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Genetic Engineering of the Cry Gene as a Source of Resistance to Insect Pest of Some Major Crops

Wedajo Gebre[1*] Barko Belachew[2]
1.Jinka University, College of Agriculture and Natural Resources, Department of Plant Science
2.Jinka University, College of Agriculture and Natural Resources, Department of Biology

Abstract
In recent times, genetic engineering has become a source of agriculture innovations, providing a new solution to the age of old problems. Transfers of genes between plant species have played an important role in crop development for many decades. Advancement field of genetic engineering have provided new technologies for gene identification and gene transfer into plants has provided the opportunity for genetically engineering insect pest resistance into agriculturally desirable cultivars without altering critical quality traits. Bt toxin gene the source of the insecticidal toxins produced in commercial transgenic plants is the soil bacterium Bacillus thuringiensis (Bt). Bacillus thuringiensis synthesizes crystalline proteins during sporulation. These crystalline proteins are highly insecticidal at very low concentrations. With the advent of molecular biology and genetic engineering, it has become possible to use Bt more effectively and rationally by introducing the cry genes of Bt in crop plants. The bacterium produces an insecticidal crystal protein (ICP: also called Cry proteins, encoded by cry genes). The toxin protein binds to specific receptors present in the midgut epithelial membranes. The disturbances in osmotic equilibrium and cell lysis lead to insect paralysis and death. Scientists have mitigated this risk through 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. Alternatively, synthetic variants of Cry genes has been employed as in the case of MON863 which expresses a synthetic Bt kumamotoensis Cry3Bb1 gene against maize rootworm, which is eight times more effective than the native, non-modified version. The success of the transgenic approach led to the development of Bt crops, transgenic crops are used worldwide to control major pests of rice, cotton, maize and soybean. Rice effective against lepidopteron pests, Cotton (Gossypium hirsutum) tolerant to lepidopteran larvae (caterpillars), maize (Zea mays) tolerant to both lepidopteran and coleopteran larvae (rootworms) and soya bean (Glycine max) both lepidopteran and coleopteran larvae have become widely used in global agriculture and have led to reductions in pesticide usage and lower production costs. To overcome resistance acquired by insects against Cry toxins different strategies were employed to modify Cry functional domains to improve their toxicity. Therefore, multiple mutations/adaptations need to be made by target pests in order to develop resistance to this robust new generation of insect resistant crops.
Keywords: Cry gene, Genetic engineering, Source of resistance to insect pest
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1. Introduction

Agricultural productivity is highly influenced by pest and diseases, known as the most harmful factor concerning the growth and productivity of crops worldwide. One way to increase the quantity and quality of food is to reduce damages caused by insects, diseases and weeds to crops. Pathogens cause losses in 10-16% of the global harvest (Chakraborty et al., 2011). This figure for pest damage is about 14-25% of the total agricultural production (DeVilliers et al., 2011). In traditional agriculture, only individuals of the same species (or eventually closely related species) can be crossbred. If in this naturally available gene pool, resistance to biotic stress does not exist, traditional breeders cannot create resistance or introgression this trait into new varieties. Therefore, it is necessary to search for alternative sources of genes in other completely unrelated species of plants or in microbial organisms. Conventional breeding methods are being used to develop the varieties more resistance to biotic stresses. At the same time these methods are time taking, resource consuming and germplasm dependent. Besides it requires evaluation at hot spot area. Sometimes the screening based on natural occurrence in the hot spot areas also does not give consistent results. A combination with plant breeding approaches will likely to be needed for the improvement of crops (Roy *et al*., 2011). On the other hand, pest management by chemicals obviously has brought about considerable protection to crop yields over the past five decades. Regrettably, extensive and very often, indiscriminate usage of chemical pesticides has resulted in environmental degradation, adverse effects on human health and other organisms, eradication of beneficial insects and development of pest-resistant insects (Wahab, 2009). At this situation tool of genetic engineering has provided humankind with unprecedented power to manipulate and develop novel crop genotypes towards a safe and sustainable agriculture in the 21st century (Bates *et al*., 2005). In recent times, genetic engineering has become a source of agriculture innovations, providing a new solution to the age of -old problems (Mittler and Blumwald, 2010; Ahmad *et al*.,

2012). Plant genes are being cloned, genetic regulatory signals deciphered and genes transferred from entirely unrelated organism to confer new agriculturally useful traits on crop plants (Josine *et al*., 2011). Recent advance in genetic engineering, Bt technology has emerged as a powerful modality for battling some of the important insect pests, It is chemical free and economically viable approach for insect pest control in plants (DeVilliers and Hoisington, 2011; Sanahuja *et al*., 2011). Negotiate exchange of this transgenic technology to the **developing countries** at easy terms and its integration with the conventional approaches for resistance breeding will ensure evergreen revolution crucial for global food security (Dhaliwal and Uchimaya, 1999). In this review we mainly discussed on role of genetic engineering in crop improvement, Bt technology and Bt crops global status, benefits and limitations.

Currently, transgenic plants with herbicide, insect pests and virus disease resistance are cultivated in more than 175.2 million hectares in the world while in 1996, only 1.7 million hectares of land were under transgenic crops. Out of the 27 countries currently contributing to the cultivation of transgenic plants, 19 are developing countries and 8 industrial. During the 1996-2012 period, cumulative economic benefits from transgenic plants were high in developing countries at US$ 47.9 billion compared to US$ 59 billion generated by industrial countries (Masoud Tohidfar and Solmaz Khosravi, 2015).

2. Genetic engineering of crop plants

Before examining GM strategies for developing insect pest tolerance in plants, it is useful to consider some of the characteristics of the insects causing the damage. The first point to make is that, where as some adult insects feed off plants and can damage crops, most of the problems are caused by insect larvae.). The major classes of insect that cause crop damage are the orders Lepidoptera (Butterflies and moths), Diptera (flies and moths), Orthoptera (grasshoppers and crickets), Homoptera (aphids) and Coleopteran (beetles) (Dhaliwal *et al*., 2010). The changing scenario of insect pest problems in agriculture as a consequence of genetic engineering technology has been well documented.

Genetic engineering of plants mostly involves the addition of genetic material (single or multiple genes) that is integrated into a recipient plant, leading to the modification of the plant's genome. The plants with modified genome are known as transgenic plants or Genetically Modified (GM) plants (Pandey *et al*., 2011). Transfers of genes between plant species have played an important role in crop development for many decades (Carriere *et al*., 2010). Plant improvement whether as a result of natural selection or the efforts of plant breeder, has always relied on upon evolving, evaluating and selecting the right combination of alleles. Useful traits such as resistance to insect pests have been transferred to crop varieties from non cultivated plants, Since 1970 (Dhaliwal and Uchimaya, 1999). Success in breeding for better adapted varieties to insect pests depends upon the concerted efforts by various research domains including plant and cell physiology, molecular biology, genetics and breeding (Isbat *et al*., 2009). Advancement field of genetic engineering have provided new technologies for gene identification and gene transfer into plants has provided the opportunity for genetically engineering insect pest resistance into agriculturally desirable cultivars without altering critical quality traits (Gulzar *et al*., 2011; Karthikeyan *et al*., 2011; Tiwari and Youngman, 2011). Moreover, transgenic research has made significant progress in crop genetic improvement and offers the prospect many advantages: not just widening the potential pool useful genes but also permitting the introduction of a number of different desirable genes at a single event and reducing the time needed to introgress introduced characters into an elite genetic background, besides introduction of molecular change by genetic engineering takes less time compared to other classical genetic methods (Behrooz *et al*., 2008). Hence, genetic engineering for developing insect pest tolerant plants, based on the introgression of genes that are known to be involved in insect pest response and putative tolerance, might prove to be a faster track towards improving crop varieties.

2.1 Bt technology and the expression Cry genes

Bt toxin gene the source of the insecticidal toxins produced in commercial transgenic plants is the soil bacterium *Bacillus thuringiensis* (Bt). *Bacillus thuringiensis* synthesizes crystalline proteins during sporulation. These crystalline proteins are highly insecticidal at very low concentrations. Moreover, Bt strains show differing specificities of insecticidal activity toward pests and constitute a large reservoir of genes encoding insecticidal proteins, which are accumulated in the crystalline inclusion bodies produced by the bacterium on sporulation (Cry proteins, Cyt proteins). The bacterium produces an insecticidal crystal protein (ICP: also called Cry proteins, encoded by cry genes). Cry proteins are one of several classes of endo-toxins produced by the sporulating bacteria. With the advent of molecular biology and genetic engineering, it has become possible to use Bt more effectively and rationally by introducing the cry genes of Bt in crop plants. The mechanism of action of the Bt cry genes has been worked out in some detail. The molecular structure of at least three different cry genes has been studied. The crystals, upon ingestion by the insect larva, are solubilized in the highly alkaline midgut into individual protoxins which vary from 133-138 kDa in molecular weight, depending upon the type of protoxin (Slater *et al*., 2009). The protoxins are acted upon by midgut proteases which cleave them into two halves, the N-

terminal half which is usually of 65-68 kDa is the toxin protein. The toxin protein fragment can be divided into three domains (domains I, II and III). The first is involved in pore formation, the second determines receptor binding and the third is involved in protection to the toxin from proteases. The toxin protein binds to specific receptors present in the midgut epithelial membranes. Upon receptor binding, the domain I insert itself into the membrane leading to the pore formation. The disturbances in osmotic equilibrium and cell lysis lead to insect paralysis and death (DeVilliers and Hoisington, 2011).

The current status of Bt technology: The first generation of insect resistant crops that were commercialized expressed single Bt Cry genes, which poses a relatively high risk that insect will evolve resistance to the toxin. In the second and third generations, scientists have mitigated this risk through 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. Alternatively, synthetic variants of Cry genes has been employed as in the case of MON863 which expresses a synthetic Bt kumamotoensis Cry3Bb1 gene against maize rootworm, which is eight times more effective than the native, non-modified version(Vaughn *et al*., 2005).

This review, summarize the application of *Bt* toxin gene technology for successful development of transgenic crops ***which are used*** control major pests.

2.2. Application of Cry gene technology in some major crops

The success of the transgenic approach led to the development of Bt crops, transgenic crops are used worldwide to control major pests of rice, cotton, maize and soybean. Rice effective against lepidopteron pests, Cotton (*Gossypium hirsutum*) tolerant to lepidopteran larvae (caterpillars), maize (*Zea mays*) tolerant to both lepidopteran and coleopteran larvae (rootworms) and soya bean (*Glycine max*) both lepidopteran and coleopteran larvae have become widely used in global agriculture and have led to reductions in pesticide usage and lower production costs (Brookes and Barfoot, 2005). The first widely planted Bt crop cultivars were maize producing Bt toxin Cry1Ab and cotton producing Bt toxin Cry1Ac (Tabashnik *et al*., 2009). While most target pest populations remain susceptible to Bt crops, field-evolved resistance has been documented in some populations of five lepidopteran pests: cereal stem borer, *Busseola fusca*, in South Africa to Bt maize producing Cry1Ab (Kruger *et al*., 2009), fall armyworm, *Spodoptera frugiperda*, in Puerto Rico to Bt maize producing Cry1F (Marvier *et al*., 2008), pink bollworm, *Pectinophora gossypiella*, in western India to Bt cotton producing Cry1Ac (Bagla, 2010), cotton bollworm, *Helicoverpa zea*, in the southeastern United States to Bt cotton producing Cry1Ac and Cry2Ab (Tabashnik *et al*., 2008a, 2009) and bollworm, *Helicoverpa punctigera*, in Australia to Bt cotton producing Cry1Ac and Cry2Ab (Downes *et al*., 2010). Field-evolved resistance was reported to be associated with increased field damage by B. fusca, S. frugiperda, P. gossypiella and H. zea (Kruger *et al*., 2009; Tabashnik *et al*., 2008b, 2009; Bagla, 2010).

2.2.1 Bt Rice

The first insect-resistant genetically engineered (IRGE) rice line expressing a ***Bt*** delta-endotoxin gene driven by the ***CaMV 35S*** promoter was developed in 1989 (Yang, H. *et al*., 1989), and so far dozens of ***Bt*** rice lines have been produced in China. These Bt rice lines can be divided into three categories, namely: (i) lines containing a single Bt gene, such as cry1Ab in the Kemingdao(KMD) and mfb-MH86 lines; cry1Ac in AC-1, E10, and E54; cry1C in T1C-19, and C-54; cry2A in T2A-1, T2A-2, T2A-3, and T2A-4; and cry9C in 9C-1, 9C-2, 9C-3, 9C-4, and 9C-5; (ii)containing a fusion Bt gene, such a sthecry1Ab/1AcfusiongeneinTT51-1(Huahui1),TT9-3,andBt Shanyou 63; and the cry1Ab/vip3H gene in G6H-1, G6H-2, G6H-3, G6H-4, G6H-5, and G6H-6; and (iii) containing stacked insecticidal genes such as cry1Ac and modified CpTI (cowpea trypsin inhibitor) in MSA, MSB, and Kefeng6 . In addition, some Bt rice lines were stacked with other types of transgenes, such as bar for herbicide tolerance, and Xa21 for disease resistance. In the development of Bt rice lines, China made great efforts for independent innovation, and also took an active part in international cooperation. For example, KMD was developed by Zhejiang University in collaboration with the University of Ottawa, and Huahui 1 and Bt Shanyou 63 were developed by Huazhong Agricultural University in collaboration with the International Rice Research Institute (Chen, *et al*., 2006). Agrobacterium and gene gun-mediated transformations are commonly used for Bt rice development, and the promoters used for driving the expression of Bt genes include ubiquitin, rice rbcS (small subunit) of ribulose-1,5-bisphosphate carboxylase/oxygenase) promoter, and Actin1 (Table 1).

Table 1. Some Insect-resistant Bt rice lines and their efficacy on target lepidopteran pests in China (Qingsong et al., 2016)

Insecticidal Proteins	Plant Lines	Promoter; Method of Transformation	Recipient Cultivar	Expression Level of Bt Protein [a]	Efficacy on Target Lepidopteran Pests	
					In Laborator	In Field
Cry1Ab	KMD1	Ubiquitin; Agrobacterium-mediated	Xiushui 11 (japonica)	3.74–7.50 μg/g in stems FW; 3.78–9.13 μg/g in leaves FW; 12.78 μg/g in pollen DW	100% for 1st- or 3rd-instar larvae of 8 lepidopteran species *; 78% (4th-instar), and 68% (nstar) f5th-i or C. medinalis	100% for C. suppressalis, S. incertulas and C. medinalis
	KMD2	Ubiquitin; Agrobacterium-mediated	Xiushui 11 (japonica)	4.32–8.84 μg/g in stems FW; 3.97–8.29 μg/g in leaves FW; 31.37 μg/g in pollen DW	100% for C. suppressalis	100% for C. suppressalis
	mfb-MH86 Ubiquitin;	Agrobacterium-mediated	Minghui 86 (indica)	9.71–34.09 μg/g in leaves DW; 7.66–18.51 μg/g in stems DW; 1.95–13.40 μg/g in roots DW	100% for C. suppressalis	-
	T1Ab-10	Ubiquitin; Agrobacterium-mediated	Minghui 63 (indica) 7.54 μg/g in leaves FW	100% for C. medinalis, 98.2%	–	100% for C. suppressalis, 98.9%–100% for S. incertulas
	-	Rice rbcS promoter; Agrobacterium	mediated Zhejing22 (japonica)	1.66–3.31 μg/g in leaves FW; 0.11–0.17 μg/g in seeds FW	–	–
	-	Actin1; Gene gun	Zhongguo 91 (japonica)	–	100% for C. suppressalis	–
	–	Ubiquitin; Agrobacterium-mediated	Zhongguo 91 (japonica)	–	>99% for C. suppressalis	-
	–	Ubiquitin; Agrobacterium mediated	Xiushui 11 (japonica)	–	100% for C. suppressalis, S. incertulas, C. medinalis, and Psara licarisalis	-
Cry1Ac	-	Ubiquitin; Agrobacterium-mediated	Xiushui 11 (japonica)	-	100% for C. suppressalis, S. incertulas, C. medinalis, and Psara licarisalis	-

Insecticidal Proteins	Plant Lines	Promoter; Method of Transformation	Recipient Cultivar	Expression Level of Bt Protein [a]	Efficacy on Target Lepidopteran Pests	
					In Laborator	In Field
	P6, H7	Ubiquitin; Agrobacterium mediated	Guangling xiangjing (japonica)	0.025%–0.10% in leaves	100% for 2nd instar C. suppressalis and C. medinalis	100% for C. medinalis
	E10, E19	Ubiquitin; Agrobacterium mediated	Wuxiangjing9 (japonica)	0.025%–0.10% in leaves	100% for 2nd instar C. suppressalis and C. medinalis	100% for C. medinalis
Cry1C	T1C-`19	Ubiquitin; Agrobacterium	Minghui 63 (indica)	Up to 3.65 μg/g in leaves DW	85%–100% for C. suppressalis	94.8%–100% for C. medinalis; 99.98%–100% for C. suppressalis
	RJ-5	Rice rbcS promoter; Agrobacterium-mediated	Zhonghua 11 (japonica)	0.87 μg/g in leaves FW; 0.0026 μg/g in endosperm FW	-	97.9% for stem borers, and 99.4% for leaf folders
	-	Agrobacterium mediated	Hanhui 3(indica)	0.46–2.11 μg/g in leaves FW	-	100% for C. medinalis
	C-6	Rice rbcS promoter; Agrobacterium mediated	Jijing 88 (japonica)	2.42 μg/g in leaves FW	-	97.1% for C. suppressalis
	C-54	Rice rbcS promoter; Agrobacterium mediated	Jili 518 (japonica)	2.27 μg/g in leaves FW	-	95.9% for C. suppressalis
Cry2A	T2A-1, T2A-2, T2A-3, T2A-4	Ubiquitin; Agrobacterium mediated	Minghui 63 (indica)	9.65–12.11 μg/g in leaves FW	100% for S. incertulas	92.5%–94.6% for S. incertulas; 95.8%–99.0% for C. medinalis
	T2A-1	Ubiquitin; Agrobacterium mediated	Minghui 63 (indica)	Up to 87.25 μg/g in leaves DW; 33.5 μg/g in pollen DW	55.6%–100% for C. suppressalis; 64.69% (1st-instar), and 64.92% (3rd-instar) for C. medinalis	95.7%–100% for C. medinalis; 99.9%–100% for C. suppressalis
	2A-1, 2A-2, 2A-3	Ubiquitin; Agrobacterium mediated	Minghui63 (indica)	109.35–138.75 μg/g in leaves FW	100% for S. incertulas	84.6%–91.7% for C. suppressalis
	B2A68	Ubiquitin; Agrobacterium mediated	D68 (indica)	10.45–26.84 μg/g in leaves FW	100% for C. suppressalis	-

2.2.2 Bt maize (maize)

Maize is the sole *Bt* crop commercially produced and sold in 5 European countries (Spain, Portogal, Romania,

the Czech Republic, and Slovania) (Koch et al. 2015) and is used for feeding livestock and as row material for the starch industry. Such countries produce approximately 173 million tones ensilage maize and 56 million tons of grain maize. A part of the *Bt* maize seeds is used for manufacturing food products, like starch, maize flakes, pop maize, canned sweet maize, maize on the cob, and maize oil, as the high heat used for producing such foods breaks down any toxins. There are rules in Europe countries that all food products made from *Bt* maize must be labeled. The USA and Canada, however, do not have such rules, and almost 75% of their manufactured maize products are made from *Bt* maize (Anonymous 2012. Cultivation of *Bt* maize started in the USA, Canada, and Europe (Spain) in 1997, and by 2009, it was commercially planted in 11 countries. It was then representing 85% of the total area of maize in USA, 84% in Canada, 83% in Argentina, 57% in South Africa, 36% in Brazil, 20% in Spain, and 19% in Philippines. In 2016, GM maize in the world (in 16 countries) reached 60.6 million ha, out of which 6 million (10%) were *Bt* maize, 7 million (11.7%) were herbicide-tolerant maize, and 47.7 million (78.7%) were combined *Bt* and herbicide-tolerant maize. The crop was produced to resist the infestation by the European maize borer, *Ostrinia nubilalis*, but later in the 2000s, it has been produced against the maize earworm, *H. zea*, and the maize rootworm, *Diabrotica virgifera* in addition to *O. nubilalis* (James 2016).

Bt maize has been transformed with either cryAb, cryAc or cryC to protect it against Ostrinia nubilalis and Sesamia nonagriodes, or with cry1F to protect it against Spodoptera frugiperda, and with cryBb, cryAb and cryAb to protect it against the rootworms of the genus Diabrotic (James, 2012). By the end of the year 2012, more than 18 million hectares were under the cultivation of Btcotton plants. Most commercially planted Bt cotton contain cry1Ac or a fusion gene of cryAc and cryAb (James, 2013). Bt potatoes protected against Leptinotarsa decemlineata have also been planted commercially in North America and Europe and contain the cry3A a gene (Coombs etal. 2002).Development of Bt maize started in the late 1980s in China, but moved relatively slowly during the initial stage. Greater progress was achieved in the past decade, especially after the initiation of the National GMO New Variety Breeding Program in 2008. To date, over a dozen Bt maize lines have been obtained (Table 2).

Table 2. Some Insect-resistant Bt maize lines and their efficacy on target lepidopteran pests in China (Qingsong et al., 2016)

Insecticidal Proteins	Plant Lines	Promoter; Method of Transformation	Recipient Cultivar	Expression Level of Bt Protein [a]	Efficacy on Target Lepidopteran Pests	
					In Laborator	In Field
Modified Cry1Ab	-	pZmUbi-1; Agrobacterium mediated	HiII	0.30–0.47 µg/g in leaves FW	78% of leaves for O. furnacalis in 5-day bioassays	0.14 survivors, 2.43 tunnels/plant, 3.64 cm tunnel length/plant
mCry1Ac	BT-799	CaMV 35S; Gene gun-mediated	Zheng 58	0.77 µg/g in leaves FW; 0.23 µg/g in silks DW; 0.30 µg/g in husks DW; 0.15 µg/g in young kernels DW; 0.059 µg/g in pollen DW	-	Leaf damage ratings (LDR) below 2 for O. furnacalis
	Zhengdan958K	-	Zhengdan 958	-	100% of whorl leaves, 83.3% of silk, 97.2% of husk, and 63.5% of young kernel for O. furnacalis	-

Insecticidal Proteins	Plant Lines	Promoter; Method of Transformation	Recipient Cultivar	Expression Level of Bt Protein [a]	Efficacy on Target Lepidopteran Pests	
					In Laborator	In Field
	BT- X	CaMV 35S;	HiII×H99	90.087–0.23 μg/g in whorl leaves FW; 0.044 μg/g in silks FW	4.7%–97.2% of whorl leaves for O. furnacalis	LDR was 1.15 for O. furnacalis
	BT- 38	CaMV 35S;	Zheng 58	0.44 μg/g in whorl leaves FW	98.6% of whorl leaves for O. furnacalis	–
	BT- 181	CaMV 35S;	Zheng 58	0.42 μg/g in whorl leaves FW	97.2% of whorl leaves for O. furnacalis	–
	BT-`105	CaMV 35S;	Chang 72	0.42 μg/g in whorl leaves FW	100% of whorl leaves for O. furnacalis	–
	–	Ubiquitin; Agrobacterium-mediated	Zhongguo 91 (japonica)	–	>99% for C. suppressalis	-
	–	Ubiquitin; Agrobacterium mediated	Xiushui 11 (japonica)	–	100% for C. suppressalis, S. incertulas, C. medinalis, and Psara licarisalis	-
Cry1AcM	C1, C2, C3	pZmUbi 1; Agrobacterium mediated	Chang 7-2	–	LDR was below 2.08, >80% of kernels, and >90% of husks for O. furnacalis	LDR was below 1.91, >80% of kernels, and >90% of husks for O. furnacalis
	Z1, Z2, Z3	pZmUbi 1, Agrobacterium mediated	Zheng 58	-	LDR was below 2.07, >80% of kernels, and >90% of husks for O. furnacali	LDR was below 1.50, >80% of kernels, and >90% of husks for O. furnacalis
	Q1, Q2, Q3	pZmUbi 1; Agrobacterium mediated	Qi 319	-	LDR was below 2.0, >80% of kernels for O. furnacalis	LDR was below 1.11, >90% of husks for O. furnacalis
	L1, L2, L3	pZmUbi 1; Agrobacterium mediated	9801	-	LDR was below 2.0, >80% of kernels for O. furnacalis	LDR was below 1.15, >90% of husks for O. furnacalis

Insecticidal Proteins	Plant Lines	Promoter; Method of Transformation	Recipient Cultivar	Expression Level of Bt Protein [a]	Efficacy on Target Lepidopteran Pests	
					In Laborator	In Field
Cry1Ah	HGK60	Ubiquitin; Agrobacterium mediated	Z 31	2.88, and 3.50 μg/g in leaves FW at 6-leaf stage, and heading stage; 3.62, and 9.98 μg/g in tassels FW at heading stage and filling stage	100% of leaves for O. furnacalis, >80% for H. armigera in 3-day bioassay	LDR was 1.29, and 2.47 for O. furnacalis, and M. separata, high resistant of kernel to H. armigera
	Q11, X8	Ubiquitin; Agrobacterium mediated	Q31×Z3	Up to 0.05% in leaves	-	LDR was 2.4 (Q11), and 3.4 (X8) for O. furnacalis
	G186	Ubiquitin; Agrobacterium mediated	Z31	Up to 1 μg/g in leaves FW	100% of leaves for O. furnacalis	LDR was 1.3 for O. furnacalis

2.2.3 Bt cotton

For cotton growers, there was a lot of pressure from pests before the introduction of *Bt* cotton. Due to synthetic insecticide resistance, farmers were losing much of their cotton because of *H. virescens* and pink bollworm, *Pectinophora gossypiella*. According to USDA, 94% of the cotton cultured in USA is genetically modified (James, 2015).A study in University of California revealed that the average cost reduction in pesticides applied in *Bt* cotton fields from 1996 to 1998 was between 25 and 65 dollars per acre; the yield estimated, in the same period, was 5% more, on average, than the traditional cotton. In addition, *Bt* cotton significantly decreased the number of foliar sprays, against other cotton pests and consequently the cost of insecticides

In 1996, Bollgard cotton (a trademark of Monsanto Company) was the first *Bt* cotton to be marketed in the USA. It was producing Cry1Ac toxin with high activity on tobacco budworm and pink bollworm. *Bt* cotton was widely adopted in the USA by farmers in the Western Cotton Belt for the pink bollworm and by farmers in the Mid-south and South-east for primarily tobacco budworm and to a lesser extent for fall armyworm, *Spodoptera frugiperda* and *S. exigua* .

Bollgard II was introduced in 2003 representing the next generation of *Bt* cotton. It was producing Cry2Ab toxin. Wide Strike cotton (a trademark of Dow Agro-sciences) was produced in 2004 containing Cry1Ac and Cry1F. Both Bollgard II and Wide Strike have better activity on a wide range of caterpillar insects than the original Bollgard (Stewart, 2007). The most recent 3rd generation of Bt cotton contained three genes: Bollgard 3 (Cry1Ac + Cry2Ab + Vip3A), Twin Link Plus (Cry1Ab + Cry2Ac + Vip3Aa19), and Wide Strike 3 (Cry1Ac + Cry1F + Vip3A) (Vyavhare, 2017).

Bt cotton is the only *Bt* crop cultivated in developing countries. In India and China, the cultivated area of *Bt* cotton increased sharply during 2006 and 2007 to reach 25 million acres (2.5 million ha). In 2016, the world total area of cotton was 35 million ha (in 18 countries), out of which 22.3 million (64%) were GM cotton. In the USA, however, the total area of cotton was 4 million ha and out of which 3.2 million ha (80%) were combined *Bt* and herbicide-tolerant cotton (James 2015).Varieties of *Bt* maize and *Bt* cotton registered in the USA were producing 18 different combinations of 11 *Bt* toxins. Each variety produces 1–6 *Bt* toxins that kill caterpillars, beetles, or both (Tabashnik et al. 2009).

3. Conclusion

Insect pests have become an integral part of agricultural crops worldwide. Globally, insects cause about 15% of direct losses to different agricultural crops as well as indirect losses owing to impaired quality of the produce. Insects also act as vectors of various plant pathogens such as bacterial, fungal, and viral**.** They significantly reduce yield and affect almost every aspect of the plants. For many years major challenge for scientists has been developing the resistant varieties against pests in plants. Plant breeders have also been successful during the last century in producing a few Insect-resistant cultivars/lines of some potential crops through conventional breeding,

but this again has utilized modest resources. However, this approach seems now inefficient due to a number of reasons and alternatively, genetic engineering for improving crop pest and disease resistance is being actively followed these days by the plant scientists, world-over. New tools and genes have been developed for use in the genetic engineering of plants to introduce effective resistance to biotic stresses and to understand the mechanisms of resistance. Recent advances in genetic engineering, *Bacillus thuringiensis* (Bt) has resulted in successful control of many economically important pests in food crops. This approach should allow increases in both productivity and quality of plants in an environmentally friendly manner, thereby reducing the use of and reliance on chemical control of pests.

The threat of development of resistance by insect pests to broad spectrum chemical insecticides has prompted research for adoption of alternative strategies. Several crop plants have been engineered with different cry genes conferring resistance against various major insect pests. Rapid adoption and commercial introduction of Bt crops led to the development of resistance by insects against Bt toxins. Major pests, such as the diamondback moth, tobacco budworm, Colorado potato beetle, Indian mealy moth, maize stalk borer, cotton bollworm and fall armyworm have shown resistance to Cry toxins. Moreover, Cry toxins are effective against lepidopterans but are ineffective against sucking pests belonging to hemiptera. Plants, in general, are known to synthesize a wide range of defense proteins against different pathogens. The insecticidal activity of plant lectins against various insects belonging to hemipteran has been well documented. Transgenic rice expressing ASAL exhibited ample resistance against sucking insects BPH, GLH and WBPH**.** Pyramided transgenic rice lines containing asal and gna lectin genes exhibited enhanced resistance to major sap-sucking insects. A number of successful fusion proteins were developed using lectin as a carrier protein. The observed increases in the mortality of insects caused by fusion proteins have been ascribed to the lectin domain, which enhanced the binding process and facilitated the entry of toxin more efficiently into the insect. GNA when fused as a carrier protein for different chimeric toxins such as, Manase-AC/GNA, SFlI/GNA, Chitinase/ GNA, ButalT/GNA and ω-ACTX-Hv1a/GNA resulted in higher toxicities against various insect pests.

To overcome resistance acquired by insects against Cry toxins different strategies were employed to modify Cry functional domains to improve their toxicity (Bravo, A. et al.2013) Different gene fusions, viz., Cry1Ca, Cry1Fb, Cry1Ba modified with Cry1Ac domain III, Cry1Ac/ricin-B, Cry1Ac/CpTI, Cry1Ac/HWTX-I, Cry1Ac/CDEP2, and Cry1Ab/ ACTX-Ar1, employed for engineering of plants bestowed with enhanced insect resistance (De Maagd, R. A.etal, 2000). In addition, it is important for agricultural oversight agencies to enhance their ability to supervise and regulate GMO biosafety, since any potential incidents associated with GMO biosafety may impair public confidence in the biosafety on GMOs.

5. References

Ahmad, P., M. Ashraf, M. Younis, X. Hu, A. Kumar, N.A. Akram and F. Al-Qurainy, 2012. Role of transgenic plants in agriculture and biopharming. Biotechnol. Adv., 30: 524-540.

Bagla, P., 2010. India: Hardy cotton-munching pests are latest blow to GM crops. Science, 327: 1439-1439.

Bates, S.L., J.Z. Zhao, R.T. Roush and A.M. Shelton, 2005. Insect resistance management in GM crops: Past, present and future. Nat. Biotechnol., 23: 57-62.

Bravo, A. et al. 2013. Evolution of Bacillus thuringiensis Cry toxins insecticidal activity. Microbial Biotech. 6, 17–26.

Behrooz, D., S. Farajnia, M. Toorchi, S. Zakerbostanabad, S. Noeparvar and C.N. Stewart Jr., 2008. DNA-delivery methods to produce transgenic plants. Biotechnology, 7: 385-402.

Brookes, G. and P. Barfoot, 2005. GM crops: The global economic and environmental impact- The first nine years 1996-2004. AgBioForum, 8: 187-196.

Carriere, Y., D.W. Crowder and B. E. Tabashnik, 2010. Evolutionary ecology of insect adaptation to Bt crops. Evol. Appl., 3: 561-573.

Chakr aborty S.&NewtonA.C.,2011. Climate change, plant diseases and food security: an overview.Plant Pathol.,60(1),1-14.

Chen, M.; Zhao, J.; Ye, G.; Fu, Q.; Shelton, A.M. Impact of insect-resistant transgenic rice on target insect pests and non-target arthropods in China. Insect Sci. 2006, 13, 409–420.

Coombs J.J. etal., 2002. Combining engineered (Bt-cry3A) and natural resistance mechanisms in potato for control of Colorado potato beetle.J. Am. Soc. Hortic. Sci.,127,62-68.

DeVilliers, S.M. and A.D. Hoisington, 2011. The trends and future of biotechnology crops for Insect pest control. Afr.J. Biotechnol., 10: 4677-4681.

De Maagd, R. A., Weemen-Hendriks, M., Stiekema, W. & Bosch, D. (2000). Bacillus thuringiensis delta-endotoxin Cry1C domain III can function as a specificity determinant for Spodoptera exigua in different, but not all, Cry1-Cry1C hybrids. Appl Environ Microbiol.66, 1559–1563 (2000).

Dhaliwal, G.S. and H. Uchimaya, 1999. Genetic engineering for disease and pest resistance in plants. Plant Biotechnol., 16: 255-261.

Dhaliwal, G.S., V. Jindal and A.K. Dhawan, 2010. Insect pest problems and crop losses: Changing trends. Indian J. Ecol., 37: 1-7.

Downes, S., R.J. Mahon, L. Rossiter, G. Kauter, T. Leven, G. Fitt and G. Baker, 2010. Adaptive management of pest resistance by Helicoverpa species (Noctuidae) in Australia to the Cry2Ab Bt toxin in Bollgard II ® cotton. Evol. Appl., 3: 574-584.

Gulzar, S.S., H.W. Shabir, G. Singh, L.K. Prem and N.B. Singh, 2011. Designing crop plants for biotic stresses using transgenic approach. Vegetos-Int. J. Plant Res., 24: 1-25.

Isbat, M., N. Zeba, S.R. Kim and C.B. Hong, 2009. A BAXinhibitor-1genein Capsicum annuum is induced under various abiotic stresses and endows multi-tolerance in transgenic tobacco. J. Plant Physiol., 166: 1685-1693.

James, C., 2011. Global Status of Commercialized Biotech/GM Crops: 2011. Vol. 43., ISAAA Brief, Ithaca, USA.

James C.,2012.Global status of commercialized biotech/ GM crops.Brief No.44. Ithaca, NY, USA: ISAAA.

James C., 2013.Global status of commercialized biotech/ GM crops. Brief No.46. Ithaca, NY, USA:ISAAA.

James, C. Global Status of Commercialized Biotech/ GMCrops: 2015. IS AAABrief,No. 51; International Service for the Acquisition of Agri-biotech Applications (ISAAA): Ithaca, NY, USA,2015.

Josine, T.L., J. Ji, G. Wang and C.F. Guan, 2011. Advances in genetic engineering for plants Abiotic stress control 2011.Afr.J.Biotechnol.,10:5402-5413.

Karthikeyan, A., M. Sudha, M. Pandiyan, N. Senthil, V.G. Shobana and P. Nagarajan, .2011. Screening of MYMV resistant mungbean (*Vigna radiata* (L.) wilczek) progenies through Agroinoculation. Int. J. Plant Pathol., 2: 115-125.

Karthikeyan, R. Valarmathi, S. Nandini and M.R. Nandhakumar, 2012. Genetically Modified Crops: Insect Resistance. *Biotechnology, 11: 119-126.*

Kruger, M., J.B.J. van Rensburg and J. van den Berg, 2009. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. Crop Prot., 28: 684-689.

Marvier, M., Y. Carriere, N. Ellstrand, P. Gepts and P. Kareiva *et al*., 2008. Harvesting data from genetically engineered crops. Science, 320: 452-453.

Masoud Tohidfar and Solmaz Khosravi, 2015.Transgenic crops with an improved resistance to biotic stresses.Biotechnol. Agron. Soc. Environ. , 62-70.

Qingsong Liu , Eric Hallerman, Yufa Peng and Yunhe Li 2016. Development of Bt Rice and Bt Maize in China and Their Efficacy in Target Pest Control. Int. J. Mol. Sci., 17, 1561; doi:10.3390/ijms17101561.

Pandey, A., M. Kamle, L.P. Yadava, M. Muthukumar and P. Kumar *et al*., 2011. Genetically modified food: It Uses, future prospects and safety assessment. Biotechnology, 10: 473-487.

Sanahuja, G., R. Banakar, R.M. Twyman, T. Capell and P. Christou, 2011. *Bacillus thuringiensis*: A century of research, development and commercial applications. Plant Biotechnol. J., 9: 283-300.

Slater, A., N.W. Scott and R.M. Fowler, 2009. GM starategies for insect resistance: *Bacillus thuringiensis*. Plant Biotechnol. Genet. Manipulation Plants, 6: 133-138.

Tabashnik, B.E., A.J. Gassmann, D.W. Crowder and Y. Carriere, 2008. Insect resistance to Bt crops: Evidence versus theory. Nat. Biotechnol., 26: 199-202.

Tabashnik, B.E., A.J. Gassmann, D.W. Crowder and Y. Carriere, 2008. Field-evolved resistance to Bt toxins. Nat. Biotechnol., 26: 1074-1076.

Tabashnik, B.E., J.B. van Rensburg and Y. Carriere, 2009. Field-evolved insect resistance to Bt crops: Sefinition, theory and data. J. Econ. Entomol., 102: 2011-2025.

Tiwari, S. and R.R. Youngman, 2011. Transgenic Bt maize hybrids and pest management in the USA alternative farming systems, biotechnology, drought stress and ecological fertilisation. Sustainable Agric. Rev., 6: 15-37.

Vaughn, T., T. Cavato, G. Brar, T. Coombe and T. DeGooyer *et al*., 2005. A method of controlling maize rootworm feeding using a bacillus thuringiensis protein expressed in transgenic maize. Crop Sci., 45: 931-938.

Wahab, S., 2009. Biotechnological approaches in the management of plant pests, diseases and weeds for Sustainable Agriculture. J. Biopesticides, 2: 115-134.

Yang, H.; Li, J.X.; Guo, S.; Chen, X.; Fan, Y. 1989.Transgenic rice plants produced by direct Up take of δ-endotoxin protein gene from Bacillus thuringiensis into rice protoplasts. Sci. Agric. Sin., 22, 1–5.