



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 145-150
Received: 19-09-2018
Accepted: 21-10-2018

Tara Yadav

Ph.D. Scholar, Division of
Entomology, Rajasthan
Agricultural Research Institute
(RARI); Durgapura, S.K.N.
Agriculture University, Jobner,
Rajasthan, India

Veer Singh

Professor, Dept. of Entomology,
Swami Keshwanand Rajasthan
Agricultural University, Bikaner,
Rajasthan, India

VS Acharya

Associate Professor, Dept. of
Entomology, Swami
Keshwanand Rajasthan
Agricultural University, Bikaner,
Rajasthan, India

Ramawtar Yadav

Ph.D. Scholar, Dept. of
Entomology, Swami
Keshwanand Rajasthan
Agricultural University, Bikaner,
Rajasthan, India

Correspondence**Tara Yadav**

Ph.D. Scholar, Division of
Entomology, Rajasthan
Agricultural Research Institute
(RARI); Durgapura, S.K.N.
Agriculture University, Jobner,
Rajasthan, India

Molecular approaches and their role in integrated pest management

Tara Yadav, Veer Singh, VS Acharya and Ramawtar Yadav

Abstract

Recent advances in plant molecular biology have opened new avenues for the production of genetically engineered plants and in the precise transfer of novel genes into crop plants from diverse sources. A series of genes has been transferred through various transformation techniques including genes for several agronomically important traits such as herbicide resistance, enzyme inhibitors (amylase inhibitors, protease inhibitors), lectin proteins, disease and insect resistance. The application of transgenic technology has resulted in useful GM insect-resistant varieties by genetic engineering (GE). Crops expressing *Cry* toxins derived from *Bacillus thuringiensis* (*Bt*) have been planted globally and are a vital tool for pest control. Molecular techniques employed for identifying and monitoring establishment and dispersal of specific biotypes of natural enemies. Production, formulation and storage of entomopathogenic fungi can be dramatically improved through biotechnology and genetic engineering.

Keywords: GM Crops, Bt, resistance, enzyme inhibitors, natural enemies.

Introduction

GMCs are plants used in agriculture, the DNA of which has been modified using genetic engineering methods. The aim is to introduce a new trait to the plant which does not occur naturally in the species. With the advent of genetic transformation techniques, it has become possible to clone and insert genes into the crop plants to confer resistance to insect pests (Bennett, 1994). The first genetically modified plant was produced in 1983, using an antibiotic-resistant tobacco plant. The first genetically modified food approved for release was the Flavr Savr tomato in 1994. China was the first country to commercialize a transgenic crop in 1993 with the introduction of virus-resistant tobacco. A series of genes has been transferred through various transformation techniques including genes for several agronomically important traits such as herbicide resistance, quality improvement, disease and insect resistance, virus resistance, slow ripening and softening (Willmitzer, 1988; Shi *et al.* 1994) [66, 56]. The introduction of transgenic technology has added a new era to pest control and becoming vital component of integrated pest management worldwide. In the past two decades, transgenic technology has been developed to generate insect-resistant crops for reducing both yield loss and pesticide utilization (Christou *et al.* 2006) [14]. Resistance to insects has been demonstrated in transgenic plants expressing genes for delta-endotoxin from *Bacillus thuringiensis* (*Bt*), protease inhibitors, α -amylase inhibitors, enzymes and plant lectins (Sharma *et al.* 2000; Padul *et al.* 2012) [54-55, 46]. *Bacillus thuringiensis* (*Bt*) insect-resistant crops are one of the most outstanding achievements in plant transgenic technology, which have achieved significant success economically and ecologically. Most of the plant derived genes produce chronic rather than toxic effects and some insect pests are not sensitive to some of these factors. The potential of plant derived genes can be realized by deploying them in combination with host plant resistance and exotic genes. Genes conferring resistance to insects have been inserted into crop plants such as maize, cotton, potato, tobacco, potatoes, rice broccoli, lettuce, walnuts, apples, alfalfa and soybean (Griffiths, 1998) [26]. The need for genetic transformation of crops to improve crop production in the developing world has been discussed by Sharma and Ortiz (2000) [54-55]. There is an urgent need to develop a scientifically sound strategy to deploy exotic genes for minimizing the extent of losses caused by insect pests. Equally important is the need for following the biosafety regulations, more responsible public debate, social attitude and better presentation of the benefits for a rational deployment of the genetically transformed plants.

Importance of Biotechnology

There is a continuing need to increase food production, particularly in the developing countries and this increase has to come from increased yields from major crops grown on

existing cultivable lands. The most important objective is to alter the amino acid compositions to improve the nutritional properties of seeds. De Lumen (1990) ^[18] has discussed some of the molecular approaches to improve the nutritional and functional properties of seeds for food purposes. Genetic engineering can be used to improve protein quality by way of increasing the proportion of a specific amino acid within a protein. Genes from bacteria such as *Bacillus thuringiensis* (*Bt*) and *Bacillus sphaericus* (Gill *et al.* 1992; Charles *et al.* 1996) ^[25, 15] have been the most successful group of organisms identified for use in genetic transformation of crops for pest control on a commercial scale. Protease inhibitors, plant lectins, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins from *Bt* and related species, and small RNA viruses can also be used alone or in combination with *Bt* genes to generate transgenic plants for pest control (Hilder and Boulter, 1999) ^[30].

Application of Biotechnology for Insect Pest Management

Biotechnology has a significant role in improving efficacy, cost-effectiveness and in expanding the markets for the bio insecticides. Molecular techniques employed for identifying and monitoring establishment and dispersal of specific biotypes of natural enemies. (Tipvadee, 2002) ^[62]. It provides opportunities for the development of insect natural enemies conferring beneficial traits such as pesticide resistance, cold hardiness and sex ratio alteration. A number of insect-specific baculoviruses (NPVs) have been modified to contain genes which, when expressed in the host insect, produce insecticidal effects (Bishop, 1989). Development and deployment of transgenic plants with insecticidal genes for pest control will lead to reduction in insecticide sprays and increased activity of natural enemies (Sharma *et al.* 2000) ^[54-55].

Gene's transfer for resistance to insects

Bacillus thuringiensis (*Bt*)

Bacillus thuringiensis is a soil inhabiting gram-positive, facultative bacterium, which produces proteinaceous crystalline inclusion bodies during sporulation. Ishiwata discovered this bacterium in 1901 from diseased silkworm (*Bombyx mori*) larvae. Berliner (1915) ^[7] isolated it from diseased larvae of *Ephetia kuhniella* and designated it as *Bacillus thuringiensis*. *Bt* toxins are highly effective for many insect pest, like lepidopterans, coleopterans, dipterans and other related species (Talukdar, 2013) ^[61]. Genetic manipulation of *Bt* genes encoding for proteins toxic to insects offers an opportunity to produce genetically modified strains with more potent. *Bt* is a potent insecticide containing crystal protein endotoxin. The *Bt*-crystal (*Cry*) insecticidal protein (δ -endotoxin) genes are highly specific and represent a class of numerous proteins with insecticidal action on larvae from various orders. *CryI* and *Cry2* are toxic for lepidopteran pests, *Cry2A* for lepidopterans and dipteran pests, and *Cry3* for coleopteran pests (Malone *et al.* 2008) ^[41]. *Bt-cry* protein is toxic to insects, but non-toxic to humans and animals. When the insect larvae feed on transgenic plant, crystals and spores are ingested into the midgut of the insect. Since the pH is alkaline in nature, so the crystals become toxic to insect midgut leading to septicaemia.

The first transgenic crop was grown in 1994 and large-scale cultivation was taken up in 1996 in USA (McLaren, 1998) ^[42] and subsequently grown in several countries including Argentina, Australia, China, Colombia, Indonesia, Mexico, South Africa, and India (James, 2011) ^[36]. The adoption of *Bt* crop varieties by farmers has been rapid reflecting the benefits

of these crops such as reduced insecticide use, lower production costs and higher yields (Brookes and Bar foot 2005) ^[10]. *Bt*-maize and *Bt*-cotton are the only insect-resistant GM crops for commercial planting in world (Ibrahim mabubu *et al.* 2016) ^[34]. The first generation of insect-resistant crops that were commercialized expressed single *Bt-Cry* genes. Each produce a single *Bt*-toxin active against important lepidopteran pests and kill a narrow set of target pests, which poses a relatively high risk that insect will evolve resistance to the toxin. This narrow range of action and concerns about the evolution of pest resistance accelerated to the development of *Bt*-crops producing more than one toxin. Second generation *Bt* crops have between two to six different toxins (Tabashnik *et al.* 2009) ^[59]. According to Gatehouse *et al.* (2008) ^[23, 41] second and third generations have mitigated this risk by stacking or pyramiding different genes such as multiple but different *Cry* genes and *Cry* genes combined with other insecticidal proteins, which target different receptors in insect pests but also provide resistance to a wider range of pests and delay evolution of resistance in pests. In comparison to single-toxin *Bt* crops, multi-toxin *Bt*-crops can be more effectively control pests and reduce crop damage, which may increase environmental and economic benefits.

The effect of *Bt* toxins on a range of lepidopteran insects has been studied including, *Helicoverpa armigera* (Estela *et al.* 2004) ^[20], *Heliothis virescens* (Ryerse, 1990) ^[49], *Ostrinia nubilalis* (Hua *et al.* 2001; Li *et al.* 2004) ^[33, 39] and *Spodoptera littoralis* (Avisar *et al.* 2004) ^[2]. VIP 3 has been isolated from *B. thuringiensis* supernatants, which is highly toxic to *Agrotis* and *Spodoptera* (Estruch *et al.* 1996) ^[19]. VIP 3A+ *Cry* 1Ab expressing line (Cot 102/67B) gives maximum mortality of susceptible and resistance strain of *Heliothis virescens* as compared to individual toxin expressing line and non *Bt* line. *Bacillus israeliensis* has been used extensively for the control of mosquitoes (Barjac de and Sotherland, 1990) ^[4]. *Bacillus thuringiensis* var. *morrisoni* and *B. israeliensis* carry four genes that encode mosquito and blackfly toxins *Cry IVA*, *Cry IVB*, *Cry IVC* and *Cry IVD* (Bechtel and Bulla, 1976) ^[5].

Resistance Management Principles for *Bt* Crops

Recently there have been reports of field resistance to *Bt* crops in pink bollworm, *Pectinophora gossypiella*, cotton bollworm (*Helicoverpa* spp. armyworm (*Spodoptera frugiperda*) and western corn rootworm, *Diabrotica virgifera virgifera* (Tabashnik *et al.* 2008) ^[60]. A refuge strategy is necessary to ensure long term resistance management. The main approach for delaying evolution of resistance to *Bt* crops is the refuge strategy. Farmers are mandated to maintain an abundance of host non-*Bt* crops as a refuge surrounding their *Bt* crops (Gould, 1998) ^[27]. Gene pyramiding strategy also play important role in insect pest resistance management. Genes, that have been used in genetic transformation of crops, are either too specific or are only mildly effective against the target insect pests. Some insect species are also insensitive to some of these genes. Therefore, to convert transgenes into an effective weapon in pest control, *e.g.*, by delaying the evolution of insect populations resistant to the target genes, it is important to deploy genes with different modes of action in the same plant. Activity of *Bt* in transgenic plants can be enhanced by serine protease inhibitors (MacIntosh *et al.* 1990) ^[40]. Activity of *Bt* can also be increased in combination with tannic acid (Gibson *et al.* 1995) ^[24]. According to Hoffmann *et al.* (1992) ^[31] tobacco plants expressing *Bacillus thuringiensis* var. *kurstaki* HD-73 delta endotoxin or cowpea trypsin inhibitor (CpTI) for their efficacy against *Helicoverpa*

zea in the field. Mortality of *H. zea* larvae was significantly higher and leaf damage significantly lower for the genotypes containing *Bacillus thuringiensis* gene compared with non-transgenic control. Transgenic sugarcane plants engineered to express either the potato proteinase inhibitor II or the snowdrop lectin gene showed increased antibiosis to larvae of sugarcane grubs (*Antitrogon consanguineus*) in glasshouse trials (Nutt *et al.* 1999) [44].

Grower education, adoption, and compliance are essential to the implementation and success of a long term resistance management strategy. *Bt* crops are to be used as part of an integrated pest management programme to enhance pest management goals. Coordinated annual performance monitoring and surveillance is necessary to detect or follow resistance development. IRM strategies should be tailored to address specific regional resistance management concerns, as appropriate. Deployment of IPM tactics with different modes of action, including conventional pesticides, *Bt* toxins expressed in crops with different modes of action, biological control methods and other control methods, it is essential for sustainable pest management goals. Continued resistance management research should be conducted to evaluate the effectiveness of and be used to modify, as necessary, IRM, strategies for *Bt* crops. Immediate and coordinated remedial action for suspected and confirmed incidents of resistance is necessary.

Alpha amylase inhibitors

The enzyme inhibitors impede digestion through their action on insect gut. Digestive enzymes alpha-amylases and proteinase, which play a key role in the digestion of plant starch and proteins (Franco *et al.* 2000). Carbohydrate metabolism in insects has been targeted through the use of α -amylase inhibitors. Amylase inhibitors from wheat (WAAI) and common bean (BAAI) have been identified. Since the metabolic energy requirement is essential for larval development, this energy is received by starch hydrolysates (Carlini, 2002) [12]. Insect pests like, *Callosobruchus maculatus* seed weevils and maxican bean weevil, *Zabrotus subfaciatus*, Red flour beetle, *Tribolium castaneum* (Herbst) are extensively starch dependent insects and utilize α -amylase for their survival (Strobl, 1998; Cinco, 2008) [58, 16]. Transgenic tobacco expressing WAAI gene has been reported to increase mortality of the lepidopteran larvae between 30 to 40% (Carbonero *et al.* 1993) [11]. Genes encoding for BAAI have been expressed in pea by the *phal* gene promoter to direct high levels of expression in seeds to increase the levels of resistance to *Collosobruchus* spp. (Shade *et al.* 1994; Schroeder *et al.* 1995) [52, 51]. Amylase inhibitors occur in many plants as part of the natural defense mechanisms. Amylase inhibitors are of great interest as potentially important tools of natural and engineered resistance against pests in transgenic plants (Ishimoto, 1989; Valencia, 2000) [35, 63].

Protease Inhibitors

Protease inhibitors inhibit the protease activity of various proteolytic enzymes, causes hyper production of digestive enzymes which enhances the loss of sulphur amino acids, which leads to stunted growth and weaker insects. (Johnson *et al.* 1989; Hilder *et al.* 1992) [37, 29, 31]. Serine proteinases have been identified in extracts from the digestive tracts of insects from many families, particularly those of Lepidoptera (Houseman, 1989) [32] and many of these enzymes are inhibited by proteinase inhibitors. Serine proteinase inhibitors have anti-nutritional effect against several lepidopteran insect

species (Applebaum, 1985) [1]. Trypsin inhibitors at 10% of the diet were toxic to larvae of the *Callosobruchus maculatus* (Gatehouse, 1983) [22] and *Manduca sexta* (Shulke, 1983) [57]. The transgenic plants were resistant against herbivorous insects such as *Callosobruchus maculatus*, *Heliothis spodoptera* and *Diabrotica* and *Tribolium sp.* (Johnson *et al.*, 1989) [37] transformed tobacco plants with gene coding tomato and potato inhibitor proteins and the transgenic plants found resistant to *M. sexta*. The possible role of protease inhibitors (PIs) in plant protection was investigated as early as 1947, when Mickel and Standish observed that the larvae of certain insects were unable to develop normally on soybean products. Subsequently the trypsin inhibitors present in soybean were shown to be toxic to the larvae of flour beetle *Tribolium confusum* (Lipker, 1954) [38].

Plant lectin

Plant lectins are a heterogeneous group of sugar binding proteins, which have a protective function against a range of organisms (Sharma *et al.* 2000) [54-55]. The most likely mechanisms underlying the entomotoxic activity of lectins involve interactions with different glycoproteins or glycan structures in insects, which may interfere with a number of physiological processes in these organisms. Since lectins possess at least one carbohydrate-binding domain and different sugar specificities and considering the variety of glycan structures in the bodies of insects (Vandenborre *et al.* 2011; Vandenborre *et al.* 2010) [64, 65], possible targets for lectin binding are numerous. Antinutritional effects are often observed as a result of lectin ingestion and could affect several biological parameters in insects, such as larval weight, larval development period, adult emergence fecundity, pupation and survival (Oliveira *et al.* 2011) [45]. The snowdrop lectin (*Galanthus nivalis* agglutinin or GNA) has received particular attention due to its toxic effects against hemipterans and other economically important insect pests. Powell *et al.* (1998) [47] showed GNA binding to cell surface carbohydrate moieties in the midgut epithelium of brown rice plant hoppers (*Nilaparvata lugens*). Lectins produce chronic effects on survival and development of insect pests belonging to different insect orders (Czapla and Lang, 1990; Habibi *et al.* 1992) [17, 28]. Transgenic tobacco expressing pea expressing pea lectin has shown adverse effects against *H. virescens* (Boulter *et al.* 1990) [9].

Secondary plant metabolites

Secondary plant metabolites such as alkaloids, steroids, foliar phenolic esters (rutin, chlorogenic acid, *etc.*) terpenoids, cyanogenic glycosides, glucosinolates, saponins, flavonoids, pyrethrins and non-protein amino acids act as potent protective chemicals. Some of the secondary plant metabolites are produced in response to insect feeding, infection by pathogens, and abiotic stress factors. These compounds are called phytoalexins (Sharma and Agarwal, 1983) [53]. Secondary metabolites present in plants apparently function as defense (toxic), which inhibits reproduction and other processes (Rattan, 2010) [48]. *Detarium microcarpum*, *Sclerocarya birrea*, *Piper guineense* as seed protectants for maize (*Sitophilus zeamais*), *Cassia nigricans* Vahl oil and the plant as grain protectants of stored wheat weevil, *Tribolium castaneum*, as well as containing biologically active compounds, that may serve as candidates for new formulations in the treatment and prevention of livestock diseases and pest management (Ayo, 2010) [3]; *Lantana camara* as grain protectants of cowpea seeds *Causus*

maculatus (Schery 1954; Champagne *et al.* 1989)^[50, 13]. *Arabidopsis* mutants deficient in linoleic acid cannot synthesise jasmonate and are susceptible to the fungal gnat (*Bradasia impatiens*). Xu *et al.* (1993)^[67] observed enhanced resistance in rice by wounding methyl jasmonate and abscisic acid in transgenic plants.

Future of transgenic crops for insect pest management

Over the next years, there is continuing need to increase food production. The implementation of agricultural machinery and food technology plays a prominent role in this increase, as well as the amplification of planted land. With the wide food supply, insect attacks will probably increase in the same proportion. Transgenic crops have clearly increased profitability for farmers in developed and developing countries. We need to pursue the management strategy that reflects the pest biology, insect plant interactions and their influence on the natural enemies to prolong the life span of the transgenics. Refugia can play an important role in resistance management and should take into account the pest complex, the insect hosts and the environment. Expression of more than one gene (gene pyramiding) and single chain antibody genes, which would be compatible with the likely trends in pesticide discovery using biology derived target based methods. Equally important is the need to follow the biosafety regulations and make this technology available to farmers, who cannot afford the high cost of seeds marketed by the private sector.

Conclusion

The continuous use of pesticides for crop protection had resulted in damaging impact on biological ecosystems. The use of target specific compounds with low persistence of intrinsic plant resistance mechanisms are safer alternative strategies for effective insect pest's management. The transgenic crops developed for insect resistance need to be compatible with other components of integrated pest management programmes for pest resistance to be durable and impact on agricultural systems. The ideal transgenic technology should be commercially feasible, environmentally benign (biodegradable), and easy to use in diverse agro-ecosystems as well as show a wide-spectrum of activity against the crop pests. It should also be harmless to the natural enemies, target the sites in insects that have developed resistance to the conventional pesticides, flexible enough to allow ready deployment of alternatives (if and when the resistance is developed by the pest), and preferably produce acute rather than chronic effects on the target insects. However, the use of biotechnology brings questions regarding the potential impact of those genetically modified organisms (GMOs) or plants to human, animal and environment. National biosafety and regulatory systems for proper management of GMOs must be in place to enable the full exploitation of biotechnology.

References

- Applebaum SW. Biochemistry of digestion. In: Kerbot, G.A. and Gilbert, L.I., eds. 1985; 4:279-311
- Avisar D, Keller M, Gazit E, Prudovsky E, Sneh B, Zilberstein A. The role of *Bacillus thuringiensis* CryIC and CryIE separate structural domains in the interaction with *Spodoptera littoralis* gut epithelial cells. J Biol Chem. 2004; 279:15779-15786.
- Ayo RG. Phytochemical Constituents and Bioactivities of the Extracts of *Cassia nigricans* Vahl. A Review. J Med. Plant Res. 2010; 4(14):1339-1348.
- Barjac de H, Sothier DJ. Bacterial control of mosquitoes and blackflies. Rutgers University Press, New Brunswick, New Jersey, USA, 1990.
- Bechtel DB, Bulla LA Jr. Electron microscope study of sporulation and parasporal crystal formation in *Bacillus thuringiensis*. Journal of Bacteriology. 1976; 127:1472-1483.
- Bennett J. DNA-based techniques for control of rice insects and diseases: Transformation, gene tagging and DNA fingerprinting. In: Rice pest science and management, Teng, K.L. Heong and K. Moody (Eds.), pp. International Rice Research Institute, Los Banos, Philippines, 1994, 147-172.
- Berliner E. Über die Schallfsuchi der Mehlmottenraupo (*Ephestia kuhniella* Zell.) und thren Erreger, *Bacillus thuringiensis* sp. Zietschfit. fur Angewandte Entomologie 1915; 2:29-56.
- Bishop DH. Genetically engineered viral insecticides-a progress report 1986-1989. Pestic. Sci. 1989; 27:173-189.
- Boulter D, Edwards GA, Gatehouse AMR, Gatehouse JA, Hilder VA. Additive protective effects of incorporating two different higher plant derived insect resistance genes in transgenic tobacco plants. Crop Protection. 1990; 9:351-354.
- Brookes G, Bar foot P. GM Crops: The Global Economic and Environmental Impact-The First Nine Years 1996-2004. Ag Bio Forum. 2005; 8:187-196.
- Carbonero P, Royo J, Diaz I, Garcia-Maroto F, Gonzalez-Hidalgo E, Gutierrez C, Casanera P. Cereal inhibitors of insect hydrolases (a-amylases and trypsin): genetic control, transgenic expression and insect pests. In: Workshop on engineering plants against pests and pathogens, 1-13 Jan 1993, G.J. Bruening, F. Garcia-Olmedo and F.J. Ponz (Eds.). Instituto Juan March de Estudios Investigaciones, Madrid, Spain, 1993.
- Carlini CR, Grossi-de-Sa MF. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon. 2002; 40:1515-1539.
- Champagne DE, Isman MB, Towers GHN. Insecticidal activity of Phytochemicals and Extracts of the Meliaceae. Am. Chem. Soc. Symp. Ser. 1989; 387:95-109.
- Christou P, Capell T, Kohli A, Gatehouse JA, Gatehouse AM. Recent developments and future prospects in insect pest control in transgenic crops. Trends in Plant Science. 2006; 11:302-308.
- Charles JF, Nielsen-LeRoux C, Delecluse A. *Bacillus sphaericus* toxins: Molecular biology and mode of action. Annual Review of Entomology. 1996; 41:451-472.
- Cinco-Moroyoqui FJ, Diaz-Malvaez FI, Alanis-Villa A, Barron-Hoyos JM, Cardenas-Lopez JL, Cortez-Rocha MO, Wong-Corral FJ. Isolation and partial characterization of three is oamylases of *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae). Comp Biochem. Physiol. 2008; 150B:153-160.
- Czapla TH, Lang BA. Effects of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and southern corn rootworm (Coleoptera: Chysomelidae). Journal of Economic Entomology. 1990; 83:2480-2485.
- Delumen BO. Molecular approaches to improving the nutritional and functional properties of plant seeds as food sources: developments and comments. Journal of Agricultural and Food Chemistry, 1990; 38:1779-1788.

19. Estruch JJ, Warren GW, Mullins MA, Nye GJ, Craig JA, Koziel MG. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proceedings, National Academy of Sciences, USA. 1996; 93:5389-5394.
20. Estela A, Escriche B, Ferré J. Interaction of *Bacillus thuringiensis* toxins with larval midgut binding sites of *Helicoverpa armigera* (Lepidoptera: Noctuidae). Appl Environ Microbiol. 2004; 70:1378-1384.
21. Franco OL, Rigden DJ, Melo FR, Grossi-De-Sá MF. Plant alpha-amylase inhibitors and their interaction with insect alpha-amylases. Eur J Biochem. 2002; 269(2): 397-412.
22. Gatehouse AMR, Boulter D. J of the Sci. of Food and Agri. 1983; 34:345- 350.
23. Gatehouse J. Biotechnological prospects for engineering insect resistant plants. Plant Physiology. 2008; 146:881-887.
24. Gibson DM, Gallo LG, Krasnoff SB, Ketchum REB. Increased efficiency of *Bacillus thuringiensis* subsp. *kurstaki* in combination with tannic acid. Journal of Economic Entomology. 1995; 88:270-277.
25. Gill SS, Cowles EA, Pietrantonio FV. The mode of action of *Bacillus thuringiensis* endotoxins. Annual Review of Entomology. 1992; 37:615-636.
26. Griffiths W. Will genetically modified crops replace agrochemicals in modern agriculture? Pesticide Outlook. 1998; 9:6-8.
27. Gould F. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. Annu. Rev. Entomol. 1998; 43:701-726.
28. Habibi J, Backus EA, Czapla TH. Effect of plant lectins on survival of potato leafhopper. Proceedings, XIX International Congress of Entomology. Beijing, China. 1992, 373.
29. Hilder VA, Gatehouse AMR, Boulter D. Transgenic plants conferring insect tolerance: protease inhibitor approach. In: Transgenic Plants, S. Kung and R. Wu (Eds), Academic Press, New York, USA. 1992, 310-338.
30. Hilder VA, Boulter D. Genetic engineering of crop plants for insect resistance - a critical review. Crop Protection. 1999; 18:177-191.
31. Hoffman MP, Zalom FG, Wilson LT, Smilanick JM, Malyj LD, Kisen J, Hilder VA, Barnes WM. Field evaluation of transgenic tobacco containing genes encoding *Bacillus thuringiensis* d-endotoxin or cowpea trypsin inhibitor: efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae). Journal of Economic Entomology. 1992; 85:2516-2522.
32. Houseman JG, Downe AER, Philogene BJR. Canadian J of Zool. 1989; 67:864-868.
33. Hua G, Masson L, Jurat-Fuentes JL, Schwab G, Adang MJ. Binding analyses of *Bacillus thuringiensis* Cry d-endotoxins using brush border membrane vesicles of *Ostrinia nubilalis*. Appl Environ Microbiol. 2001; 67:872-879.
34. Ibrahim Mabubu J, Hua NH. Advances of transgenic *Bt*-crops in insect pest management: An overview. Journal of Entomology and Zoology Studies. 2016; 4(3):48-52.
35. Ishimoto M, Kitamura K. Growth inhibitory effects of an amylase inhibitor from kidney bean, *Phaseolus vulgaris* (L.) on three species of bruchids (Coleoptera: Bruchidae). Appl. Entomo. I Zoo. I, 1989; 24:281-286.
36. James C. Global status of commercialized biotech/GM crops: ISAAA: Ithaca NY, USA, editor. ISAAA, 2011, 41.
37. Johnson R, Narvez J, Am G, Ryan CA. Natl. Acad. Sci. USA. 1989; 86:9871-9875.
38. Lipker H, Fraenkel GS, Liener IE. J of the Sci. of Food and Agriculture. 1954; 2:410-415.
39. Li HR, Oppert B, Higgins RA, Huang FN, Zhu KY, Buschman LL. Comparative analysis of proteinase activities of *Bacillus thuringiensis*-resist. Ant and susceptible *Ostrinia nubilalis* (Lepidoptera: Crambidae). Insect Biochem Mol Biol. 2004; 34: 753-762.
40. MacIntosh SC, Kishore GM, Perlak FJ, Marrone PG, Stone TB, Sims SR, Fuchs RL. Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. Journal of Agriculture and Food Chemistry, 1990; 38: 50-58.
41. Malone LA, Gatehouse AMR, Barratt BIP. Beyond *Bt*: Alternative strategies for insect-resistant genetically modified crops. In: Romeis J, Shelton AM, Kennedy GG, eds. Integration of Insect- Resistant Genetically Modified Crops with IPM Programs. Springer, Berlin, Germany. 2008, 357-417.
42. McLaren JS. The success of transgenic crops in the USA. Pesticide Outlook, 1998; 9:36-41.
43. Mickel C E, Standish J. University of Minnesota Agricultural Experimental Station. Technical Bulletin. 1947; 178:1-20.
44. Nutt KA, Allsopp PG, Mc Ghie TK, Shepherd KM, Joyce PA, Taylo GO, McQualter RB, *et al* . Transgenic sugarcane with increased resistance to cane grubs. Proceedings of the 1999 Conference of the Australian Society of Sugar Cane Technologists, Townsville, 27-30 April 1999, Brisbane, Australia. 1999, 171-176.
45. Oliveira CFR, Luz LA, Paiva PMG, Coelho LCBB, Marangoni S, Macedo MLR. Evaluation of seed coagulant *Moringa oleifera* lectin (cMoL) as a bioinsecticidal tool with potential for the control of insects. Process Biochem. 2011; 46:498-504.
46. Padul MV, Chougale AD, Dama LB, Gadge PP, Shaikh FK, Chopde RM, Vadane SS. α - amylase from sugarcane wooly aphid (*Ceratovacuna lanigera*). Trends in Biotechnology Research Journal, 2012; 1(1):49-52.
47. Powell KS, Spence J, Bharathi M, Gatehouse JA, Gatehouse AMR. Immunohistochemical and developmental studies to elucidate the mechanism of action of the snowdrop lectin on the rice brown plant hopper, *Nilaparvata lugens* (Stal). J Insect Physiol. 1998; 44:529-539.
48. Rattan RS. Mechanism of Action of Insecticidal Secondary Metabolites of Plant Origin. Crop Protect. 2010; 29(9):913-920.
49. Ryerse, JS, Beck JR, Lavrik PB. Light microscope immunolocalization of *Bacillus thuringiensis* *kurstaki* delta-endotoxin in the midgut and Malpighian tubules of the tobacco budworm, *Heliothis virescens*. J Invertebr Pathol. 1990; 56:86-90.
50. Schery RW. Plants for Man. Publ. George Allen and Unwin Ltd., London, 1954, 564.
51. Schroeder HE, Gollasch S, Moore A, Tabe LM, Craig S, Hardie DC, Chrispeels MJ, Spencer D, Higgins TJV. Bean alpha-amylase inhibitor confers resistance to pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum* L.). Plant Physiology. 1995; 107:1233-1239.

52. Shade RE, Schroeder HE, Pueyo JJ, Tabe LM, Murdock LL, Higgins TJV, Chrispeels MJ. Transgenic pea seeds expressing α -amylase inhibitor of the common bean are resistant to bruchid beetles. *Bio Technology*. 1994; 12:793-796.
53. Sharma HC, Agarwal RA. Role of some chemical components and leaf hairs in varietal resistance in cotton to jassid, *Amrasca biguttula buguttula* Ishida. *Journal of Entomological Research*. 1983; 7:145-149.
54. Sharma HC, Sharma KK, Seetharama N, Ortiz R. Prospects for using transgenic resistance to insects in crop improvement. *Journal of Biotechnology*. 2000; 3(2):76-95.
55. Sharma KK, Ortiz R. Program for the application of the genetic engineering for crop improvement in the semi-arid tropics. *In Vitro Cellular and Developmental Biology (Plant)*. 2000; 36(2):83-92.
56. Shi Y, Wang MB, Powell KS, Van Dame E, Hilder VA, Gatehouse AMR, Boulter D, Gatehouse JA. Use of the rice sucrose synthase-I promoter to direct phloem-specific expression of (3-g) beta glucuronidase and snowdrop lectin genes in transgenic tobacco plants. *Journal of Experimental Botany*. 1994; 45:623-631.
57. Shulke RH, Murdock LL. *Env. Ento*. 1983; 12:787-791.
58. Strobl S, Maskos K, Wiegand G, Huber R, Gomis-Ruth FX, Glockshuber R. A novel strategy for inhibition of α -amylases: yellow meal worm α -amylase in complex with the ragi bifunctional inhibitor at 2.5 Å resolution, *Structure*. 1998; 6:911-921.
59. Tabashnik BE, Van Rensburg JBJ, Carrière Y. Field-evolved insect resistance to *Bt* crops: definition, theory, and data. *J Econ Entomol*. 2009; 102:2011-2025.
60. Tabashnik BE, Gassmann AJ, Crowder DW, Carrière Y. Insect resistance to *Bt* crops: evidence versus theory. *Nat Biotechnol*. 2008; 26:199-202.
61. Talukdar D. Modern biotechnological approaches in insect research. *Int. Res. J of Science & Engineering*. 2013; 1(3):71-78
62. Tipvadee A. Biotechnology for insect control. *Proc. Sat. Forum*, 2002, 74-85.
63. Valencia A, Bustillo AE, Ossa GE, Chrispeels MJ. Amylases of the coffee berry borer (*Hypothenemus hampei*) and their inhibition by two plant amylase inhibitors, *Insect Biochem. Mol Biol*. 2000; 30:207-213.
64. Vandenborre G, Smagghe G, Ghesquière B, Menschaert G, Rao RN, Gevaert K, Van Damme EJM. Diversity in protein glycosylation among insect species. *PLoS One* 6: e16682. 2011
65. Vandenborre G, Van Damme EJM, Ghesquière B, Menschaert G, Hamshou M, Rao RN, Gevaert K, *et al*. Glycosylation signatures in *Drosophila*: Fishing with lectins. *J Proteome Res*. 2010; 9: 3235–3242.
66. Willmitzer L. The use of transgenic plants to study plant gene expression. *Trends in Genetics*, 1988; 4:13-18.
67. Xu D, McElroy D, Thoraburg RW, Wu R. Systemic induction of a potato pin 2 promoter by wounding methyl jasmonate and abscisic acid in transgenic rice plants. *Plant Molecular Biology*. 1993; 22: 573-588.