultural practices and use of resistant varieties (Adenle, A.A. 2015). However, IPM has various drawbacks which come from identifying the best method to implement in a given strategy. These drawbacks have forced the workers and researchers for the development of biotechnological approach for IPM.

II. IPM VIS A VIS BIOTECHNOLOGY

Biotechnology is an interdisciplinary area of biological science that utilizes biological systems of organisms to create products or processes for targeted use (CBD,1992). In relation to insect pest management, it is described as the intentional and calculated manipulation of biological system of organisms to get the desired insect pest management. Biological world is replete with instances of capabilities that could be exploited for sustained insect pest control. Biotechnology is a cascade of technologies that involve living organisms and their molecular agents to make processes, outcomes or functioning for the benefit of mankind (Ales and Pokormy, 2012; Cano *et.al*; 2017). The most promising and sustainable technologies include molecular breeding techniques, recombinant DNA technology, genetic engineering and bioprocess engineering etc. (Fermin-Munoz *et.al*; 2000; Leonard *et. al*; 2003).

III. INFLUENCE OF BIOTECHNOLOGY IN ENHANCING AGRICULTURAL PRODUCTION

The development of agricultural technology in recent times has revolved between green revolution and gene revolution. The green revolution that has extended from 1944-1994 and gene revolution from 1995 to present day (Ghanian, *et al*, 2016; Ayesha and Raza, 2017) has been the edifice of modern agriculture. Apart from higher yields, green revolution necessitated the requirement of agrochemicals, mechanization, fertilizers and irrigation. In addition to these high yielding varieties that were mostly developed through traditional plant

breeding practices, the insect pest problem in these varieties is quite high due to their susceptible nature. However due to ever increasing global population and demand for more food, gene revolution supported by modern biotechnological tools paved the way for elimination of barriers of conventional plant breeding by virtue of genetic engineering (Ghanian, *et al*,2016). Desirable traits in genes are identified and exploited more effectively by gene technology and their subsequent introduction in plants with precision (Robert, 2006; Jim, 2010; Naseer, 2014; Khan *et al*; 2018).

IV. GENETICALLY ENGINEERED BIOLOGICAL PESTICIDES

1. **Bioagents in IPM:** Insect natural enemies used for classical or augmentative release is the backbone of traditional biological control in IPM. Keeping laboratory reared bioagents in the best of quality is difficult due to probable genetic changes triggered by accidental selection, founder effects in breeding and genetic drift (Hopper *et al*;1993). With the advent of cutting-edge biotechnological approaches, new DNA based method for observing any genetic variations like mitochondrial DNA analysis, ribosomal DNA analysis, Random amplified polymorphic DNA (RAPD- PCR) and restriction fragment length polymorphism (RFLP) is being utilized (Edwards and Hoy,1993; Attathom.T, 2002). Genetic improvement has opened a new era of efficacy in bioagents. Transgenic techniques offer a vast array of techniques to establish and manifest exotic genes or mask the function of existing genes so as to get the characters of interest in subsequent generations (Allathom.T, 2002). Establishment of genetic material into insect germ cells could be employed by techniques like electroporation, microinjections and biolistics (Atkinson *et al;* 2001). Inserting DNA into insect germ cells can be best accomplished by micro injection method like in *Drosophila melanogaster*. Without the help of transposable element vector, the DNA can be microinjected to the gravid females through cuticle by a technique called maternal microinjection that has been successfully demonstrated in various insects (Presnail and Hoy, 1992; Atkinson *et al;* 2001; Attathom.T, 2002; Barratt *et al*; 2018; Enyiukwu *et al*; 2016). The four transposable element vectors from *Drosophila hydei, Musca Domestica, Drosophila Mauritiana* and *Trichoplusia ni* namely Minos, Hermes. MOSI and piggy Bac respectively have been developed for the various generations of transgenic insects (Barzman *et al;* 2015; Johan, 2017; Melaku, A. 2022)

   Apart from these, resistance genes for insecticides could be potentially exploited to enhance the suitability of bioagents in the present scenario of IPM.The available genes include parathion hydrolase gene from *Pseudomonas diminuta*, an acetyl cholinesterase gene from *Drosophila melanogaster and Anopheles stephensi* (Stevens *et al*; 2012, Jaiswal *et al*; 2018).A gene isolated from culex mosquito and named as esterase B1 that impart resistance to organophosphorus insecticides (Guleria and Tiku, 2009; Ivana, *et al*, 2011). Heat and cold resistance gene incorporation in natural enemies could impart adaptability in them to endure broader range of climate changes and offer themselves as efficient bioagents (Hoy,1996).

2. **Insect Pathogens:** Insect pathogens constitute a major segment of biocontrol which in recent years have received wide spread attention due to negative effects of synthetic pesticides. This paper will deal with the bacteria and transgenics separately. Here we will focus on other insect pathogens like entomopathogenic nematodes, viruses, fungi etc.

3. **Entomopathogenic nematodes:** Nematodes have a great advantage for the control of insect pests through inoculative and inundative releases. They are considered second to bacteria as far as their commercial importance is concerned. The three important families of nematodes that can be utilized as bio- insecticides are Rhabditidae, Steinernematidae and Heterorhabditidae (Attathom.T, 2002). The mortality of insects is due to the symbiotic bacterium associated with the nematodes. The entomopathogenic nematodes are best utilized against soil dwelling insects and borers, while as there is not much success against other insects. Biotechnological techniques like AFLP, RFLP, RAPD-PCR and satellite DNA analysis have been exploited to ascertain genetic diversity and offer a screening tool to sort out useful strains (Ivana *et al*; 2011). Susceptibility to abiotic factors in nematodes and to enhance genetic traits to overcome that stress would be a giant leap in biotechnological approach vis-a-vis entomopathogenic nematodes (Harrison and Bonning,1998). Heat shock protein genes (Hsp70A) have been engineered to express in *Heterorhabditis elegans* (Hashmi *et al*; 1998).

4. **Insect Pathogenic viruses:** Nucleopolyhedroviruses (NPV's) have been mostly utilized as microbial insecticides for the management of caterpillars. Formulation and spraying of NPV's in the field is as same as the chemical insecticides (Melaku, A 2022).There are some of the limitations with NPV's use that render it incapable as compared to other bioagents like, slow speed of mortality in host, low efficacious under field conditions, narrow host range and expensive mass production (Badu *et al*; 2017; Peng *et al;*2017).There have been several attempts to genetically engineer NPV's like deletion of genes that express products responsible for host survival and incorporation of genes that express an insecticidal protein (Attathom.T, 2002). Deletion of the ecdysteroid UDP-glycosyltransferase (EGT) gene of *Autographa Californica* NPVX resulted in less feeding of army worm (*Spodoptera frugiperda*) that led to death of larvae by 30% earlier than larvae that were exposed to wild type ACNPV(O'Reilly and Miller 1991; Alan K. *et al*; 2016; Gangwar, 2017). Baculoviruses genetic engineering has employed the polyhedron or p10 promoters and production of recombinant baculovirus is accomplished by the polyhedrin gene by exotic genes. Once recombinant being successful, the p10 polyhedrin promoter arose the expression of the exotic gene to equivalent levels to those of p10 in wild type virus (Miller, 1995).

   Insect metabolism is controlled by various hormones like morphogenesis and reproduction as they are suitable candidates for engineered Baculoviruses. The hormones that could be exploited for genetic engineering of baculovirus are: diuretic hormone (DH) that control water balance, eclosion hormone that control ecdysis, prothoracicotropic hormone (PTTH) that trigger moulting process and allatostatins and allatotropins that control the release of juvenile hormone (Adenle A. A,2015; Kirk, 2017; Herman *et al*; 1995) experimented the introduction of scorpion toxin genes Aa1T and Lqb1T into Baculoviruses followed by infection into larvae of *Helicoverpa virescens* expressed 5-10-fold level of activity. Insect cell cultures in conjunction with baculovirus expression system have opened the utility of insect viruses as efficient biocides that necessitate the evolution of more effective and economic viral insect control agents.

5. **Fungal biocontrol agents:** The most effective insect pathogenic fungi are *Bauveria bassiana* and *Metarhizium anisopliae* that have exhibited insecticidal property over a

wide range of insects (Flexner and Belnavis,1998). However, there are myriad of constraints that fall into biotic and abiotic categories that limit the ability of these fungi to infect their host. These include temperature, host behavior, desiccation, age of host and UV light. Genetic advances in formulation and production could contribute to the viability under extremes of climate of these mycoinsecticides (Hidayat, *et al*, 2018; Barratt *et al*, 2018). Genes have been cloned from *Metarhizium anisopliae* that are related to appressorium formation and help in the cuticle penetration and confer virulence. Gene Pr1 encoding protease responsible for insect cuticle penetration had been incorporated in the genome of *Metarhizium anisopliae*. Mortality in the recombinant strain infected larvae was recorded to be 25% sooner and reduction of damage by 40% (St. Leger *et al;*1996)

6. **Genetically engineered plants:** There is a vast array of biological compounds present in plants or other organisms that offer them natural defense against insect pests. These compounds mostly proteins could be exploited for imparting host plant resistance through their introduction to plants either by conventional breeding of sexually compatible plants or by modern biotechnological methods. By transferring genetic material across genus barrier, transgenic plants have been developed that contain toxic pesticidal compounds against insects which the plants could not produce on their own. This plant incorporated protectants (PIP's) from other organisms have modified the genetic makeup of plants that produce toxic protein which cause insect mortality and transgenics have been developed for corn, cotton, potatoes to list a few. Biotechnological approaches like gene transformation, gene editing, marker assisted selection, RNA interference, somaclonal variation, embryo culture, protoplast fusion help to develop host plants that are resistant to insect pests (Talakayala *et al;*2020).

7. **Gene transformation:** Genetically engineered plants for resistance against herbivores entails insertion of desired DNA segment into plant genome to impart resistance against destructive insect pests.The gene that is incorporated through recombinant technology encodes a specific protein with toxicity to insect pests and resistance is conferred to host plants against some specific pests (Gatehouse,2013).The genetically engineered plants have been developed and tested against myriad of insect pests belonging to different orders (Birkett and Pickett, 2014). Insecticidal crystalline proteins(ICP's) from *Bacillus thuringiensis* is incorporated in genetically modified crops have been extensively used in agriculture since their advent in 1996 (Abbas, 2018).The transfer of cry gene involves the insertion of targeted DNA sequence or genes into desired plants via mediated transformation by Agrobacterium or particle bombardment (Juturu *et al*; 2015).Other strategies to protect plants from the ravages of insect pests include lectins, protease inhibitors, alpha-amylase inhibitors, chitinase and chitinase like proteins have been successfully explored. Lectins present in plants bind to carbohydrates in the midgut of insects leading to the interruption of digestive process (Vandenborre *et al*; 2011). Genetic engineering techniques have also been exploited to insert protease inhibitors in plants that make insects incapable of digesting their food (Singh *et al*; 2020). Transgenic plants encoding alpha-amylase inhibitors have been developed that proved resistant to various insect orders like hemipterans, Dipterans, Lepidopterans and Coleopterans. Chitin degrading proteins like chitinase that degrade the gut lining of insects have been successfully inserted into host plants and depict excellent insecticidal action (Pritam *et al*; 2022). These genetically modified plants have been elaborated as under:

8. ***Bt* transgenic plants:** More than 30 years ago first transgenic plant resistant to insects was produced and various resistance genes of different origin were found and used for plant transformation. Gene from one organism transferred to induce or exhibit a change in other organism in the laboratory conditions form the genetically modified organism (Tipvadee A. 2002). The use of cry (*Bt*) genes encoding endotoxins from bacterium *Bacillus thuringiensis*is a well modified technology of transgenic plant production (Sharma *et al*; 2000). *Bt* is a soil inhabiting spore forming, rod shaped gram-positive facultative bacterium producing ample amount of proteinaceous crystalline inclusion bodies (cry proteins). These cry proteins crystallize intracellularly at sporulation stage and are toxic to larvae of various insects of order viz; Coleoptera, Lepidoptera and Diptera (Lacey and Goettel, 1995). Strains of *Bt* produce a variety of crystal toxins. In 1901 Ishiwata discovered *Bt* from diseased silkworm (*Bombyx mori*) larvae. Around 400 genes from a wide range of *Bt* has been identified encoding toxins (Crickmore *et al*; 1998). Some of the cry genes engineered into plants are cry1Aa, cry1Ab, cry1Ac, cry1Ba, cry1 H, cry2Aa, cry3A, cry6A, cry9c, cry1F (Malone *et al;*2008). They have distinctive insecticidal spectrum and affect larvae of different orders like coleoptera, Lepidoptera and Diptera. However, *Bt* toxins are not toxic to humans, wildlife and natural enemies with a great opportunity for biocontrol. The various lepidopteran insects are found effective against *Bt* viz; *Bombyx mori*, *Helicoverpa armigera, Heliothis virescens, Manduca sexta, Ostrinia nubilalis, Plutella Xylostella, Sesamia nonagrioides, Spodoptera exigua, Spodoptera frugiperda, Spodoptera littoralis* (Stevens *et.al;* 2012, Hua *et al;* 2001, Ryerse,1990, Avisar *et al*; 2004). *Bt* cotton and maize are the only insect resistant genetically modified crops for commercial planting (Ibrahim and Hua 2016). Insect resistant transgenic plants were commercially used in mid-1990 with the introduction of genetically modified corn, potato and cotton expressing genes encoding the ϭ- endotoxin from *Bt.* Garlic, onion, and leek are the crops which have natural resistance to insects. The concept of cry protein was not new as *Bt* formulations (Dipel) have been used for around five decades commercially to control insect pests mainly lepidoptera (Gatehouse *et al*; 2011). Bollgard I (BG I) containing cry1Ac belonged to the first generation *Bt* cotton was released in 2002 to contain the bollworms in cotton growing areas of India. In 2006 Bollgard II (BG II) belonging to the second-generation *Bt* cotton was released with pyramided traits containing cry1Ac and cry2Ab and is now grown in 95% of the total India's cotton sowing area (Pritam *et al*; 2022). BG had only cry 1Ac alone, but BG II contains multiple toxins, cry1Ac and cry2Ab and offer greater ability for containing bollworms (Carriere *et al*; 2015).

Bangladesh and Latin America have permitted the cultivation of transgenic cotton and brinjal cultivars and *Bt* soybean expressing cry1Ac + cry1Ab cleared for cultivation in2014 (Koch *et al;* 2015). Tomato plant incorporated with synthetic cry1Ab gene expressed resistance to tomato leaf miner, *Tuta absoluta* with cent percent mortality (Soliman *et al;* 2021). Mortality in rice leaf folders have been caused by insecticidal protein cry2A incorporated in rice lines (var. Bg94–1) (Gunasekara *et al;* 2017). Combined transgene cry1Ac and cry2Aa exhibited larval mortality in the range of 80%–100% to *H. armigera* in transgenic pigeon pea lines (Ghosh *et al;* 2017). Rice lines constructed through transgenics have expressed the cry2AX1 gene demonstrating toxicity to rice leaf folder (*Cnaphalocrocis medinalis*) and rice yellow stem borer (*Scirpophaga incertulas*) (Rajadurai *et al;* 2018). Lepidopteran insect *Spodoptera litura* encountered resistance in sweet potatoes expressing Cry1Aa gene (Zhong *et al;* 2019). Agrobacterium transformation technique employed for transferring cry1Aa gene in non-edible castor

showed resistance against *Achaea janata* (semi-looper) and *S. litura* (Muddanuru *et al;* 2019). *Holotrichia parallela*, a Coleopteran pest did not survive on transgenic soybean encoding cry8-like gene from *B. thuringenesis* (Qin *et al;* 2019). The expression of cry gene in different crops has been summarized in Table 1. Cry gene technologies in crops have exhibited excellent resistance in host plants against harmful insects but other problems like resistance to insecticidal toxic proteins, outbreak of secondary pest, new biotypes evolution, environmental concerns on transgenic genes, less knowledge on non-target organisms and transgenic food biosafety has limited its scope.

Table 1. Crop wise list of transgenic genes imparting resistance to insects

S.N	Crop	Transgene	Insect controlled	References
1.	Cotton	*cry1Ab + Nptll*	*Helicoverpa armigera*	Khan *et al.* (2011)
		cry1Ab	*Heliothis sp*	Khan *et al.* (2013)
		cry2AX	*Helicoverpa armigera*	Sakthi *et al.* (2015)
		cry1Aa	*Anthamous grandis*	Ribeiro *et al.* (2017)
		cry1AC + cry2 Ab	*Spodoptera litura*	Siddiqui e*t al*. (2019)
		cry2AX1	*Helicoverpa armigera*	Jadhav *et al*. (2020)
2.	Chickpea	*cryIIAa*	*Helicoverpa armigera*	Sawardekar *et al.* (2017)
3.	Castor	*cry1AC*	*Achaea janata, Spodoptera litura*	Muddanuru *et al.* (2019)
4.	Pigeon pea	*cry1AC* + c*ry2 Aa*	*Helicoverpa armigera*	Ghosh *et al.* (2017)
		cry2Aa	*Helicoverpa armigera*	Baburao and Sumangala (2018),Singh *et al.* (2018)
5.	Rice	*cry 1a(b)*	*Chilo suppressalis, Cnaphalocrocis medinalis*	Fujimoto *et al.* (1993)
		cry 1 a(b)	*Scirpophaga incertulas & Chilo suppressalis*	Wunn *et al.* (1996)
		cry 1 a(b)	*Scirpophaga incertulas, Cnaphalocrocis medinalis*	Ghareyazie *et al.* (1997)
		cry 1a(c)	*Scirpophaga incertulas*	Nayak *et al.* (1997)
		cry 1a(b)/cry1a (c)	*Scirpophaga incertulas*	Tu *et al.* (2000)
		cry 2a/cry 1a(c)	*Cnaphalocrocis medinalis, Scirpophaga incertulas*	Maqbool *et al.* (2001)
		cry2A	*Cnephalocrosis medinalis*	Gunasekara *et al.* (2017)
		cry2AX1	*Scirpophaga incertulas, Cnaphalocrocis medinalis*	Rajadurai *et al.* (2018)
6.	Soyabean	*cry 8 like*	*Holtrichia panallele*	Qin *et al.* (2019)
7.	Sweet Potato	*cry1Aa*	*Spodoptera litura*	Zhong *et al.* (2019)

8.	Tomato	*cry1Ac*	*Tuta absoluta*	Selale *et al.* (2017)
		cry1Ab	*Tuta absoluta*	Soliman *et al.* (2021)

9. Mode of action: The mode of action of *Bt* is given by two ways:

- Pore formation model.
- Signal transduction model

In Pore formation model the larvae are killed by osmotic shock of their midgut cells. This model is the accepted one for more than two decades. First the protein toxins must be secreted from the host. The toxins bind to primary receptors of the columnar cells of midgut epithelium. Cadherin like proteins are the main receptors for cry toxin in lepidopterans and their binding site depends on the structure of cry toxin. This facilitates proteolytic cleavage of toxin and promotes formation of oligomers. They then interact with secondary receptors in the midgut larval membrane. After secondary receptor binding, the toxin creates pores by inserting into the membrane which leads to disruption of membrane causing electrolyte imbalance which leads to septicemia and death of insect by starvation (Anderson *et al*; 2016).

Signal transduction processes are protein driven events. In this pathway cry toxins trigger a signaling cascade pathway. The model is different from pore formation in that it lacks formation of oligomers, secondary receptors on the pore formation in membrane. In the signal transduction model binding to cadherin receptor initiates a Mg^{2+}dependent signal cascade pathway and include a guanine nucleotide binding protein, protein kinase A and adenylyl cyclase and later results in the death of cells. The four steps of signal transduction are signal molecule binds to receptor→activates a protein→create second messenger →create a response. It means a cell detects a signaling molecule from outside the cell. When the molecule binds the receptor, it changes a receptor protein in some way and finally the signal triggers a specific cellular response.

Recently the reports of field resistance to *Bt* cotton against pink bollworm (*Pectinophore gossypiella*), army worm (*spodoptera frugiperda*), corn root worm (*Diaabrotica virgifera virgifera*), cotton bollworm (*Helicoverpa armigera*). The common method of resistance is the disruption of binding of *Bt* toxin in midgut membrane and can be caused by mutation in the receptor that blocks binding or change in the receptor and its expression. The transgenic plants resistant to insects will be a major component of sustainable pest management.

10. Carbohydrate binding proteins: Lectins known as one of entomotoxic compounds are basically proteins that bind to carbohydrates in insect gut giving them insecticidal properties. They offer good insect control and can be found in the plants belonging to Poaceae, Solanaceae and Fabaceae, with high legume seeds having high amount of lectins. Lectins help the plants to store proteins in their seeds and have good involvement in insect defense. Plant lectins from various plant sources have been identified to the members of order Lepidoptera, Coleoptera and Homoptera (Gatehouse *et al*; 1984, Czapla and Lang,1990; Powell *et al*; 1993; Sauvion *et al*;1996). Feeding inhibition increased mortality, reduced fecundity, enhancement of developmental period, reduction in larval weight and adult emergence are some of the impacts of lectin on insect biology

(Hopper *et al*; 1993). Lectins have affinity towards glycoproteins and glycolipids present in insect cell membrane (Camaroti *et al;*2017).

Allium sativum leaf agglutinin and Galanthus nivalis lectin (GNA) in transgenic rice impart resistance against brown plant hopper (BPH), green leaf hopper (GLH) and white backed plant hopper (WBPH) (Bharathi *et al;* 2011). Snowdrop lectin (GNA) in transgenic rice demonstrate high level of resistance against sucking insect pests (Sudhakar *et al;*1998). *Brassica juncea* transgenic plants containing lentil lectin (LL) and chickpea protease inhibitor (CPPI) demonstrated resistance to sucking insects (Rani *et al*; 2017). Agrobacterium mediated mannose binding lectin demonstrated resistance to wheat aphid (Duan *et al;* 2018). Chitin binding rhizome lectins (MVRL)obtained from *Microgramma vacciniifolia* exhibited feeding and nutrition effects on the adults of *Sitophilus zeamais* (Albuquerque *et al*; 2020). Crops with transgenic lectin genes have been summarized in Table 2.

Table 2: Crop wise list of lectin genes imparting resistance to insects

S.N	Crop	Transgene	Insect controlled	References
1.	Common bean	Arcelin	*Callosobruchus chinensis*	Hilda *et al.* (2022)
		Arcelin-5, Leucoagglutinin, Erythroagglutinin	*Callosobruchus chinensis*	Caroline *et al.* (2022)
2.	Cotton	Insect gut binding lectin from *Sclerotium rolfsii*	*Aphis gossypii* and *Spodoptera litura*	Vanti *et al.* (2018)
3.	Cowpea	Arcl on APA locus from *Phaeselous vulgaris*	*Callosobruchus chinensis*	Grazziotin *et al.* (2020)
4.	Mustard	*Colocasia esculenta* tuber agglutinin (CEA)+ Galanthus nivalis agglutinin (GNA)	*Lipaphis erysimi*	Das *et al.* (2018)
5.	Potato	Hv1a/GNA	*Myzus persicae* and *Sitobio navenae*	Nakasu *et al*. (2014)
		Galanthus nivalis agglutinin (GNA)	*Myzus persicae*	Mi *et al.* (2017)
6.	Rice	Snowdrop lectin (*Galanthus nivalis* agglutinin; GNA)	*Sap sucking pests*	Sudhakar *et al*. (1998)
		Allium sativum leaf agglutinin (ASAL) and Galanthus nivalis lectin (GNA)	*Nilaparvata lugens, Sogatella furcifera* and *Nephotettix nigropictus*	Bharathi *et al.* (2011)
7.	Rice, Sugarcane	Snowdrop lectin (*Galanthus nivalis* agglutinin; GNA)	*Eoreumaloftini*(Dyar)and *Diatraea saccharalis*	Setamou *et al.* (2002)

8.	Rice and Potato	GNA-spider-venom toxin I (SFI1)	*Nilaparvata lugens* and *Myzus persicae)*	Down *et al.* (2006)
9.	Stored grains	*M. urundeuva* leaf lectin (MuLL)	*Sitophilus zeamais*	Napoleao *et al.* (2013)
		Schinus terebinthifolius leaf lectin (SteLL)	*Sitophilus zeamais*	De Santana Souza *et al.* (2018)
		Microgramma vacciniifolia rhizome lectin (MvRL)	*Sitophilus. zeamais*	Albuquerque *et al.* (2020)
		Water-soluble *Moringa oleifera* lectin (WSMoL)	*Sitophilus zeamais*	De Oliveira *et al.* (2020)
		Polygonum persicaria L. (PPA) Lectin	*Sitophilus oryzae*	Khoobdel *et al* (2022)
10.	Tomato	GNA-neuropeptide-allatostatin	*Lacanobia oleracea*	Fitches *et al.* (2004)
		GNA-lepidopteran-specific toxin (ButalT)	*Lacanobia oleracea*	Trung *et al.* (2006)
11.	Wheat	Snowdrop lectin (*Galanthus nivalis* agglutinin; GNA)	*Sitobionavenae*	Stoger *et al.* (1999)
		Pinellia pedatisecta agglutinin (PPA)	*Metopolophium dirhodum, Schizaphis graminum, Rhopalosiphum padi,* and*Sitobio navenae*	Duan *et al.* (2018)

The leaf lectin (MuLL) obtained from *M. urunteuva* affects the digestive enzymes of *S. zeamais* (Napoleao *et.al;* 2013). Lectins as biocides have tremendous potential in transgenic world but due to their known toxicity to mammals, their role in transgenic plants should be exercised with caution.

11. Protease Inhibitors: Inhibitors obtained from various plants that forestall the activity of digestive proteases in insects are known as protease inhibitors (Haq *et al*; 2004, Macedo & Freire 2011, Zhu-Salzman and Zeng, 2015). In insects' proteolysis is accomplished by digestive proteases that are rendered ineffective by protease inhibitors (PI's) and result in decreased fecundity, longer developmental periods and increased mortality due to non-availability of essential amino acids. Serpins and cystatins are well documented plant inhibitors against insect pests. Serpins are inhibitors of serin proteases and Cystatins are inhibitors of Cysteine proteases (Irving *et al*; 2002). Trypsin inhibitors from legumes forestall a wide range of proteases in insects and have demonstrated insecticidal activity to a myriad of insects (Macedo *et al;* 2004, Sharma, 2012). Rice cultivars were inserted with PI's genes to offer defense against stem borers (Duan *et al*; 2018; Xu *et al;*1996). Increased mortality was found in insect pests when PI's were given through transgenic

plants or artificial diet (Gatehouse, 2011). Protease inhibitors might become a new weapon to fight against insects but due to adaptive behavior of insects with respect to co evolution with host plants, these molecules have not yielded their true potential.

12. **α-Amylase inhibitors:** One of the important digestive enzymes for the digestion of carbohydrates is α -amylase. In insects digestion is affected by the α -amylase inhibitors that are present in seeds and vegetative organs of plants and observed to control various herbivores (Chrispeels *et al*; 1998). α -amylase inhibitor expressed by seeds of *phaseolus vulgaris* impacted the overall life cycle of *callosobruchus chinensis* (Ishimoto and Kitamura,1989; Shade *et al*;1994). The inhibitor aAI-1, expressed by transgenic pea demonstrated elevated resistance against pea weevil (*Bruchus pisorum* and cowpea weevil *callosobruchus maculates* (Morton *et al*; 2000). Wheat α -amylase inhibitors effectively control the damage of stored wheat pest *Rhizopertha dominica* (Priya *et al*;2010). Tobacco plant incorporated with a wheat gene expressing α-amylase inhibitor exhibited resistance traits against *spodoptera* and *Agrotis spp* (Jaiswal *et al* ;2018). Cotyledons of *Phaseolus coccineus* containing a gene aAI-Pc1 producing ∝-amylase inhibitors have great scope in transgenics.

13. **Chitin degrading enzymes:** The hydrolytic enzymes have the ability to inhibit the formation of chitin are called as insect chitinases. Chitin protects the insects from harsh environmental conditions and natural enemies and is present in exoskeleton and peritrophic membrane (Chen *et al*; 2018).The process of chitin hydrolysis brought about by chitinase is important for ecdysis and various plants exhibit this enzyme to degrade the chitin in insects (Oyeleye and Normi, 2018). Genetically modified crop like maize expressing chitinase enzyme exhibited resistance against *Sesamia cretica* (Osman *et al*; 2016). Insect chitinase as biocides in transgenic host plants has been of unique importance due to their inhibition on the development of insects and if their role as agrochemical is harnessed will revolutionize the transgenic host plant process

14. **Genome edited crops:** Development of insect resistance is a pre adaptive phenomenon and insect exhibiting resistance to transgenic *Bt* has evolved the agricultural scientists to develop gene editing techniques to fight insect resistance. Gene editing has become a new edifice of insect pest management in today's agriculture. The gene editing or genome editing involves any of the three steps like replacing, inserting or deleting particular DNA bases in a target specific DNA sequence to bring out desired effect in a gene without any foreign element (Bortesi and Fisher, 2015). These techniques have the ability to alter an organism's DNA (Belfort and Benocora, 2014) and has opened myriad of opportunities in insect pest management through enhancing plant resistance to insect pests. In these techniques targeted genome sequence is cut at certain sections by nuclease enzymes. The most popular genome editing tool is clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (Ricroch *et al*; 2017). The two main pests of the CRISPR/Cas9 system are Ca9 protein and single guide RNA's (Sg RNA's) that can be easily engineered. Genome editing brought about by Cas9 requires a process of DNA cleavage followed by DNA repair. CRISPR-Cas9 technology not only allows replacing, regulating or removing genes in plants but in animals as well and providing heritable and desired modifications (Ricroch *et al*; 2017).Multiplexing or double standard breaks (DSB's) at many locations in a genome that can be employed to bring desired effects in multiple genes in a single process is the hallmark of CRISPR-Cas9 technology (Li *et al*,2013).This

technique has evolved as an effective tool for producing insect pest resistance in plants. Insects use visual appearances gustatory senses and ovipositional sites to find host plants (Larsson *et al*; 2004). Plant secondary metabolite mixtures can be changed by gene editing that would result in less or no herbivory. In a study transgenic tobacco plants leaves changed to red due to over production of anthocyanin pigmentation that reduced the attack of *Spodoptera litura* and *Helicoverpa armigera* (Malone *et al;*2009). The function of SFABCC2 a cry1F gene responsible for imparting resistance to *Spodoptera frugiperda* can be studied by (CRISPR) / Cas9 gene editing technique (Ming *et al*; 2021). The mutated cytochrome P450 gene CYP71A that converts tryptamine to serotoxin showed high levels of salicylic acid in insect resistant rice plants (Lu *et al*; 2018). The next generation insect resistant plants could be developed through CRISPR gene editing method as it has the ability to alter only specific gene.

15. **Marker assisted selection:** Phenotypic selection with the help of molecular markers in crop improvement is known as marker assisted selection (MAS). The individuals are selected not on their phenotype traits but on their genotype traits. The efficiency of conventional plant breeding has been enhanced by the identification of various molecular markers such as single nucleotide polymorphism (SNP). The most advanced method for introduction of desired genes into desired crop in appropriate combination are the molecular marker techniques. Resistance was enhanced in Minghiri 63 and its derived hybrids against Brown plant hopper (BPH) through introduction of BPH14 and BPH15 with the half of marker assisted selection (Hu *et al*;2012) .Rice susceptible cultivar C418 was crossed with a brown plant hopper *Nilaparvata lugens* resistance gene BPH2 derived from rice line, ASD7 and evaluated through marker assisted selection (Li-Hong *et al*; 2006) pyramiding of two BPH resistant genes BPH3 and BPH27 introgressed into rice cultivars demonstrated elevated resistance to BPH, was accomplished by marker assisted pyramiding (Liu *et al*; 2016). Biotypes 2 and 3 of BPH encountered resistance in rice cultivars developed through marker assisted selection (Shabanimofred *et al*; 2015). In rice chromosome 12,two resistant genes BPH 1 and BPH2 to BPH had been inserted successfully trough marker assisted gene pyramiding (Sharma *et al*; 2012).The benefits of marker assisted selection in imparting resistance to plants is due to its flexibility like cost effective and quick than phenotypic assays, compatible with seedling materials, evaluation of multiple markers on single DNA strand and less effect of biotic conditions. The marker assisted selection holds future of biotechnology in host plant resistance.

16. **Host plant resistance through RNA interference:** RNA interference RNAi or post transcriptional gene silencing (PTGS) is a method of gene suppression by suppressing specific sequences. It is a unique method brought about by double stranded RNA (dsRNA) by gene silencing mechanisms at cellular level. Undesirable genes are repressed when dsRNA is infected into a cell (Kamthan *et al*; 2015). Ingestion of dsRNA into the insect pest is the fulcrum of RNAi tactic for the pest management. The dsRNA upon ingestion expresses and disperses throughout the insect body (Katoch *et al;*2013). The sap sucking insects could be controlled by RNAi techniques that easily feed on transgenic crops. Specific gene silencing is utilized by dsRNA's in plants with the aim to develop disease resistance. Rice knockdown lines TT51(cryAb and cryAc) and TIC-19(cry1Ac) provided resistance to *C. Suppressalis* (Qiu *et al;*2017). Maize with silenced genes dvvgr and dvbol reduced the fertility and larval feeding in western corn root worm *D. Virgifera* (Niu *et al*; 2017). Greater mortality in soyabean aphid aphis glycines was achieved by

nano carrier formulation of ds RNA targeted the TREH, ATPD, ATPE and CHSI genes (Yan *et al*; 2020). Inhibition of juvenile hormone methyl transferase (JHMT) in *Helicoverpa armigera* was observed in transgenic cotton lines that had BT toxin combined with RNAi (Ni *et al*;2017). Reduced larval weight and length was observed in yellow rice stem borer by knockdown of acetylcholine esterase gene (AchE) in rice lines (Kola *et al*;2019). Larval stunting and mortality was seen in western corn rootworm *Diabrotica virgifera virgifera* as a result of ingestion of (ds)RNSAs in an artificial diet that caused RNA interference (Baum *et al*; 2007). Gene knockdown and enhanced mortality of sucking and borer insects brought about by spraying of dsRNA in maize (Li *et al*; 2015). In order to bring resistance in tobacco and tomato chitinase gene (HacHI) was silenced that produced HI-RNAi induced abnormalities in *Helicoverpa armigera* (Mamta *et al*; 2016). Phenolic glucoside malonyl transferase encoding gene that detoxifies phenolic glycosides was targeted by dsRNA in tomato plants, resulted in complete resistance to the tobacco whitefly, *Bemisia tabaci* (Xia *et al*; 2021). Chloroplast expressed dsRNA caused mortality to the tobacco hornworm, tobacco whitefly and Colorado potato beetle (Dong *et al*; 2020). The transgenics developed through RNAi interference is presented in Table 3.

Table 3. Crop wise list of transgenics developed through RNA interference for insect resistance.

S.N	Crop	Silenced gene	Insect controlled	References
1.	Cotton	Juvenile hormone methyl transferase (JHMT)	*Helicoverpa armigera*	Ni *et al.* (2017)
2.	Maize	Suppression of target mRNA	*Diabrotica virgifera virgifera*	Baum *et al.* (2007)
		hunchback (hb) and brahma (brm) gene	*Diabrotica v. virgifera*	Khajuria *et al.* (2015)
		dsRNA-Spray	*Lepidopteran*	Li *et al.* (2015)
		Dvvgr, dvbol	*Diabrotica virgifera virgifera LeConte*	Niu *et al.* (2017)
3.	Potato	*β-actin* gene	*Leptinotarsa decemlineata*	Zhang *et al.* (2015)
		ECR gene	*Leptinotarsa decemlineata*	Hussain *et al.* (2019)
	Rice	Aminopeptidase N genes *APN1+APN2*	*C. suppressalis*	Qiu *et al.* (2017)
		AchE-Acetylcholine esterase	*Scirpophaga incertulas*	Kola *et al.* (2019)
4.	Soyabean	SpbPO-dsRNA	*Leguminivora glycinivorella*	Meng *et al.* (2017)
		TREH, ATPD, ATPE, CHSI	*Aphis glycines*	Yan *et al.* (2020)
5.	Tomato, Tobacco	Chitinase gene-*HaCHI*	*H. armigera*	Mamta *et al.* (2016)
6.	Tobacco	*v-ATPaseA* gene	*Manduca sexta*	Burke *et al.* (2019)
		BtACTB gene	*Bemisia tabaci*	Dong *et al.* (2020)
		Phenolic glucoside	*Bemisia tabaci*	Xia *et al.* (2021)

		malonyltransferase		
		Sl 102 immune gene	*Spodoptera littoralis*	Di Lelio *et al.* (2022)

RNAi technology is proving to be in silencing or knocking down target genes in various insects and develop insect resistant plants, however the real challenge lies in the better uptake of dsRNA by the insect body. There are two ways of transport of dsRNA for its efficient utilization in the field. In one method high concentration synthesis of dsRNA is achieved and then sprayed to target crops as a conventional spray to kill the insect pests. The method is also known as spray induced gene silencing (SIGS). The (HIGS) host induced gene silencing where in crop genome is involved for the transgenic expression of dsRNA (Christiaens *et al*;2020a). However, in both the methods gene silencing is the common approach. The ability of the dsRNA to be taken up by the insects determines the success of this technology. Some novel spray formulations based on nano materials is being undertaken to stabilize and improve uptake of dsRNA (Christiaens *et al*;2020b)

V. CONCLUSION

Ever since the green revolution began to provide its dividends insect pests become a dominant figure in agriculture. Management of insect pests is a global concern and farmers rely on the use of pesticides to contain them. The negative effects of pesticides on humans and other non-target organisms compel us to look for other pest management options. The development of plants that are resistant to insect pests provide a suitable alternative in this regard. The identification of resistant insect in plants has been achieved with great success, but to breed a crop plant with traditional breeding process slow and cumbersome due to snarl traits at multiple loci. New technological interventions with cutting edge biotechnological tools have opened a new dimension to pest control. These interventions offer protection against invasive and non-invasive crop pests across all cultivable plants by employing new molecules editing genes so that their expression and pattern changes and exploiting genes that are insecticidal in nature. By exploiting delta endotoxin, lectins, protease inhibitors, gene editing, development of ideal host plant resistant traits has become a reality. Genome editing by CRISPR/cas9 along with RNA interference has lifted the barriers of crop breeding for insect resistance. Modern biotechnological tools have come as a blessing for mankind in the fight against insect pests that has proven to be environmentally safe, cost effective and compatible with other pest management practices. Silencing plant genes to the advantage host plants has been accomplished with advances in RNAi and CRISPR techniques.

There are numerous advantages of biotechnological techniques as far as management of insect pests are concerned, but at the same time it is of paramount importance to take cognizance of potential impacts on environmental externalities. Hazards associated with the adoption of resistant plants to insects should be considered especially for developing nations where regulatory mechanisms are feeble or absent. By striking a balance between negative and positive impacts, biotechnology exhibit a unique combination of science from lab to land and can make a meaningful contribution to the society in terms of improved nutritional quality and pest and disease resistance in crop plants.

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