ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 11 (3): 119-126, 2012
ISSN 1682-296X / DOI: 10.3923/biotech.2012.119.126

Genetically Modified Crops: Insect Resistance

[1]A. Karthikeyan, [2]R. Valarmathi, [2]S. Nandini and [3]M.R. Nandhakumar
[1]Centre for Plant Molecular Biology, Department of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641-003, India
[2]Centre for Plant Protection Studies, Department of Entomology, Tamil Nadu Agricultural University, Coimbatore-641-003, India
[3]Department of Agronomy, Directorate of Crop Management, Tamil Nadu Agricultural University, Coimbatore-641-003, India

Abstract: Insect pests have become an integral part of agricultural crops worldwide. 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.

Key words: *Bacillus thuringiensis* (Bt), cry proteins, insect resistance, genetic engineering, transgenic plants

INTRODUCTION

Agricultural productivity is highly influenced by pest and diseases, known as the most harmful factor concerning the growth and productivity of crops worldwide. 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 (Wani and Sanghera, 2010; 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 (Hilder and Boulter, 1999; Gatehouse, 2008; 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

Corresponding Author: A. Karthikeyan, Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore-641-003, India

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.

Major pests in food crops: 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. They cause serious economic losses in many major crops by reducing yield. Food crops of the world are damaged by more than 10,000 species of insects less than 10% of the total identified pest species are generally considered as major pests (Dhaliwal *et al*., 2007). List of important pests of major crops are given in Table 1. 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. Detailed role of genetic engineering in crop improvement is discussed below.

Genetic engineering of crop plants: 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). Transfer 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 (Bhatnagar-Mathur *et al*., 2008; 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 (Cassells and Doyle, 2003; Christou *et al*., 2006; 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.

Table 1: List of important insect pests in food crops

Insect pests	Scientific name	Order	Family	Crops
American bollworm	*Helicoverpa armigera*	Lepidoptera	Noctuidae	Cotton
Brown plant hopper	*Nilaparvata lugens*	Hemiptera	Delphacidae	Rice
Diamond back moth	*Plutella xylostella*	Lepidoptera	Plutellidae	Cauliflower and cabbage
Fruit borer	*Helicoverpa armigera*	Lepidoptera	Noctuidae	Tomato
Fruit fly	*Bactrocera* sp.	Diptera	Tenthredinidae	Fruits and vegetables
Gall midge	*Orseolia oryzae*	Diptera	Cecidomyiidae	Rice
Gram pod borer	*Helicoverpa armigera*	Lepidoptera	Noctuidae	Chickpea and pigeon pea
Green leafhopper	*Nephotettix* sp.	Hemiptera	Cicadellidae	Rice
Leaf miner	*Aproaerema modicella*	Lepidoptera	Gelechiidae	Groundnut
Mealy bug	*Several species*	Hemiptera	Pseudococcidae	Several field and horticultural crops
Mustard aphid	*Lipaphls erysimi*	Hemiptera	Aphididae	Mustard
Pink stem borer	*Sesamia inferens*	Lepidoptera	Noctuidae	Wheat
Pyrilla	*Pyrilla perpusilla*	Hemiptera	Lophophidae	Sugarcane and rice
Shoot and fruit borer	*Leucinodes orbonalis*	Lepidoptera	Pyralidae	Brinjal
Thrips	*Several species*	Thysanoptera	Thripidae	Groundnut, cotton, chilies, roses, grapes and citrus
Top borer	*Scirpophaga nivella*	Lepidoptera	Pyralidae	Sugarcane
Tuber moth	*Phthorimaea operculella*	Lepidoptera	Gelechiidae	Potato
Yellow stem borer	*Scirpophaga incertulas*	Lepidoptera	Noctuidae	Rice
Whitefly	*Bemisia tabaci*	Hemiptera	Aleyrodidae	tobacco
Wheat aphid	*Macrosiphum miscanthi*	Hemiptera	Aphididae	Wheat, barley, oats

Table 2: List of important ICP proteins (Slater *et al*., 2009)

Cry protein	Protein size (kDa)	Susceptible insect class
Cry1A(a-i)	133	Lepidoptera
Cry1B(a-g)	140	Lepidoptera
Cry1C(a, b)	133-134	Lepidoptera
Cry1D(a, b)	131-132	Lepidoptera
Cry1E(a, b)	133-134	Lepidoptera
Cry1F(a, b)	132-134	Lepidoptera
Cry1G(a-c)	132-133	Lepidoptera
Cry1H(a, b)	133	Lepidoptera
Cry1I(a-d)	81	Lepidoptera
Cry1J(a-d)	133	Lepidoptera
Cry1Ka	137	Lepidoptera
Cry1La	133	Lepidoptera
Cry2A(a-e)	71	Lepidoptera
Cry3Aa, Cry3B(a, b), Cry3Ca	73-75	Coleoptera
Cry4Aa, Cry4Ba	135,128	Diptera
Cry5A(a, b)	142-152	Nematodes
Cry5Ac	135	Hymenoptera
Cry5Ba	140	Hymenoptera
Cry6Aa, Cry6Ba	143	Nematodes
Cry7A(a, b)	129-130	Coleopteran
Cry8Aa, Cry8B(a-c), Cry8(Ca-Ha)	131	Coleopteran
Cry9Aa, Cry9B(a, b), Cry9Ca, Cry9D(a, b), Cry9E(a, d)	130	Lepidoptera
Cry10Aa	78	Diptera
Cry11Aa	72	Diptera
Cry11B(a, b)	81	Diptera

***Bacillus thuringiensis*:** Bt toxin gene the source of the insecticidal toxins produced in commercial transgenic plants is the soil bacterium *Bacillus thuringiensis* (Bt). It was discovered by Ishiwaki in 1901 in diseased silkworms. Further research on Bt by Steinhaus (1951) lead to renewed interest in biopesticides and as a result, the more potent products such as Thuricide a and Dipela were introduced (Bravo *et al*., 2007; Federici *et al*., 2010; Sanahuja *et al*., 2011). It was subsequently classified and named after its isolation from the gut of diseased flour moth larvae in thurienberg, by Ernst Berliner. The ubiquitous nature of *Bacillus thuringiensis* (Bt) is now being mirrored in major crops plants that have been engineered through recombinant DNA to carry genes responsible for producing these crystal proteins and providing host plant resistance to major pests (Ranjekar *et al*., 2003; DeVilliers and Hoisington, 2011). *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) or expressed during bacterial growth (Vip proteins) (Federici *et al*., 2010; Sanahuja *et al*., 2011). The bacterium produces an insecticidal crystal protein (ICP: also called Cry proteins, encoded by cry genes). Cry proteins are one of several classes of endotoxins produced by the sporulating bacteria: Hence they were originally classified as -endotoxins, to distinguish them from the other classes of and endotoxins (Ranjekar *et al*., 2003; Slater *et al*., 2009). With the advent of molecular biology and genetic engineering, it has become possible to use Bt more effectively and rationally by introducing the ICPs of Bt in crop plants. List of important ICP proteins given in Table 2.

Bt technology: *Bacillus thuringiensis* is a gram-positive aerobic, sporulating bacterium, which produces proteinaceous crystalline inclusion bodies during sporulation. There are several subspecies of this bacterium, which are effective against lepidopteran, dipteran and coleopteran insects (Tabashnik *et al*., 2008b). The mechanism of action of the Bt ICPs has been worked out in some detail. The molecular structure of at least three different ICPs 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) (Ranjekar *et al*., 2003; DeVilliers and Hoisington, 2011). 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 (Christou *et al*., 2006; Gatehouse, 2008). Alternatively, synthetic variants of Cry genes has been employed as in the case of MON863 which expresses a synthetic Bt kumamotoensis Cry3Bb1 gene against corn rootworm, which is eight times more effective than the native, non-modified version (Vaughn *et al*., 2005). 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.

Bt crops: The success of the transgenic approach led to the development of Bt crops, transgenic crops are used worldwide to control major pests of cotton, corn and soybean. 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 (Toenniessen *et al*., 2003; Brookes and Barfoot, 2005). The first widely planted Bt crop cultivars were corn 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 corn producing Cry1Ab (Kruger *et al*., 2009), fall armyworm, *Spodoptera frugiperda*, in Puerto Rico to Bt corn 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 (Luttrell *et al*., 2004; 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 (Matten *et al*., 2008; Kruger *et al*., 2009; Tabashnik *et al*., 2008b, 2009; Bagla, 2010).

Global status and Benefits of Bt crops: Genetically, engineering crop resistance to insect pests offer the potential of a user friendly environment and consumer friendly method of crop protection to meet the demands of sustainable agriculture in the 21st century. Biotech crops, including those that are Genetically Modified (GM) with *Bacillus thuringiensis* (Bt) endotoxins for insect resistance, have been cultivated commercially and adopted in steadily increasing numbers of countries over the past 15 years. Biotech crops being cultivated globally include soybean, maize, cotton, canola, squash, papaya, sugar beet and tomato. Almost all of the global biotech crop area derives from soybean, corn, cotton and canola (Brookes and Barfoot, 2010). In 2011, biotech crops soybeans accounted for the largest share (52%), followed by corn (30%), cotton (13%) and canola (5%) (Fig. 1). GM crops have been grown commercially since 1996. In 2011, 16.7 million farmers across 29 countries (ten industrialized countries and 19 developing countries planted 160 million hectares of biotech crops. 90% or 15 million were small and resource poor farmers in developing countries (James, 2011). The US had the largest share of global biotech crop plantings in 2011 (69 million ha), followed by Brazil (30.3 M ha). The other main countries planting biotech crops in 2011 were, Argentina (23.7 M ha), India (10.6 M ha) and Canada (10.4 M ha). Global area of biotech crops in 2011: by Country (Table 3). (Brookes and Barfoot, 2010) reported 725 approvals for commercial cultivation had been granted for 155 events in 24 crops and 57 countries globally have granted regulatory approvals for biotech crops for import for food and feed use and for release in to the environment since 1996 incl.

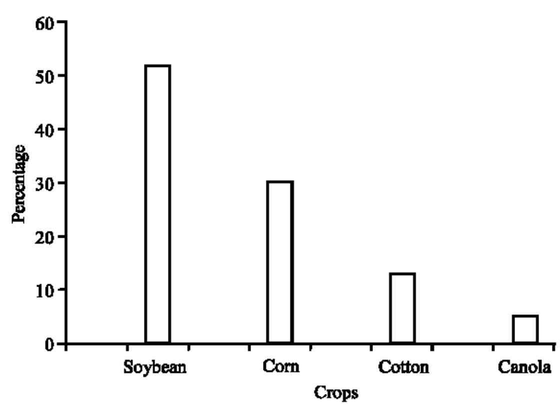

Fig. 1: Global biotech crops

Table 3: Global area of biotech crops in 2011: by country (James, 2011)

Rank	Country	Total area (m ha)	Biotech crops
1	USA	69.0	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash
2	Brazil	30.3	Soybean, maize, cotton
3	Argentina	23.7	Soybean, maize, cotton
4	India	10.6	Cotton
5	Canada	10.4	Canola, maize, soybean, sugar beet
6	China	3.9	Cotton, papaya, poplar, tomato, sweet pepper
7	Paraguay	2.8	Soybean
8	Pakistan	2.6	Cotton
9	South Africa	2.3	Maize, soybean, cotton
10	Uruguay	1.3	Soybean, maize
11	Bolivia	0.9	Soybean
12	Australia*	0.7	Cotton, canola
13	Philippines	0.6	Maize
14	Myanmar	0.3	Cotton
15	Burkina Faso	0.3	Cotton
16	Mexico	0.2	Cotton, soybean
17	Spain	0.1	Maize
18	Colombia	<0.1	Cotton
19	Chile	<0.1	Maize, soybean, canola
20	Honduras	<0.1	Maize
21	Portugal	<0.1	Maize
22	Czech Republic	<0.1	Maize

Japan, USA, Canada, South Korea, Mexico, Australia, Philippines, The European union, New Zealand and China. In 2011 the five lead developing countries in biotech crops are India and China in Asia, Brazil and Argentina in Latin America and South Africa on the continent of Africa, which together represent 40% of the global population, which could reach 10.1 billion by 2100. Six EU countries planted a record 114,490 hectares of biotech Bt maize, up 26% from 2010 and an additional two countries planted the biotech potato Amflora . Africa made steady progress with regulation. South Africa, Burkina Faso and Egypt, together planted a record. 2.5 million hectares; three more countries, Kenya, Nigeria and Uganda conducted field trials (James, 2011). Moreover, global scientific and regulatory authorities found biotech crops as safe as conventional crops and stated that foods from biotech crops are thoroughly evaluated through comprehensive testing for food, feed and environmental safety (James, 2009). The first generation of Bt crops has been extraordinarily successful, Bt crops offer advantages such as in-built protection against pests and other stresses, over hybrid crops and are cultivated as any other conventional crops (Brookes and Barfoot, 2010). Although, there is much debate both politically and publically concerning the environmental impact of genetically engineered crops, it is clear that Bt crops have provided immense environmental benefits. According to the recent survey of global impact of biotech crops for the period From 1996-2010, biotech crops contributed to Food Security, Sustainability and Climate Change by: increasing crop production valued at US$78.4 billion; providing a better environment, by saving 443 million kg a.i. of pesticides; a saving of 8.4 % in pesticides, Which is equivalent to a 16.1% reduction in the associated environmental impact of pesticides use on these crops, as measured by the Environmental Impact Quotient (EIQ). In 2010 alone reducing CO_2 emissions by 19 billion kg, equivalent to taking 9 million cars off the road; conserving biodiversity by saving 91 million hectares of land and helped alleviate poverty by helping 15.0 million small farmers who are some of the poorest people in the world (James, 2011). Biotech crops are essential but are not a panacea and adherence to good farming practices such as rotations and resistance management, are a must for biotech crops as they are for conventional crops, global value of biotech seed alone was valued at ~US$13 billion in 2011, with the end product of commercial grain from biotech crops valued at ~US$160 billion per year (James, 2011).

Limitations: Bt crops are not a panacea for solving all the pest problems. There are some genuine or perceived concerns. The major limitations of transgenic plants secondary pests are not controlled in the absence of sprays for the major pests, need to control the secondary pests through chemical sprays will kill the natural enemies and thus offset one of the advantages of transgenics, cost of producing and deployment of transgenics may be very high, proximity to sprayed fields will reduce the benefits of transgenics, insect migration may reduce the effectiveness of transgenics, development of resistance in insect populations may limit the usefulness of transgenics.

Perspectives: Transgenic crops play a central role in protecting the crop from its major insect pests. The production of insect tolerant plants has been major success for scientists. At the same time efficacy of transgenic crops depend on very much on whether they are viewed from the perspective of chemical pesticides or from that of no additional protective intervention. Even the best current transgenics do not perform as spectacularly as chemicals. There are many insect pests which are not susceptible to currently available range of ICP genes. Many serious pests of local, crop-specific importance have received little or no attention from this technology. There is a need to broaden the pool of genes which are available to cover these pests which are currently untreatable. Transgenic crops are used worldwide to control major pests. Development of strategies to delay the evolution of pest resistance to Bt crops requires an understanding of factors affecting responses to natural selection, which include variation in survival on Bt crops, heritability of resistance and fitness advantages associated with resistance mutations. The two main strategies adopted for delaying resistance are the refuge and pyramid strategies. Both can reduce heritability of resistance, but pyramids can also delay resistance by reducing genetic variation for resistance. One of the major challenge for scientists is accessibility of these products is relatively restricted, In some developed countries, this has been a result of vocal opposition to transgenic crops itself; but in many instances, in both developed and developing countries, it is more a case of potential economic returns not being sufficient to make the introduction of engineered crop varieties commercially viable.

CONCLUSION

Many exciting insights have emerged from recent research on plant genetic engineering. The advantages of successfully engineering plants for insect resistant response are evident. There is no doubt that the use of insect pests (Bt) resistance genes, singly and in combination, has been successful in practice, aside from social and environmental concerns. Overall, the evidence strongly suggests that in both developed and developing countries, the adoption of transgenic crops can increase the farmer's income. The increase in income to small-scale farmers in developing countries can have a direct impact on poverty alleviation and quality of life, a key component of sustainable development. In the developing world, a change in attitude by governments, non-governmental organizations and the public at large is needed for insect-resistant transgenic crops to be able to benefit all the world's population, not just a few. However, it is to be hope that encourage progress described above is maintained and developed so as to make significant contribution towards redressing the balance between world food productions and world food requirements in future.

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.

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.

Bhatnagar-Mathur, P., V. Vadez and K.K. Sharma, 2008. Transgenic approaches for abiotic stress tolerance in plants: Retrospect and prospects. Plant Cell Rep., 27: 411-424.

Bravo, A., S.S. Gill and M. Soberon, 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon, 49: 423-435.

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.

Cassells, A.C. and B.M. Doyle, 2003. Genetic engineering and mutation breeding for tolerance to abiotic and biotic stresses: Science, technology and safety. Bulg. J. Plant Physiol., 30: 52-82.

Christou, P., T. Capell, A. Kohli, J. Gatehouse and A. Gatehouse, 2006. Recent developments and future prospects in insect pest control in transgenic crops. Trends. Plant Sci., 11: 302-308.

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.

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

Dhaliwal, G.S., A.K. Dhawan and R. Singh, 2007. Biodiversity and ecological agriculture: Issues and perspectives. Indian J. Ecol., 34: 100-109.

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.

Federici, A.B., H.W. Park and D.K. Bideshi, 2010. Overview of the basic biology of *Bacillus thuringiensis* with emphasis on genetic engineering of bacterial larvicides for mosquito control. Open Toxinol. J., 3: 83-100.

Gatehouse, J.A., 2008. Biotechnological prospects for engineering insect-resistant plants. Plant Physiol., 146: 881-887.

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.

Hilder, V.A. and D. Boulter, 1999. Genetic engineering of crop plants for insect resistance-a critical review. Crop Prot., 18: 177-191.

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

James, C., 2009. Global Status of Commercialized Biotech/GM Crops: 2011. Vol. 41., ISAAA Brief, Ithaca, USA..

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

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.

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.

Luttrell, R.G., I. Ali, K.C. Allen, S.Y. Young and A. Szalanski *et al*., 2004. Resistance to Bt in Arkansas populations of cotton bollworm. Proceedings of the 2004 Beltwide Cotton Conferences, January 5-9, 2004, National Cotton Council of America, Memphis, pp: 5-9.

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

Matten, S.R., G.P. Head and H.D. Quemada, 2008. How Governmental Regulation can Help or Hinder the Integration of BT CROPS into IPM Programs. In: Integration of Insect-resistant Genetically Modified Crops within IPM Programs, Romeis, J., A.M. Shelton and G.G. Kennedy (Eds.). Springer, New York, pp: 27-39.

Mittler, R. and E. Blumwald, 2010. Genetic engineering for modern agriculture: challenges and perspectives. Annu. Rev. Plant Biol., 61: 443-462.

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.

Ranjekar, P.K., A. Patankar, V. Gupta, R. Bhatnagar, J. Bentur and P.A. Kumar, 2003. Genetic engineering of crop plants for insect resistance. Curr. Sci., 84: 321-329.

Roy, B., S.K. Noren, A.B. Mandal and A.K. Basu, 2011. Genetic engineering for Abiotic stress tolerance in agricultural crops. Biotechnology, 10: 1-22.

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.

Steinhaus, E.A., 1951. Possible use of *Bacillus thuringiensis* as an aid in the biological control of the alfalfa caterpillar. Hilgardia, 20: 359-381.

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

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

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 corn hybrids and pest management in the USA alternative farming systems, biotechnology, drought stress and ecological fertilisation. Sustainable Agric. Rev., 6: 15-37.

Toenniessen, G.H., J.C. O'Toole and J. DeVries, 2003. Advances in plant biotechnology and its adoption in developing countries. Curr. Opin. Plant Biotechnol., 6: 191-198.

Vaughn, T., T. Cavato, G. Brar, T. Coombe and T. DeGooyer *et al*., 2005. A method of controlling corn 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.

Wani, S.H. and G.S. Sanghera, 2010. Genetic engineering for viral disease management in plants. Not. Sci. Biol., 2: 20-28.