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Review Article

Transgenic Pest Resistance

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Abstract: After the first report of vegetative insecticidal proteins (VIP) from *Bacillus thuringiensis* there was an influx of scientific research to introduce insect resistance in crop plants using *Bt* or *Cry* genes. In this article most of the work happened on insect resistance including other insecticidal genes other than *Cry* is reviewed. However, transgenic plants including RNAi technology is not included.

Introduction

Legumes are used for their chemicals, timber, as cooking fuel, forage crops, pasture crops, cover crops, green manures, for feed and, most importantly food for vegetatarian people in the world. They are grown in the Semi Arid Tropics particularly as rain fed crops and hence are subject to biotic and abiotic stress factors.

Biotic stress is mainly due to insect herbivores, bacteria, viruses and fungi. In the tropics the key factor limiting the yield of pulse crop is the insect pest since decades (Singh and Emden, 1979). The most economically important pests are those, which attack the plant at flowering and fruiting stages. Nevertheless, the pests that attack during the young stages of the plants and during storage are also equally important. Most plants have inherent defense system against most of them that has evolved over a period of time. But this is hardly of any use as the pathogens and the insect herbivores co-evolve for their survival. Plant breeders have been trying hard to develop the crops with higher level of resistance against the pathogens and insect pests since decades through hybridization, but without significant success. Carnivore natural enemies that suppress herbivore populations can protect plants ecologically but at the same time thwart opportunities for selection of herbivore resistance. In other words the effectiveness of defense external to the plant in suppressing herbivores can prevent the evolution of internal resistance to herbivory (Strong and Larsson, 1992).

The pests on legumes are leaf hoppers (*Empoasca*), distributed widely in tropics and sub tropics that attack the legume plants at the seedling stage; aphids (*Aphis*) that deplete the assimilates by the removal of sap, and alsoact as vectors for viruses; bean flies (*Melanagromyza*) a widely distributed seedling pest; a large number of beetles - foliage beetle (*Ootheca*), a vector for yellow mosaic virus, striped foliage beetle (*Luperodes*), blister beetles (*Mylabris* and *Coryna*) that damage flowers and foliage, bean beetle (*Epilachna*, *Lagria* and *Chrysolagria*); weevils – striped bean weevil (*Alcidodes*), pod weevil (*Piezotrachelus* and *Apion*); thrips (*Megalurothrips*) and pod sucking bugs (*Nezara, Anoplocnemis, Riptorus* and *Acanthomia*) have been identified world wide causing damage mainly to the foliage and flowers resulting in stunted growth, late

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flowering and fruiting (Singh and Emden, 1979). However, the most widely distributed and most important foliage, flower and pod borers are those belonging to the order *Lepidoptera*. These have attracted a great deal of attention in recent years because of their wide host range, the severity of damage they cause to crops and their resistance to commonly used chemical insecticides. *Helicoverpa armigera*, *Maruca testulalis*, *Cydia tychora*, *Matsumuraeses phaseoli*, *Etiella zinckenella*, and *Spodoptera littoralis* (Singh and Emden, 1979) are few of them of which *Helicoverpa* and *Spodoptera* spp. are the most notorious ones.

With the advent of genetic transformation techniques, it has become possible to insert genes that confer resistance to insects into the host plant genome. However, protocols for regeneration and transformation are not routinely available for grain legumes. Biotechnology in association with the conventional crop improvement programs can make a drastic difference in the food production (Ortiz, 1998). The major difference between conventional breeding and biotechnology lies in the speed, precision, reliability and scope rather than in goals or process (Sharma and Ortiz, 2000). Among the biological pesticides, bacteria such as *Bacillus* species have been the most successful for use in pest control on a commercial scale. Insecticidal and anti-feedant genes like *Bt* genes, trypsin, protease and amylase inhibitors (enzyme inhibitors), enzymes, lectins, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins could be used singly or in combination for efficient pest control. Synthesised scorpion insect toxin AaHIT gene was also used for resistance to cottonbollworm (Wu *et al.,* 2008) but in this review the subject would be restricted to the above mentioned genes.

It is not surprising that insect resistant crops were the first to hit the market among the transgenic plants, because of the booming price of insecticides and pesticides and the intensity of crop loss due to insects and concern over the pollution caused to the environment. Among the insect resistant transgenics, the plants/crops harboring *Cry*genes are in fairly advance stage in Plant Biotechnology.

Genes from Bacillus thuringiensis

Bacillus thuringiensis is a gram-positive spore forming soil bacterium, which forms parasporal crystals during sporulation with unique insecticidal property. The parasporal crystals consist of one or more or truncated δ-endotoxin (pro-toxin sub units) or Insecticidal Crystal Proteins (ICP) of approximately 138 kDa and the active toxin that shows effective insecticidal properties is about 25 to 135 kDa. Ingestion of the insecticidal crystal protein by the target insect is essential for the toxin to be effective. Inside the gut of the insect, the pro-toxin breaks down to active form in presence of the high pH and gut enzymes, that binds to the gut membrane where it creates ion channels or 'pores' leading to leakage of mid-gut content, paralysis and death. In some insects, it leads to a loss of appetite and stops feeding ultimately leading to death. The high specificity of the protein is due to high-affinity binding sites in the brush border membrane of target insect mid-gut (Hofmann *et al.,* 1998) that is lacking in human beings or ruminates.

There are also vegetative insecticidal proteins (VIP) isolated from the *B. thuringiensis* and do not belong to δ-endotoxin family. The protein is expressed at the vegetative phase of the growth starting at mid-log phase unlike Cry proteins. A list of Vip proteins can be retrieved from the web site http://btnomenclature. info/ (Crickmore *et al.*, 2016). A number of VIPs proteins have been identified and characterized against insects. As Cry and Cyt (cytolytic) proteins, VIPs are also very specific to the order of insects; Vip3A was found to be effective against Lepidopteran insects such as *Spodoptera exigua, Spodoptera litura* and *Helicoverpa armigera* (Estruch *et al.*, 1996; Chen *et al.*, 2002); Vip3V has been cloned and characterized by Doss *et al.* (2002) was found to work against Lepidopteran insects and it did not show any adverse effect on silk worms and mosquitoes. Vip1 and 2 have activity againsts insects belonging to order *Coleoptera* (Pitkin *et al.*, 2005). Yu *et al.* (1997) explained that the basic mode of action of Vip proteins lies in the lysis of mid gut epithelium of the susceptible insect larvae.

There are 341 *cry* genes so far identified of which, only a few have so far found application in technological plant breeding. The Cry and Cyt proteins are classified according to protein structural homologies. Revision of nomenclature of proteins was done by Crickmore *et al.* (1998) and the list of new names of all the insecticidal *cry* genes can be found at the website http://btnomenclature. info/ (Crickmore *et al.*, 2016). These δ-endotoxins are designated as *Cry I*, *Cry II*, *Cry III, Cry IV*, which are fatal to Lepidopteran, Dipteran and Lepidopteran, Coleopteran and Dipteran insects respectively and *cyt* genes and Cyt proteins, which are significantly different in structure and function from the *cry* genes. Fore more information on the *Cry* and *Cyt* genes with respect to the types and their insecticdal protperties, a reference can be made to Hofte and Whiteley (1989), Schnepf *et al*. (1998). The host range of some of Cry and Cyt proteins are given in **Table 1**. Other than those mentioned in the table, fusion genes were also developed for better expression, toxicity and host range in plants based on these genes; for example: *Cry1B-Cry1Ab* (Bohorova *et al.*, 2001), *Cry I E-C* (Singh *et al.*, 2004), *Cry2A** (Chen *et al.*, 2005).

Starting with the first successful demonstration of the power of Cry proteins in transgenic plants inducing insect resistance by Vaeck *et al*. (1987) and Barton *et al*. (1987) in tobacco trailed by Perlak *et al*. (1991), Carozzi *et al*. (1992), Koziel *et al.* (1993) in tobacco and maize, there are umpteen number of successful examples of transgenic plants for insect resistance in many crop plant speices across monocots and dicots. However, insect resistant legume transgenic plants carrying Cry proteins are not too many in number. The usage of *Cry* genes in legumes started in 1996 with the introduction of *Cry IC* in alfalfa for Spodoptera resistance by Strizhov and group followed by Ozias Akins and co workers (Singsit *et al.*, 1997), who have developed peanut transgenic plants carrying *Cry I ac* for resistance against lesser corn stalk borer. Lesser corn stalk borer enhances the invasion of aflatoxin producing fungal species, *Aspergillus niger* on peanut seed. This has resulted in complete to partial protection in peanut. Subsequently, S. K. Sen's group deomonstrated resistance to pod borer, Heliothis in transgenic chickpea carrying *Cry I ac* (Kar *et al.*, 1997). However, this pace was not sustained in legumes.

Transgenic plants expressing *Cry* genes produce the protein continuously; through all the phases of plant growth, though to varying extents, giving them protection and the problem of narrow specificity can be overcome by expressing them properly singly or in combination other insect resistance conferring proteins having with different specificities, gene stacking/ pyramiding (de Maagd *et al.*, 1999; Estruch *et al.*, 1997; Broderick *et al.*, 2000; Sharma *et al.*, 2000; Datta *et al.*,2002; Zhao *et al.*, 2003; Moar, 2003; Hilder, 2003; Beena *et al.*, 2008; Arvinth *et al.*, 2010). Stacking also helps in delaying development of resistance towards the toxins (Zhao *et al.*, 2003). Abdeen *et al.* (2005) suggested that using two dissimilar genes rendered stronger resistance to transgenic plants. Cao *et al.*, (2002a) demonstrated that two *Cry* genes (*Cry1Ac* and *Cry 1C*) pyramided in transgenic broccoli plants imparted resistance against diamond back moth that is resistant to *Cry1A* and *Cry1C* individually in transgenic plants. Use of more than two genes for resistance against yellow stem borer and sap sucking insects has been established by Ramesh *et al.*, (2004) by introducing *Cry IAb* + *CryIAc* + *GNA* into indica rice using super binary vectors of *Agrobacterium*. Transgenic rice plants exhibited significant levels of protection against the target insects. Potentiality of using other genes along with *Cry* genes has been demonstrated (Liang *et al.*, 1994; Fan *et al.*, 1999; Down *et al.*, 2001; Lawrence and Novak, 2001; Gujar *et al.*, 2004; Zhang *et al.*, 2004; Loc *et al.*, 2002; Mehlo *et al.*, 2005; Beena *et al.*, 2008). While Abdeen *et al.*, (2005) could not achieve 100% kill of the insect pest when transformed tomato plants with two different classes of Proteinase inhibitors (Potato protease inhibitor (*PI-II*) and Carboxypeptidase inhibitor (*CPI*)), Wang *et al.* (2005a) produced insect resistant *Brassica napus* by transforming it with chitinase (*Chi*) and scorpion Insect Toxin gene (*Bmk IT*), and Maqbool *et al.* (2001) combined *Cry1Ac* and *Cry2A* with *GNA* to develop resistance against three pests in rice viz., rice leaf folder, brown hopper and yellow stem borer. Datta *et al.* (2002) combined three entirely different genes conferring resistance to bacterial blight, yellow stem borer and sheath blight (*Xa2I* + *Bt* + chitinase), and produced stable transgenic rice lines resistant to all the three. This shows that stacking genes in a transgenic crop plant is technologically viable and sound for achieving stronger effect in one trait or satisfactory effect in multiple traints depending on the genes deployed. There are also reports on combining QTLs with *Cry1Ac* for better and sustainable lepidopteran insect resistance (Walker *et al.*, 2002). Haidi *et al.* (1996), Chen *et al.* (1998) and Campbell *et al.* (2000) have recorded multiple gene integrations (up to 13) with different plasmid vectors using biolistics method of transformation

When transgenic plants and other strategies were compared for the potential ecological and human health consequences including effects on non-target organisms, food safety, and the development of resistant insect populations in insect management, the risks were definitely lower and beneficial than any other strategies deployed (Betz *et al.*, 2000; Shelton *et al.*, 2002, Romeis *et al.*, 2006) and can be successfully utilized in IPM. There are other reports that clearly demonstrate safety in using *Cry* and other genes for insect resistance in crop plants (Girard *et al.*, 1998; Wraight *et al.*, 2000; Gatehouse *et al.*,2002; Hellmich *et al.*, 2001; Sears *et al.*, 2001; Tabashnik *et al.*, 2003; Dutton *et al.*, 2003; Cowgill and Atkinson, 2003; Hanley *et al.*, 2003; Wei-Xiang *et al.*, 2004; Schuler *et al.*, 2004; Bashir *et al.*, 2004; Huang *et al.*, 2005; Turlings *et al.*, 2005). Carriere *et al.* (2003) have shown in a ten-year study on *Bt* cotton in Arizona region that these transgenic plants helped long-term suppression of the pests and reduced the use of insecticide sprays considerably. Apart from this, there was no resistance developed by the insect against the introduced transgene encoded protein. There are only two insect species, which developed resistance towards *Bt* insecticide sprays under commercial situations. As Shelton (2002) suggested, it is necessary to have optimal and consistent expression of the Cry and other proteins to achieve desisrable effects in the transgenic plants, and delay the development of resistance in the insect and it is also likely that there are fewer genes in insects for resistance than estimated and recessive genes may be involved which might need more generations to develop resistance. Liu *et al.* (2001) observed that there is no cross resistance towards other insecticidal genes in diamond black moth that had evolved resistance against *Cry 1C*.

Since the list of transgenic plants carring the different *Cry* genes singly or in combination with other genes is exhaustive, only some significant examples in other crops along with legume transgenic plants have been listed in Table 2.

Enzymes and Enzyme Inhibitors Conferring Insect resistance

The enzymes and enzyme inhibitoros that confer insect resistance would include protease inhibitors, chitinase, cholesterol oxidase, polyphenol oxidase, ehancin etc.

Plant proteinase inhibitors are mostly polypeptides or proteins, which occur in a variety of plants that have been evolved as a defense mechanism against herbivores. They inhibit the synthesis of proteins thereby affecting proteolysis of protein into amino acids, which in turn affects the growth of the insect larvae. At the same time, they enhance pernicious hyper production of proteinase activity that again leads to scarcity of amino acid supply for insect growth and development (Gujar *et al.,* 2000). According to Koiwa *et al*. (1997), protease inhibitors are an important element of the plant defense response to insect predation and may also act to restrict infection by some nematodes. They are generally classified according to the class of proteases they inhibit. Four types of proteases have been identified as serine, cystein, aspartic, or metallo-proteases (Koiwa *et al.,* 1997; Lawrence and Koundal, 2002). Some plant serine protease inhibitors are bifunctional possessing trypsin and α-amylase inhibitors activity. These proteinase inhibitors are insecticidal at high concentrations. Phytocystatins are cysteine proteinase inhibitors of plant origin that occur in a variety of higher plants including rice (Abe *et al.,* 1987; Kondo *et al.,* 1989, 1990) and corn (Abe *et al.,* 1992). Production of these inhibitors is highly regulated by a signal transduction pathway that is initiated by predation and transduced as a wound response. For more detailed information on enzyme inhibitors, please refer to Ryan (1990), Ussuf *et al.* (2001), De Leo *et al.* (2002), Oleveira *et al.* (2003), Valueva and Mosolov (2004).

Chitin is a linear homopolymer of β (1-4) linked 2-deoxy-2-acetamido-D-glucopyranosyl residues and is a major component of the exoskeleton and alimentary canal of insects. Together with proteinases in the moulting fluid, endo-splitting chitinases and exo-splitting β-N-acetylglucosaminidases break down the unsclerotized layers of the old cuticle prior to shedding of the sclerotized portion or exuvium. Transcripts of moulting fluid chitinase are seen only in epidermal and gut tissue of 5^{th} instar larvae between 5 and 7 days after moulting and just prior to pupal transformation (Ding *et al.,*1998a). This tight developmental regulation suggests that this chitinase could be detrimental to insect growth if presented in the diet at an inappropriate time. Transgenic plants expressing chitinase show enhanced protection against insects. Van der Westhuizen *et al.* (1998) analyzed the differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation and suggested that peroxidase and chitinase have crucial roles in insect resistance. There are also reports showing that chitin synthesis inhibitors (CSI) applied externally also gives 100% mortality in pests (Cohen, 1993; Wilson and Cryan, 1997; Kostyukovsky and Trostanetsy, 2006).

Cholesterol oxidase is a monomeric bacterial flavoenzyme that catalyzes the oxidation and isomerization of cholesterol to ketosteroids and hydrogen peroxide and is widely used in clinical assays for determining levels of total serum cholesterol. This enzyme is produced by phylogenetically diverse group of microorganisms and usually occurs as a secreted protein. Cholesterol oxidases have been isolated from several sources including members of the genera *Streptomyces*, *Pseudomonas*, *Schyzophyllum* and *Rhodococcus*. It has potent insecticidal activity against cotton boll weevil, *Anthonomus grandis* (Corbin *et al.* 2001). Upon ingestion, this enzyme causes developmental arrest and death of the larvae (Purcell *et al.*, 1993) and also marked decrease in the adult female fecundity (Greenplate *et al.*, 1995). It shows moderate mortality when used against some of the lepidopteran insects (Greenplate *et al.*, 1997). It was Purcell *et al.* (1993, 1994), who found that the filtrate of *Streptomyces* cultures killed the boll weevil larvae and this has been attributed to the oxidation of cholesterol in the mid gut epithelial membrane resulting in physical and functional disruption of the membrane; and that the active component that caused the mortality was cholesterol oxidase. Shen et al. (1997) reported that mild toxicity of cholesterol oxidase on Lepidopteran insects was due to general susceptibility to cholesterol oxidase. Santos *et al*. (2002) showed that the emergence and viability of cotton boll weevil larvae (*Anthonomus grandis*) was acutely affected when fed with artificial diet containing cholesterol oxidase.

Polyphenol oxidase oxidizes phenols by catalyzing the oxidation of the *o*-diphenols to *o*-diquinones, as well as hydroxylation of monophenols. This activity of the enzyme increases in response to different types of stress, both abiotic and biotic, mostly due to physiological injury (Rivero *et al.*, 2001). Felton *et al.* (1992) reported that polyphenol oxidase significantly reduced protein quality in *Spodoptera exigua* larvae, which influenced the larval growth rate. Polyphenol oxidase cDNAs have been cloned from sugarcane, apple, grape, potato etc. and upon characterization of this cNDA, Constabel *et al.* (1995) proposed the role of defense for these genes. Upon chewing and feeding, the interaction of polyphenol oxidase with phenolic substrates generates *o*-quinones and these highly reactive conpounds covalently modify free amino and sulfhydryl groups in dietary proteins within the insect. Constabel *et al.* (2000) have also cloned a polyphenol oxidase gene from poplar expressed in response to wounding and herbivory.

Enhancin gene from baculoviruses encodes baculovirus metalloproteases that can degrade mucin in the mid guts of insects (Lepore *et al.*, 1996; Wang and Granados, 1997). Disruption of peritrophic membrane increases viral infection and leads to faster larval mortality (Wang *et al.*, 1994). Hayakawa *et al*. (2000) and Cao *et al.* (2002b) have shown that it is effective against larvae of *Spodoptera exigua* and *Trichoplusia ni* in transgenic tobacco.

Protease and amylase inhibitors, chitinases and other enzymes deployed in raising transgenic plants including legumes, that resisted herbivores are listed below in Table 3 including the legume transgenic plants.

Other secondary metabolites with insecticidal activity

Oleoresins, lectins, phytoalexins (antimicrobial secondary metabolites) etc. are other secondary metabolites induced by wounding are shown to have increased effect against herbivore insects and are used in transgenic plants for developing insect resistance.

Oleoresin is a complex mixture of terpenoids, consisting of a turpentine (monoterpene and sesquiterpene) and rosin (diterpene) fraction. The turpentine fraction contains a range of insect and microbial toxins such as limonene and 3-carene and other biologically active agents that often act synergistically to discourage insect predation. Turpentine also acts as the solvent for transporting the higher molecular weight diterpenoid resin acids (rosin fraction) to the site of injury. Upon exposure to the atmosphere, the volatile turpentine evaporates leaving a semi crystalline mass of resin acids that oxidatively polymerize to form a hardened barrier that seals the wound, often trapping insect invaders and microbial pathogens in the matrix (Phillips and Croteau, 1999).

Host plants play a key role in the production and use of sex pheromones by herbivorous insects through larval or adult sequestration of chemically active compounds and pheromone precursors. Some of the plant semiochemicals/ volatiles have inhibitory or repellent effects that interrupt insect responses to pheromones and attract predators and parasitoids to the attacking species after herbivory injury (Reddy and Guerrero, 2004). The synergism between insect pheromones and plant odors can increase attraction of natural enemies, offering new strategies for IPM. Altering the plant volatile compounds will make it difficult for the phytophagous insects to locate their host plants for oviposition. In the same way nectarines in the nectar of the angiosperms are also found to have defense role against non-pollinating insects and other air borne microbes (Carter and Thornburg, 2004).

Lectins act almost like *Cry* genes where they bind to the gut surfaces of the insect larvae causing lesions and death of the larvae. They show detrimental effects on homopteran insects and aphids (Wang *et al.,* 2005b). Lectins belong to a broad group of bioactive peptides called defensins that elicit a variety of responses including plant defence against a range of factors including environmental stress, predation by insects, and infestations by bacteria, fungi, and nematodes. Many agricultural plants lack lectin defenses, however, making them susceptible to yield losses from pest infestations. The insertion of specific lectin genes into crop plants gives protection against many pestilent pests attacking them. Most commonly used lectins in transgenic studies are GNA (*Galanthus nivalis* agglutinin) and Con A (Jack bean: *Canavalia ensiformis* lectin). Lectins are favored over other pesticidal plant compounds because of their low toxicity to humans and domesticated animals at levels that are effective against insect larvae, and GNA does not appear to damage other organisms. Chrispeels and Raikhel (1991) made a comprehensive review on lectins and their role in plant defense.

Avidin is a water-soluble tetrameric glycoprotein isolated originally from chicken egg white. It strongly binds to vitamin biotin, which is an essential compound for insects, which they acquire from dietary sources. Biotin is a co-factor of major carboxylases involved in gluconeogenesis, lipogenesis, fatty acid and amino acid catabolism. It causes mortality of a wide range of pests if mixed with artificial diets and fed (Morgan *et al.,* 1993; Markwick *et al.,* 2001 and Burgess *et al.,* 2002). Transgenic plants, developed for resistance against various insects using secondary metabolites are given in Table 4.

Each insecticidal gene is unique. It is important to assess the activity of the protein encoded by a gene against a particular insect and in a particular crop. Different genes for the proteins with insecticidal activity that have been characterized from different species are summarized in the Table 5.

Conclusions and Future

The efficacy of insect resistance engineered by deployment of the various insect resisting gene products, the proteins has been well documented and the level of cultivation of insect resistant transgenic crops cultivated on 16. 2 million hectares with Bt crops including soybean and 10. 1 million hectares on the stacked herbicide tolerance and insect resistance bears testimony to the concept of insect resistance through genetic engineering (James, 2005). The technology should be used judiciously in tackling insect problems. The most important insect(s) attacking a given legume crop need to be identified and the genes for controlling them need to be ascertained. There are about 341 *Cry* genes reported and it is imperative to study the relative efficiencies of the individual Cry proteins against target insects in legumes. A thorough study is needed in this direction for identifying effective Cry proteins for deploying the corresponding genes in legume transgenic plants for insect resistance. Compared to other crops, the legumes, barring soybean which has received attention of the various laboratories and Multinational Companies on a very large scale because of the importance world wide, have started receiving attention of late and the desired transformaiotn protocols for the recalcitrant legume are standardized. With this scenario, more insect resistant legume transgenic crops are expexted to be developed in the near furture.

Apart from the *Cry* genes, the bacterium produces other proteins, which are essentially insecticidal in nature. Plants also try to defend themselves against the invading insects by producing different molecules and proteins that have a direct bearing on the insects feeding on them. It could be through various morphological, biochemical and or molecular mechanisms. The biochemical mechanisms against insects are of wide ranges which are dynamic. More and more such anti-insect proteins and molecules need to be identified for their utilization in genetic engineering. The larvae of insect pests have essentially large guts as they spend their whole life, till they pupate, feeding on the host plants. Most of the mechanisms described in this article targets the mid gut of the larvae and stops them from feeding. As the pro-toxins will be converted to active toxin only in the mid guts of the target larvae, even as the plant dies and degenerates, it will not be converted to active toxin; hence do not leave any toxin traces in the ecosystem to be biomagnified at a higher level. Occurrence of resistance alleles in the target insect populations should be assessed so that strategies such as pyramiding of two or more similar or dissimilar genes can be deployed to overcome the resistance. Over expression of a single gene also could be considered for which, lethal concentration of the protein should be measured.

Technology has changed and newer methods of engineering resistance have been discovered. Use of double stranded RNA in the forms of artificial micro RNA, small RNA, and tasi-RNA is taking over the normal gene stacking mechanisms against insects which are acting as pests and also as vectors for many devastating diseases, which are not elaborated in this review. Among the still existing intense debates whether or not transgenic plants itself and toxins and antibiotic resistance genes they carry are harmful in a long run to human kind as well as to other harmless useful insects, *Bt* still remains a very good candidate for crop improvement against insect pests.

Table 1. Host range of some Cry and Cyt proteins: (Please refer to Hofte and Whiteley (1989), Schnepf *et al*. (1998)

Cry protein	Acc. No.	Insect
CrylA(a)	M11250	Lepidoptera
CrylA(b)	M13898	Lepidoptera
CrylA©	M11068	Lepidoptera
CrylB(a)	X06711	Lepidoptera and Coleoptera
Cry1B©	AAQ52387	Lepidoptera
CrylC(a)	X07518	Lepidoptera
CrylD(a)	X54160	Lepidoptera
CrylE(a)	X53985	Lepidoptera
CrylF(b)	173895	Lepidoptera
Cry1G(a)	Y09326	Lepidoptera
Cry1I(a)	X62821	Lepidoptera
Cry1I(b)	U07642	Lepidoptera
Cry1I(d)	AF047579	Lepidoptera
Cry1K(a)	U35780	Lepidoptra
CryIIA(a)	M23723	Lepidoptera and Diptera
CryIIA(b)	M23724	Lepidoptera
CryIIA©	AAQ52385	Lepidoptera
CryIIIA(a)	Y00420	Coleoptera
CryIIIB(a)	X17123	Coleoptera
CryIIIC(a)	X59797	Coleoptera
CryIVA(a)	Y00423	Diptera
CryIVB(a)	X07423	Diptera
CryIVC	--	Diptera
CryIVD	--	Diptera
CryVA©	134543	Coleoptera and Lepidoptera
CryIXA(a)	X58120	Lepidoptera
CryIXB(a)	X75019	--
CryXA(a)	M12662	Diptera
CryXIA(a)	M22860	Diptera
CryXIB(a)	X86902	Diptera
CryXIB(b)	AF017416	Diptera
CryXVA(a)	M76442	Lepidoptera
Cry20A(a)	U82513	Diptera
Cry26A(a)	AF122897	--
Cry38A(a)	AAK64559	Siphonoptera and Coleoptera
CytIA(a)	X03182	Diptera
CytIA(b)	X98793	Diptera
CytIC(a)	AL731825	Diptera
CytIIA(a)	Z14147	Diptera
CytIIB(a)	U52043	Diptera
CytIIC(a)	AAK50455	

Table 2. A curtailed list of transgenic plants hosting *Cry* genes; legume transgenes emphasized in bold letters

***Bt* gene**	**Plant**	**Insect**	**Reference**
Cry2AX1	rice	Rice leaffolder (*Cnaphalocrosis medinalis*)	Manikandan *et al.*, 2016
Cry1Ac	Sugarcane	Sugarcane borer (*Diatrea saccharalis*)	Gao *et al.*, 2016
Cry1la12	Cotton	Fall armyworm (*Spodoptera frugiperda*) Cotton boll weevil (*Anthonomus grandis*)	de Oliveira *et al.*, 2016
Cry1Ac + Cry2A	Cotton	American boll worm *Heliothis* sp	Puspito *et al.*, 2015
Cry1Ac and *Cry2A*	Cotton	American boll worm *Heliothis* sp	Muzafar *et al.*, 2015
Cry1Ac	Tomato	*Helicoverpa armigera*	Koul *et al.*, 2014; 2015
Cry1C	Maize	*Ostrinia furnacalis*	Du *et al.*, 2014
Cry1Aa3	Sugarcane	Sugarcane borer	Kalunke *et al.*, 2009
Cry 1 E-C	**Peanut**	***Spodoptera litura***	**Beena *et al.*, 2008**
Cry1Ac	Sugarcane	Sugarcane borer	Weng *et al.*, 2006
Cry 1 E-C	**Pigeon pea**	***Spodoptera litura***	**Surekha and Beena *et al.*, 2005**
Cry3Bb1	Maize	Cut root-worm (*Diabrotica* spp.)	Vaughn *et al.*, 2005
Cry1Ac	Sorghum	Spotted stem borer (*Chilo partellus*)	Girijashankar *et al.*, 2005
*Cry2A**	Indica rice	Lepidopteran pests	Chen *et al.*, 2005
***Cry 1 Ab* protoxin**	**Soybean**	**Velvet bean caterpillar**	**Dufourmantel *et al.*, 2005**
Cry I A(c)	**Chick pea**	***Helicoverpa armigera***	**Sanyal *et al.*, 2005**
Cry 1 A(b)	Pusa Broccoli KTS-1	Diamond back moth (*Plutella xylostella*)	Viswakarma *et al.*, 2004
Cry1 Aa10	Oil seed rape	Diamond back moth (*Plutella xylostella*)	Huo *et al.*, 2003
Cry 2Ab	Cotton	Pink boll worm	Tabashnik *et al.*, 2002
Cry 1 A(b)	Cauliflower	Diamond back moth (*Plutella xylostella*)	Chakrabarty *et al.*, 2002
Cry 1 A(b)	Cabbage	Diamond back moth (*Plutella xylostella*)	Bhattacharya *et al.*, 2002
Cry9Aa	Tobacco, Potato, Cauliflower and Turnip	Diamond back moth (*Plutella xylostella*)	Kuvshinov *et al.*, 2001
Cry V	Potato	*Phthorimaea operculella* and *Symmetrischema tangolias*	Douches *et al.*, 2000; Madkour *et al.*, 2000; Lagnaoui *et al.*, 2000
Cry1 Ab3	Cabbage	Diamond back moth (*Plutella xylostella*)	Jin *et al.*, 2000
Cry2A	Rice	Yellow stem borer and rice leaf folder	Maqbool *et al.*, 1998
CryIA(b)	Sugarcane	Sugarcane borer (*Diatraea saccharalis* F.)	Arencibia *et al.*, 1997
CryIIIB	Eggplant	Colorado potato beetle (*Lepinotarsa decemlineata* Say)	Arpaia *et al.*, 1997

Cry 1 A(c)	**Chick pea**	**Pod borer (*Heliothis armigera*)**	**Kar *et al.*, 1997**
Cry 1 A(c)	**Peanut**	**Lesser Corn Stalk Borer (LCB) *Elasmopalpus lignosellus***	**Singsit *et al.*, 1997**
Cry 1 C	**Alfalfa and tobacco**	***Spodoptera***	**Strizhov *et al.*, 1996**
Cry 1Ac	*Brassica napus*	Diamondback moth (*Plutella xylostella* L.) and cabbage looper (*Tricholusia ni* Hübner)	Stewart *et al.*, 1996a
Cry 1 Ac	**Soybean**	***Helicoverpa zea, Pseudoplusia includens, Heliothis virescens,* and *Anticarsia gemmatalis***	**Stewart *et al.*, 1996b**
CryIIIA	Potato	*Leptinotarsa decemlineata*	Perlak *et al.*, 1993
Cry1A(b)	Rice	stemborer (*Chilo suppressalis*) and leaffolder (*Cnaphalocrosis medinalis*)	Fujimoto *et al.*, 1993
HD-1 (cry1A(b)) and *HD-73 (cry1A(c))*	Cotton	*Trichoplusia ni* and *Heliothis zea*	Perlak *et al.*, 1990
Bt protein	Tomato	*Helicoverpa zea, Manduca sexta, Keiferia hypersicella*	Delannay *et al.*, 1989

Table 3. List of enzymes and enzyme inhibitors used for developing transgenic plants; legume transgenes highlighted in bold face

Plant	Gene	Insect	Order	Reference
Cotton	Pin I from *Solanum tuberosum* and Pin II inhibitor from *Nicotiana alata*	*Helicoverpa* spp.	Lepidoptera	Dunse *et al.*, 2010
Tobacco	Proteinase inhibitor from *Solanum americanum*	*Helicoverpa armigera* and *Spodoptera litura*	Lepidoptera	Luo *et al.*, 2009
Tomato	Potato protease inhibitor (*PI-II*) + Carboxypeptidase inhibitor (*CPI*)	Tomato fruit worm (*Heliothis 11bsolete*) and serpentine leafminer (*Liriomyza trifolii*)	Lepidoptera; Diptera	Abdeen *et al.*, 2005
Chick pea	**Bean α-amylase inhibitor 1**	***Callosobruchus* spp.**	**Coleoptera**	**Sarmah *et al.*, 2004**
Pisum sativum	**α-amylase inhibitor**	**Pea weevil (*Brochus pisorum*)**	**Coleoptera**	**de Sausa-Majer *et al.*, 2004**
Sugarcane	Soybean proteinase inhibitor	*Diatrea saccharalis*	Lepidoptera	Falco and Silvia-Filho., 2003
Rice	Trypsin inhibitor from Barley	*Sitophylus oryzae*	Coleoptera	Alfonso-Rubi *et al.*, 2003
Tobacco	Enhancin (*Tn-En*) and (*Ha-En*)	*Trichoplusia ni*	Diptera	Cao *et al.*, 2002b

Potato	Trypsin inhibitor	*Lacanobia oleracea*	Hymenoptera	Bell *et al.*, 2001
White poplar	***Arabidopsis* proteinase inhibitor (*Atcys*)**	**Chrysomelid beetle (*Chrysomela populi*)**	**Coleoptera**	**Delledone *et al.*, 2001**
Pea	**α-amylase inhibitor 1**	**Pea weevil**	**Coleoptera**	**Morton *et al.*, 2000**
Tobacco	*Autographa californica* nucleopolyhedrovirus Enhancin	*Spodoptera exigua*	Lepidoptera	Hayakawa *et al.*, 2000
Potato and Tobacco	Soybean Kunitz C-II and PI-IV serene protease inhibitors	*Spodoptera littoralis*	Lepidoptera	Marchetti *et al.*, 2000
Rice	Bean trypsin inhibitor (*WTI-1B*)	Rice stem borer (*Chilo suppressalis*)	Lepidoptera	Mochizuki *et al.*, 1999
Tobacco and Peas	*Nicotiana alata* protease inhibitor	*Helicoverpa armigera*	Lepidoptera	Charity *et al.*, 1999
Wheat	Barely trypsin inhibitor (*BTI-Cme*)	Angoumois grain moth (*Sitotroga cerealella*)	Lepidoptera	Altpeter *et al.*, 1999
Rice	Soybean Kunitz trypsin inhibitor	*Nilaparvata lugens* Stal	Hemiptera	Lee *et al.*, 1999
Tobacco	Insect (*Madhuca sexta*) chitinase (EC 3. 2. 1. 14)	Tobacco bud worm (*Heliothis virescens*)	Lepidoptera	Ding *et al.*, 1998a
Bean	**Bean α-amylase inhibitor**	**Bean weevil**	**Coleoptera**	**Grossi de Sa *et al.*, 1997**
Rice	Cowpea Trypsin inhibitor (*CpTi*)	Striped stem borer (*Chilo suppressalis*) and pink stem borer (*Sesamia inferens*)	Lepidoptera	Xu *et al.*, 1996
Azuki bean	**Common bean α-amylase inhibitor**	**Bruchid (*Zabrotes subfasciatus*)**	**Coleoptera**	**Ishimoto *et al.*, 1996**
Pea	**Bean α-amylase inhibitor**	**Bean weevil (*Bruchus pisorum*)**	**Coleoptera**	**Schroeder *et al.*, 1995**
Cotton	*Manduca sexta* Protease inhibitor	*Bemisia tabaci*	Hemiptera	Thomas *et al.*, 1995
Alfalfa	***Manduca sexta* Antielastase proteinase inhibitor**	**Thrips**	**Thysanoptera**	**Thomas *et al.*, 1994**

Table 4. Transgenic plants developed using genes producing insecticidal secondary metabolites; Fabacean plant highlighted in bold

Plant	Gene	Insect	Order	Reference
Cotton	*Allium sativum* agglutinin	Jassid (*Amrasca devastans*) and Whitefly (*Bemisia tabaci*)	Hemiptera	Vajhala *et al.*, 2013
Rice	*Dioscorea batatus* tuber lectin 1	Brown plant hopper	Hemiptera	Yoshimura *et al.*, 2012
Rice	*Allium sativum (asal)* + *Galanthus nivalis (gna)*	*Nephotetix virescens, Nilaparvata lugens, Sagotella furcifera*	Hemiptera	Bharathi *et al.*, 2011
Chickpea	*Allium sativum (asal)*	***Aphis craccivora***	**Hemiptera**	**Chakraborti *et al.*, 2009**
Rice	*Allium sativum (asal)*	Sap sucking insects	Hemiptera	Yarasi *et al.*, 2008
Cotton	*Amaranthus codatus* agglutinin	Aphid	Hemiptera	Wu *et al.*, 2006
Tobacco	*Allium sativum* lectin	Peach potato aphid (*Myzus perscicae*)	Hemiptera	Dutta *et al.*, 2005
Maize	Snow-drop lectin (*GNA*)	Corn borer	Lepidoptera	Wang *et al.*, 2005c
Maize	Snow-drop lectin (*GNA*)	Corn leaf aphid (*Rhopalosiphum maidis*)	Hemiptera	Wang *et al.*, 2005b
Tepary bean	**Arcelins (*arc5* or *arc1*)**	**Mexican bean weevil**	**Coleoptera**	**Zambre *et al.*, 2005**
Populus	**Polyphenol oxidase**	**Forest tent caterpillar**	**lepidoptera**	**Wang and Constabel, 2004**
Rice	Snow-drop lectin (*GNA*)	White backed plant hopper (*Sogatella furcifera*), brown plant hopper and green leaf hopper	Hemiptera	Nagadhara *et al.*, 2004
Rice	Snow-drop lectin (*GNA*)	Brown plant hopper and green leaf hopper	Hemiptera	Nagadhara *et al.*, 2003
Rapeseed	Pea lectin	Pollen beetle (*Meligethes aeneus*)	Coleoptera	Melander *et al.*, 2003
Sugarcane	Snow-drop lectin (*GNA*)	Mexican rice borer (*Eoreuma loftini*)	Lepidoptera	Wachtel *et al.*, 2003
Tobacco and apple	Avidin and Streptavidin	Potato tuber moth (*Phthorimaea operculella*) and light brown apple moth (*Epiphyas postvittana*)	Lepidoptera	Markwick *et al.*, 2003

Tobacco	*Helianthus tuberosus* agglutinin	Peach-potato aphid (*Myzus persicae*)	Hemiptera	Chang *et al.*, 2003
Tobacco	*Pinellia ternata* agglutinin	Aphid	Hemiptera	Yao *et al.*, 2003
Mustard	Wheat germ agglutinin	Aphid	Hemiptera	Kanrar et al. 2002
Sugarcane	Lectin (*GNA*)	Mexican rice borer (*Eoreuma loftini*) Sugarcane borer (*Diatraea saccharalis*)	Lepidoptera	Setamou *et al.*, 2002
Rice	Lectin (*GNA*)	Brown plant hopper(*Nilaparvata lugens*)	Hemiptera	Tang *et al.*, 2001
Tobacco	Cholesterol oxidase	Cotton boll weevil (*Anthonomus grandis grandis*)	Lepidoptera	Corbin *et al.*, 2001
Maize	Avidin	Storage pests	-	Kramer *et al.*, 2000
Potato	Lectin (*Con A*)	Tomato moth (*Lacanobia oleracea*)and peach potato aphid (*Myzus persicae*)	Hemiptera	Gatehouse *et al.*, 1999
Wheat	Lectin (*GNA*)	Grain aphid (*Sitobion avenae*)	Hemiptera	Stoger *et al.*, 1999
Rice	Lectin (*GNA*)	Brown plant hopper(*Nilaparvata lugens*)	Hemiptera	Rao *et al.*, 1998
Potato	Lectin (*GNA*)	Tomato moth (*Lacanobia oleracea*)	Hemiptera	Gatehouse *et al.*, 1997
Rice	Lectin (*GNA*)	(Brown plant hopper) *Nilaparvata lugens*	Hemiptera	Gatehouse *et al.*, 1996
Grand fir	Oleoresin	Bark beetle	Coleoptera	Steele, 1995

Table 5. Some genes characterized for resistance against pests

Origin	**Gene**	**Insect**	**Order**	**Reference**
Chemical/Soybean	Serine proteinase inhibitor	*Eurygaster integriceps*	Hemiptera	Saadati and Bandani, 2011
Corn	α-amylase inhibitor	*Sitophilus zeamais*	Coleoptera	Marsaro Junior *et al.*, 2005
Rice	*Bph 1*	Brown plant hopper (*Nilaparvata lugens*)	Hemiptera	Kim and Sohn., 2005
Capsicum Annum	Proteinase inhibitors (*CapA1* and *Cap A2*)	*Helicoverpa armigera*	Lepidoptera	Tamhane *et al.*, 2005
Chick pea	**Trypsin inhibitor**	***Helicoverpa armigera***	**Lepidoptera**	**Srinivasan *et al.*, 2005**
Jackbean, Snowdrop	*Con A*, *GNA* (lectins)	*Lacanobia oleracea*	Hymenoptera	Bell *et al.*, 2004

Jackbean, Snowdrop	Lectin (*Con A*)	Pea aphid (*Acyrthosiphon pisum*)	Hemiptera	Suavion *et al.*, 2004
Poecilanthe parviflora seeds	Trypsin inhibitor	*Diatraea saccharalis, Anagasta kuehniella, Spodoptera frugiperda*, and *Corcyra cephalonica*	Lepidoptera	Garcia *et al.*, 2004
Talisia esculenta	Lectin (*TEL*)	*Callosobruchus maculatus*	Coleoptera	Macedo *et al.*, 2004
Tobacco	Cembratrienols (CBTols)	Aphids	Hemiptera	Wang *et al.*, 2004
Vigna unguiculata	**Proteinase inhibitor (vicilins)**	**Sugar cane stalk borer (*Diatraea saccharalis*)**	**Lepidoptera**	**Mota *et al.*, 2003**
Winged bean)	Trypsin inhibitor	*Helicoverpa armigera*	Lepidoptera	Giri *et al.*, 2003
Pinellia ternata	Lectin (*PTA*)	Peach potato aphids	Hemiptera	Yao *et al.*, 2003
Koelreuteria paniculata	Lectin	*Callosobruchus maculates* and *Anagasta kuehniella*	Coleoptera Lepidoptera	Macedo *et al.*,2003
Streptomyces sp.	Cholesterol oxidase (E. C1. 1. 3. 6)	Cotton boll weevil larvae	Lepidoptera	Santos *et al.*, 2002
Jackbean, Snowdrop	Lectins (*Con A, GNA*)	*Lacanobia oleracea*	Lepidoptera	Fitches *et al.*, 2001
Soybean	**Proteinase inhibitor**	**Sugarcane borer (*Diatraea saccharalis*)**	**Lepidoptera**	**Pompermayer *et al.*, 2001**
Snowdrop, Jack bean and *Psophocarpus tetragonolobus*	Lectins (*GNA, Con A, PTA*)	Plant hoppers(*Tarophagus proserpina* and *Nilaparvatha lugens*)	Hemiptera	Powell 2001
Phaseolus vulgaris	**α-amylase inhibitor**	**Coffee berry borer (*Hypothenemus hampei*)**	**Coleoptera**	**Valencia *et al.*, 2000**
Phaseolus vulgaris	**α-amylase-like**	**Cowpea weevil (*Callosobruchus maculatus*), azuki bean weevil (*C. chinensis*)**	**Coleoptera**	**Ishimoto *et al.*, 1999**
Kidney bean (*Phaseolus vulgaris*)	**Arcelin-1 (lectin-like protein)**	**Bruchids**	**Coleoptera**	**Fabre *et al.*, 1998**
Griffonia simplicifolia	**N-acetylglucosamine-specific lectin**	**Cowpea weevil (*Callosobruchus maculatus*)**	**Coleoptera**	**Zhu *et al.*, 1996**
Streptomyces sp.	Cholesterol oxidase (E. C1. 1. 3. 6)	Cotton boll weevil larvae	Coleoptera	Purcell *et al.*, 1993; 1994

References

Abdeen, A., Virgos, A., Olivella, E., Villanueva, J., Avile X., Gabbara, R. and Prat, S. (2005). Multiple insect resistance in transgenic tomato plants over-expressing two families of plant proteinase inhibitors. *Plant Mol. Biol*. **57**: 189-202.

Abe, K., Emori, Y., Kondo, H., Suzuki, K. and Arai, S. (1987). Molecular cloning of a cysteine proteinase inhibitor of rice (oryzacystatin). *J. Biol. Chem*. **262**: 16793-16797.

Abe, M., Abe, K., Kuroda, M. and Arai, S. (1992). Corn kernel cysteine proteinase inhibitor as a novel cystatin superfamily member of plant origin: molecular cloning and expression studies. *Eur. J. Biochem*. **209**: 933-937.

Alfonso-Rubi, J., Ortego, F., Castanera, P., Carbonero, P. and Dyazi, I. (2003). Transgenic expression of trypsin inhibitor CMe from barley in *indica* and *japonica* rice confers resistance to the rice weevil *Sitophilus oryzae*. *Trans. Res.* **12**: 23-31.

Altpeter, F., Diaz I., McAuslane, H., Gaddour, K., Carbonero, P. and Vasil, I. K. (1999). Increased insect resistance in transgenic wheat stably expressing trypsin inhibitor CMe. *Mol. Breed*. **5**: 53-63.

Arencibia, A., Vázquez, R. I., Prieto D., Tellez,P., Carmona, E. R., Coego, A., Hernandez, L., De la Riva, G. A. and Selman Housein, G. (1997). Transgenic sugarcane plants resistant to stem borer attack. *Mol. Breed*. **3:** 247 – 252.

Arpaia, S., Mennella, G., Onofaro, V., Perri, E., Sunseri, F. and Rotino, G. L. (1997). Production of transgenic eggplant (*Solanum melongena* L.) resistant to colarado potato beetle (*Leptinotarsa decemlineata* Say). *Theor. Appl. Genet*. **95**: 329-334.

Arvinth, S., Arun, S., Selvakesavan, R. K. Srikanth, J., Mukundan, N., Ananda, Kumar, P., Premachandran, M. N. and Subramonian, N. (2010). Genetic transformation and pyramiding of aprotinin-expressing sugarcane with *cry1Ab* for shoot borer (*Chilo infuscatellus*) resistance. *Plant Cell Rep*. **29**: 383 – 386.

Barton, K. A., Whiteley, H. R. and Yang, N. S. (1987). *Bacillus thuringiensis* δ-endotoxin expressed in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. *Plant Physiol.* **85**: 1103-1109

Bashir, K., Husnain, T., Fatima, T., Latif, Z., Mehdi, S. A. and Riazuddin, S. (2004). Field evaluation and risk assessment of transgenic indica basmati rice. *Mol. Breed.* **13(4):** 301-312.

Beena,M. R., Tuli, R., Gupta, A. D. and Kirti, P. B. (2008). Transgenic peanut (*Arachis hypogaea* L.) plants expressing *cryIEC* and rice chitinase cDNA (*Chi 11*) exhibit resistance against insect pest *Spodoptera litura* and fungal pathogen *Phaeoisariopsis personata*. *Transg. Plant J.* **2(2)**: 157-164.

Bell, H. A., Fitches, E. C., Down, R. E., Ford, L., Marris, G. C., Edwards, J. P., Gatehouse J. A. and Gatehouse, A. M. (2001). Effect of dietary cowpea trypsin inhibitor (CpTI) on the growth and development of the tomato moth *Lacanobia oleracea*(Lepidoptera: Noctuidae) and on the success of the gregarious ectoparasitoid *Eulophus pennicornis* (Hymenoptera: Eulophidae). *Pest Manag. Sci*. **57(1):** 57-65.

Bell, H. A., Smith, A. E., Marris, G. C., Edwards,J. P., Gatehouse, A. M. (2004). Oral toxicity and impact on fecundity of three insecticidal proteins on the gregarious ectoparasitoid Eulophus pennicornis (Hymenoptera: Eulophidae). *Agri. Forest Entomol* . **6**: 215-222.

Betz, F. S., Hammond, B. G. and Fuchs, R. L. (2000). Safety and advantages of *Bacillus thuringiensis*- protected plants to control insect pests. Reg. *Toxicol. Pharmacol.* **32**: 156-173.

Bharathi, Y., Vijayakumar, S., Pasalu, I. C., Balachandran, S. M., Reddy, V. D., Rao K. V. (2011). Pyramided rice lines harbouring*Allium sativum* (*asal*) and *Galanthus nivalis* (*gna*) lectin genes impart enhanced resistance against major sap-sucking pests. *J. Biotechnol.* **152**: 63-71.

Bhattacharya, A. R., Bhat, S. R., Kirti, P. B. and Chopra, V. L. (2002). Development of insect resistant cabbage plants expressing a synthetic *cry* 1 A (b) gene from *Bacillus thuringiensis*. *Curr. Sci.* **83**: 146-150.

Bohorova, N., Frutos, R., Royer, M., Estanol P., Pacheco, M., Rascon, Q., McLean S. and Hoisington, D. (2001). Novel synthetic *Bacillus thuringiensis* cry1B gene and the cry 1B-cry1A translational fusion confer resistance to south western corn borer, sugarcane borer and fall army worm in tropical maize. *Theor. Appl. Genet.* **103**: 817-826.

Broderick, N. A., Goodman, R. M., Raffa, K. F. and Handelsman, J. (2000). Synergy between zwittermicin A and *Bacillus thuringiensis* subsp. *Kurstaki* against gypsy moth (Lepidoptera: Lymantriidae) *Environ. Entomol.* **29**: 101-107.

Burgess, E. P., Malone, L. A., Christeller, J. T., Lester, M. T., Murray, C., Philip, B. A., Phung, M. M. and Tregidga, E. L. (2002). Avidin expressed in transgenic tobacco leaves confers resistance to two noctuid pests, *Helicoverpa armigera* and *Spodoptera litura. Trans. Res.* **11**: 185-198

Campbell, B. T., Baenziger, P. S., Mitra, A., Sato,S. and Clemente, T. (2000). Inheritance of multiple transgenes in wheat. *Crop Sci.* **40**: 1133-1141.

Cao, J., Ibrahin, H., Gracia, J. J., Mason, H., Granados, R. R. and Earle, E. D. (2002b). Transgenic tobacco plants carrying a baculovirus enhancing gene slow the development and increase the mortality of *Trichoplusia ni* larvae. *Plant Cell Rep.* **21**: 244-250.

Cao, J., Zhao, J. Z., Tang, J. D., Shelton, A. M. and Earle, E. D. (2002a). Broccoli plants with pyramided cry1Ac and cry1C bt genes control diamondback moths resistant to cry1A and cry1C proteins. *Theor. Appl. Genet.* **105**: 258-264.

Carozzi, N. B., Warren, G. W., Desai N., Jayne,S. M., Lotstein R., Rice D. A., Evola, S. and Koziel, M. G. (1992). Expression of a chimeric CaMV 35S *Bacillus thuringiensis*insecticidal protein gene in transgenic tobacco. *Plant Mol. Biol.* **20**: 539-548.

Carriere, Y., Ellers-Kirk, C., Sisterson, M., Antilla, L., Whitlow, M., Dennehy,T. J. and Tabashnik B. E. (2003). Long term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. *PNAS.* **100**: 1519-1523.

Carter, C. and Thornburg, R. W. (2004). Is the nectar redox cycle a floral defense against microbial attack? *Trend. Plant Sci.* **9**: 320-324.

Chakrabarty, R., Viswakarma, N., Bhat, S. R., Kirti, P. B. and Chopra, V. L. (2002). *Agrobacterium* mediated genetic transformation of cauliflower: optimization of protocol and development of *Bt* transgenic cauliflower. *J Biosci.* **27**: 495-502.

Chakraborti, D., Sarkar, A., Mondal, H. A., Das,S. (2009). Tissue specific expression of *Allium sativum* leaf agglutinin (ASAL) in important pulse crop chickpea (*Cicer arietinum* L.) to resist the phloem feeding *Aphis craccivora*. *Transgen. Res.* **18**: 529-544.

Chang, T., Chen, L, Chen, S, Cai, H, Liu, X, Xiao,G. and Zhu, Z. (2003).

Transformation of tobacco with genes encoding *Helianthus tuberosus* agglutinin (HTA) confers resistance to peach-potato aphid (*Myzus persicae*). *Trans. Res.* **12**: 607-614.

Charity, J. A., Anderson, M. A., Bittisnich,D. J., Whitecross, M. and Higgins,T. J. (1999). Transgenic tobacco and peasexpressing a proteinase inhibitor from *Nicotiana alata* have increased insect resistance. *Mol. Breed.* **5**: 357-365.

Chen, H., Tang, W., Xu, C., Li, X., Lin, Y. and Zhang, Q. (2005). Transgenic indica riceplants harboring a synthetic Cry2A* gene of *Bacillus thuringiensis* exhibit enhanced resistance against lepidopteran pests. *Theor. Appl. Genet.* **111**: 1330-1337.

Chen, J. W., Tang, L. X., Tang, M. J., Shi, Y. X. and Pang, Y. (2002). Cloning and expressionproduct of vip3 gene from *Bacillus* thuringiensis and analysis of insecticidal activity. Sheng Wu Gong Cheng ZXue. *Bao.* **18:** 687-692.

Chen, L. P., Marmey, P., Taylor, N. J., Brizard,J., Espinosa, C., Cruz, P., Huet, H., Zhang,S., de Kochko, A., Beachy, R. N. and Faquet, C. M. (1998). Expression and inheritance of multiple transgenes in rice plants. *Nat. Biotechnol.* **16**: 1060-1064.

Chrispeels, M. J. and Raikhel, N. V. (1991). Lectins, lectin genes and their role in plant defense. *The Plant Cell.* **3:** 1-9.

Cohen, E. (1993). Chitin synthesis and degradation as targets for pesticide action. Arch. Insect Biochem. *Physiol.* **22**: 245-261.

Constabel, C. P., Bergey, D. R. and Ryan, C. A. (1995). Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. *PNAS.* **92**: 407-411

Constabel, C. P., Yip, L., Patton, J. J. and Christopher, M. E. (2000). Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. *Plant Physiol.* **124**: 285-295

Corbin, D. R., Grebenok, R. J., Ohnmeiss, T. E., Greenplate, J. T. and Purcell, J. P. (2001). Expression and chloroplast targeting of cholesterol oxidase in transgenic tobacco plants. *Plant Physiol.* **126**: 1116-1128.

Cowgill, S. E. and Atkinson, H. J. (2003). A sequential approach to risk assessment of the transgenic plants expressing protease inhibitors: effects on non target herbivorous insects. *Trans. Res.* **12**: 439-449.

Crickmore, N., Zeigler, D. R., Feitelson, J., Schnepf, E., Van Rie J., Lereclus, D, Baum, J. and Dean, D. H. (1998). Revision of the nomenclature for the *bacillus thuringiensis* pesticidal Crystal Proteins. Microbiol. *Mol. Biol. Rev.* **62(3):** 807-813.

Crickmore, N., Baum, J., Bravo, A., Lereclus,D., Sampson, K., Schnepf, E., Sun, M. and Zeigler,D. R. (2016). *Bacillus thuringiensis*toxin nomenclature Available online at http://btnomenclature. info.

Datta, K., Baisakh, N., Thet, K. M., Tu, J. and Datta, S. K. (2002). Pyramiding transgenes for multiple resistancein rice against bacterial blight, yellow stem borer and sheath blight. *Theor. Appl. Genet.* **106:** 1-8.

Leo, F., Volpicella, M., Licciulli, F., Liuni, S., Gallerani, R. and Ceci, L. R. (2002). Plant PIs: a data base for plant protease inhibitors and their genes. *Nuc. Acid Res.* **30**: 347-348.

Maagd, R. A., Bosch, D. and Stiekema,W. (1999). *Bacillus thuringiensis* toxin-mediated insect resistance in plants. *Trends Plants Sci.* **4**: 9-13.

Oliveira, R. S., Oliveira-Neto, O. B., Moura, H. F., Macedo, L., Arraes, F. B., Lucena,W. A., Lourenco-Ressutti, I. T., Deus Barbosa, A. A., Silvia, M. C. and Grossi,

M. F. (2016). Transgenic cotton plants eypressing Cry1la12 toxin conger resistance to fall armyworm (*Spodoptera frugiperda*) and cotton boll weevil (*Anthonomus grandis*). *Front. Plant Sci.* **7:** 165

Sousa-Majer, M. J., Turner, N. C., Hardie,D. C., Morton, R. L., Lamont, B. and Higgins, T. J. (2004). Response to water deficit and high temperature of transgenic peas (*Pisum sativum* L.) containing a seed specific α-amylase inhibitor and the subsequent effects on pea weevil (*Bruchus pisorum* L.) survival. *J Exp. Bot.* **55(396)**: 497-505.

Delannay, X., Vallee, B. J., Proksch, R. K., Fuchs,R. L., Sims, S. R., Greenplate,J. T., Marrone, P. G., Dodson, R. B., Augustine, J. J., Layton, J. G. and Fischhoff, D. A. (1989). Field Performance of Transgenic Tomato Plants Expressing the *Bacillus thuringiensis var. kurstaki* Insect Control Protein. Bio/Technol. **7:** 1265-1269

Delledonne, M., Allegro, G., Belenghi, B., Balestrassi, A., Picco, F., Levine, A., Zelasco, S., Calligari, P. and Confalonieri, M. (2001). Transformation of white poplar (*Populus alba* L.) with a novel *Arabidopsis thaliana* cysteine proteinase inhibitor and analysis of insect pest resistance. *Mol. Breed*. **7**: 35-42.

Ding, L. C., Hu, C., Yeh, K. W. and Wang, P. J. (1998). Developments of insect-resistant. transgenic cauliflower plants expressing the trypsin inhibitor gene isolated from local sweet potato. *Plant Cell Rep.* **17**: 854-860.

Ding, X., Gopalakrishnan, B., Johnson, L. B., White, F. F., Wang, X., Morgan, T. D., Kramer, K. J. and Muthukrishnan, S. (1998). Insect resistance of transgenic tobacco expressing an insect chitinase gene. *Trans. Res.* **7:** 77-84.

Doss, V. A., Kumar, K. A., Jayakumar, R. and Sekar, V. (2002). Cloning and expression of the vegetative insecticidal protein (vip3V) gene of *Bacillus thuringiensis* in *Escherichia coli*. *Prot. Expres.* **26**: 82-88.

Douches, D., Pett, W. and Grafius, E. (2000). Potato transformation for development of potato tuber moth resistance. *Ann. Tech. Rep*. **27:** 32-37.

Down, R. E., Ford, L., Bedford, S. J., Gatehouse,L. N., Newell, C., Gatehouse, J. A. and Gatehouse, A. M. (2001). Influence of plantdevelopment and environment on transgene expression in potato and consequences for insect resistance. Trans. Res. **10:** 223-236.

Du, D., Geng, C., Zhang, X., Zhang, Z., Zheng,Y., Zhang, F., Lin, Y. and Qiu, F. (2014). Transgenic maize lines expressing a *cry1C*gene are resistant to insect pests. *Plant Mol. Biol. Rep.* **32:** 549.

Dufourmantel, N., Tissot, G., Goutorbe, F., Garcon, F., Muhr, C., Jansens, S., Pelissier, B., Peltier, G. and Dubald,M. (2005). Generation and analysis ofsoybean plastid transformants expressing *Bacillus thuringiensis*Cry1Ab protoxin. *Plant Mol. Biol.* **58**: 659-668.

Dunse, K. M., Stevens,J. A., Lay, F. T., Gaspar,Y. M., Heath, R. L., and Anderson,M. A. (2010). Coexpression of potato type I and II protease inhibitors give cotton plants protection against insect damage in the field. *PNAS.* **107(34):** 15011-15015.

Dutta, I., Saha, P., Majumder, P., Sarkar, A., Chakraborti, D., Banerjee, S. and Das,S. (2005). The efficacy of a novel insecticidal protein, *Allium sativum* leaf lectin (ASAL), against homopteran insects monitored in transgenic tobacco. *Plant Biotech. J.* **3:** 601-611.

Dutton, A., Romeis, J., Bigler, F. (2003). Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *Bio Control.* **48(6):** 611-636.

Estruch, J. J., Carozzi, N. B., Desai, N., Duck,N. B., Warren, G. W. and Koziel, M. G. (1997). Transgenic plants. An emerging approach to pest control. *Nat. Biotech.* **15**: 137-141.

Estruch, J. J., Warren, G. W., Marth, A. M., Nye,G. J., Craig, J. A., and Koziel, M. G. (1996). Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *PNAS.* **93**: 5389-5394.

Fabre, C., Ausse, H., Mourey, L., Koninkx, J., Riviea, M., Hendriks, H., Puzo, G., Samama, J. P. and Rouge, P. (1998). Characterisation and sugar binding properties of arcelin 1, an insecticidal lectin-like protein isolated from kidney bean (*Phaseolus vulgaris* L. cv. Raz-2) seeds. *Biochem J.* **329**: 551-560.

Falco, M. C. and Silvia-Filho, M. C. (2003). Expression of soybean proteinase inhibitors in transgenic sugarcane plants: effects on natural defense against *Diatrea saccharalis*. Plant *Physiol. Biochem.* **41:** 761-766.

Fan, X., Shi X., Zhao, J., Zhao, R. and Fan, Y. (1999). Insecticidal activity of transgenic tobacco plants expressing both Bt and CpTI genes on cotton bollworm (*Helicoverpa armigera*). *Chin. J Biotechnol.* **15(1):** 1-5.

Felton, G. W., Donato, K. K., Broadway, R. M. and Duffey, S. S. (1992). Impact of oxidized plant phenolics on the nutritional quality of dietar protein to a noctuid herbivore, *Spodoptera exigua. J. Insect Physiol.* **38**: 277-285.

Fitches, E., Woodhouse, S. D., Edwards, J. P., Gatehouse J. A. (2001). In vitro and in vivo binding of snowdrop (*Galanthus nivalis* agglutinin; GNA) and jackbean (*Canavalia ensiformis;* Con A) lectins within tomato moth (*Lacanobia oleracea*) larvae; mechanisms of insecticidal action. *J. Insect Physiol.* **47(7):** 777-787

Fujimoto, H., Itoh, K., Yamamot, M., Kyozuka,J. and Shimamoto, K. (1993). Insect resistant rice generated by introduction of a modified delta endotoxin gene of *Bacillus thuringiensis*. *Bio. Technol.* **11**: 1151-1155.

Gao, S., Yingying, Y., Chunfeng, W., Jinlong, G., Dinggang, Z., Qibin, W., Yachun, S., Liping, X. and Youxiong, Q. (2016). Transgenic sugarcane with a *cry1Ac*gene exhibited better phenotypic traits and enhanced resistance against sugarcane borer. *PLoS ONE.* **11(4):** 15 - 29.

Gatehouse, A. M., Davison, G. M., Newell, C. A., Merryweather, A., Hamilton, W. D., Burgess, E. P., Gilbert, R. J. and Gatehouse, J. A. (1997). Transgenic potato plants with enhanced resistance to the tomato moth *Lacanobia oleracea*: growth room trials. *Mol. Breed.* **3**: 49-63.

Gatehouse, A. M., Davison, G. M., Stewart,J. N., Gatehouse, L. N., Kumar, A., Geoghegan, I. E., Birch, A. N. and Gatehouse, J. A. (1999). Concanavalin Ainhibits development of tomato moth (*Lacanobia oleracea*) and peach-potato aphid (*Myzuz persicae*) when expressed in transgenic potato plants. *Mol. Breed.* **5**: 153-165.

Gatehouse, A. M., Ferry, N. and Remaeker, R. J. (2002). The case of the monarch butterfly: a verdict is returned. *Trends Gen.* **18**: 249-251

Gatehouse, J. A., Powell, K. and Edmonds, H. (1996). Genetic engineering of rice for resistance to homopteran insect pests. Rice genetics III. Proceedings of the Third International Rice Genetics Symposium, 16-20 Oct, Manila (Philippines): *IRRI.* **12:** 189-200

Girard, C., Picard-Nizou, A. L., Grallien, E., Zaccomer, B., Jouanin, L. and Pham-Delegue, M. H. (1998). Effects of proteinase inhibitor ingestion on survival, learning abilities and digestive proteinases of the honeybee. *Trans. Res.* **7**: 239-246

Giri, A. P., Harsulkara, A. M., Kua, M. S., Gupta,V. S., Deshpande, V. V., Ranjekar, P. K. and Franceschia,V. R . (2003). Identi. cation of potent inhibitors of *Helicoverpa armigera* gut proteinases from winged bean seeds. *Phytochem.* **63**: 523–532

Girijashankar, V., Sharma, H. C., Sharma,K. K., Swathisree, V., Prasad, L. S., Bhat, B. V., Royer, M., Secundo,B. S., Narasu,M. L., Altosaar, I. and Seetharama, N. (2005). Development of transgenic sorghum for insect resistance against spotted stem borer (*Chilo partellus*). *Plant Cell Rep.* **24**: 513-522.

Gracia, V. A., Freire, M. G., Novello, J. C., Marangoni, S., Macedo, M. L. (2004). Trypsin inhibitor from *Poecelanthe parviflora* seeds: purification, characterization and activity against pest protease. *Protein J.* **23(5):** 343-350.

Greenplate, J. T., Corbin, D. R. and Purcell,J. P. (1997). *Cholesterol oxidase: potent boll weevil larvicidal and oostatic agent suitable for transgenic cotton development.* In: Proceedings of the Beltwide cotton conference, National Cotton Council of America, New Orleans, pp. 877-880.

Greenplate, J. T., Duck N. B., Pershing, J. C. and Purcell, J. P. (1995). Cholesterol oxidase: an oostatic and larvicidal agent active against the cotton boll weevil, *grandis*. Entomol. *Exp. Appl.* **74**: 253-258.

Grossi, M. F., Mirkov, T. E., Ishimoto, M., Coluccil, G., Bateman, K. S. and Chrispeels, M. J. (1997). Molecular characterization of a bean α -amylase inhibitor that inhibits the α-amylase of the Mexican bean weevil *Zabrotes subfasciatus. Planta.* **203**: 295-303.

Gujar, G. T., Kumari, A., Kalia, V. and Chandrashekar, K. (2000). Spatial and temporal variation in susceptibility of the American bollworm, *Helicoverpa armigera* (Hubner) to *Bacillus thuringiensis* var. *kurstaki* in India. *Curr. Sci.* **78**: 995-1001

Gujar, T., Kalia, V., Kumari, A., Prasad, T. V. (2004). Potentiation of insecticidal activity of *Bacillus thuringiensis* subsp. kurstaki HD-1 by proteinase inhibitors in the American bollworm, *Helicoverpa armigera* (Hubner). Indian *J. Exp. Biol.* **42(2):** 157-163.

Haidi, M. Z., McCullen, M. D. and Finer, J. J. (1996). Transformation of 12 different plasmids in to soybean via particle bombardment. *Plant Cell Rep.* **15:** 500-505.

Hanley, A. V., Huang, Z. Y. and Pett, W. (2003). Effects of dietary transgenic *Bt*corn pollen on larvae of *Apis mellifera*and *Galleria mellonella. J. Apicult. Res.* **42(4):** 77-81.

Hayakawa, F., Shijimoto, E., Mori, M., Kaido M., Furusawa, I., Miyata, S., Sano, Y., Matsumoto, T., Hashimoto, Y. and Granados, R. R. (2000). Enhancement of baculovirus infection in *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae with *Autographa californica* nucleopoly-hedrovirus enhancing gene. *Appl. Entomol. Zool.* **35**: 163-170

Hellmich, R. L., Siegfried, B. D., Sears, M. K., Stanely-Horn, D. E., Daniels, M. J., Spencer, T., Bidne, K. G. and Lewis L. C. (2001). Monarch larvae sensitive to*Bacillus thuringiensis* purified proteins and pollen. PNAS. Early edition 1-6.

Hilder, V. A. (2003). GM plants and protection against insects – alternative strategies based on gene technology. *Acta Agric. Scand. Sect. Soil Plant Sci. Suppl.* **1:** 34-40.

Hofmann, C., Vanderbruggen, H., Hofte, H., Van, Rie J., Jansens, S. and Van Mellaert, H. (1988). Specificity of *Bacillus thuringiensis* δ-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midgets. *PNAS*. **85:** 7844-7848.

Hofte, H. and Whiteley, H. R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev*. **53(2):** 242-255.

Huang, J., Hu, R., Rozelle, S. and Pray, C. (2005). Insect-resistant GM rice in farmer's fields: Assessing productivity and health effects in China. *Sci.* **308**: 688-690.

Huo, B. K., Zhou, Y. H., Wan, L. H., Zhang, Z. L., Shen, G. F., Chen, Z. H. and Hu, Z. M. (2003). Protoplast transformation in oil seed rape. *Trans. Res*. **12**: 111-114.

Hymowitz, T. (1990). Grain legumes. In: J. Janick and J. E. Simon (eds.), Advances in new crops. Timber Press, Portland, OR., pp-. 54-57.

Iannacone, R., Grieco, P. D. and Cellini, F. (1997). Specific sequence modifications of a cry 3B endotoxin gene result in high levels of expression and insect resistance. *Plant Mol. Biol*. **34(3):** 485-496.

Irie, K., Hosoyama, H., Takeuchi, T., Iwabuchi,K., Watanabe, H., Abe,M., Abe, K. and Arai, S. (1996). Transgenic rice established to express corn cystatin exhibits strong inhibitory activity against insect gut proteinases. *Plant Mol. Biol.* **30**: 149-157.

Ishimoto, M., Sato, T., Chrispeels, M. J. and Kitamura, K. (1996). Bruchid resistance of transgenic azuki bean expressing seed α-amylase inhibitor of common bean. *Entomol. Exp. Appl.* **79**: 309-315.

Ishimoto, M., Yamada, T. and Kaga, A. (1999). Insecticidal activity of an alpha-amylase inhibitor – like protein resembling putative precursor of alpha amylase inhibitor in the common bean *Phaseolus vulgaris* L. *Biochim. Biophys Acta*. **15(1):** 104-112.

James, C. (2005). *Global status of commercialized Biotech/GM crops*-2005. Brief 34, ISAAA, Ithaca, New York.

Jin, R. G., Liu, Y. B., Tabashnik, B. E. and Borthakur, D. (2000). Development of transgenic cabbage (*Brassica oleracea*var. *capitata*) for insect resistance by *Agrobacterium tumefaciens*-mediated transformation. In Vitro Cell. *Dev. Biol. Plant*. **36(4):** 231-237.

Kalunke, R. M., Kolg,e A. M., Babu, K. H. Prasad,D. T. (2009). *Agrobacterium* mediated transformation o sugarcane for borer resistance using *Cry1Aa3* gene and one-step regeneration of transgenic plants. *Sugar Tech.* **11:** 355-358.

Kanrar, S., Venkateswari, J., Kirti, P. B. and Chopra V. L . (2002). Transgenic Indian mustard (*Brassica juncea*) for resistance to aphid (*Lipaphis erysimi*). *Plant Cell Rep.* **20:** 976-981.

Kar, S., Basu, D., Das, S., Ramkrishnan, N. A., Mukherjee, P., Nayak, P. and Sen,S. K. (1997). Expression of cry 1 A(c) gene of *Bacillus thuringiensis* in transgenic chick pea plants inhibits development of podborer (*Heliothis armigera*) larvae. Trans. Res. **6:** 177-185.

Kim, S. M. and Sohn, J. K. (2005). Identification of a rice gene (Bph 1) conferring resistance to brown plant hopper (*Nilaparvata lugens* Stal.) using STS markers. *Mol. Cells.* **20**: 30-34.

Koiwa, H., Bressan, R. A. and Hasegawa, P. M. (997). Regulation of protease inhibitors and plant defense. *Trends Plant Sci.* **2:** 379-384.

Kondo, H., Abe K., Nishimura, I., Watanabe, H., Emori, Y. and Arai, S. (1990). Two distinct cystatin species in rice seeds with different specificities against cysteine proteinases: molecular cloning, expression and biochemical studies on oryzacystatin II. *J. Biol. Chem*. **265:** 15832-15837.

Kondo, H., Emori, Y., Abe, K., Suzuki, K. and Arai, S. (1989). Cloning and sequence analysis of the genomic DNA fragment encoding oryzacystatin. *Gene.* **81**: 259-265.

Kostyukovsky, M. and Trostanestsky, A. (2006). The effect of a new chitin synthesis inhibitor, novaluron, on various developmental stages of *Tribolium castaneum* (Herbst). *J. Stored Prod. Res.* **42:** 136-148.

Koul, B., Yadav, R., Sanyal, I. and Amla, D. V. (2015). Comparative performance of modified full-length and truncated *Bacillus thuringinensis cry1Ac* genes in transgenic tomato. *Spring. Plus.* **4:** 203- 208.

Koul, B., Srivastava, S., Sanyal, I., Tripathi,B., Sharma, V. and Amla, D. V. (2014). Transgenic tomato line expressing modified *Bacillus thuringiensis cry1Ab*gene showing complete resistance to two lepidopteran pests. *Spring. Plus***3:** 84 – 87.

Koziel, M., Beland ,G. L., Bowman, C., Carozzi,N. B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., Pherson,K. M., Meghji, M., Merlin, E.,Rhodes, R., Warren, G. W., Wright, M. and Evola, S. (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio. Technol*. **11:** 194-200.

Kramer, K. J., Morgan, T. D., Thorne, J. E., Dowell, F. E., Bailey, M. and Howard, J. A. (2000). Transgenic avidin maize is resistantto storage insect pests. *Nat. Biotech.* **18**: 670-674.

Kreft, S., Ravnikar, M., Punger, J., Umek, A., Kregar, I. and Borut,T. (1997). Jasmonic acid inducible aspartic proteinase inhibitors from potato. *Phytochem.* **44(6):**1001-1006

Kuvshinov, V., Koivu, K., Kanerva, A. and Pehu, E. (2001). Transgenic crop plants expressing synthetic *cry* 9Aa gene are protected against insect damage. *Plant Sci.* **160**: 341-353.

Lagnaoui, A., Canedo, V. and Douches, D. (2000). Evaluation of Bt CryV transgenic potatoes on two species of potato tuber moth, *Phthorimaea operculella* (Zeller) and *Symmetrischema tangolias* (Gyen) in Peru. *ASPB Ann. Tech. Rep.* A40-A47.

Lawrence, P. K. and Koundal, K. R. (2002). Plant protease inhibitors in control of phytophagous insects. *J. Biotech.* **5(1):** 12-16.

Lawrence, S. D. and Novak, N. G. (2001). A rapid method for the production and characterization of recombinant insecticidal proteins in plants. *Mol. Breed.* **8:** 139-146.

Lee, S. I., Lee, S. H., Koo, J. C., Chun, H. J., Lim,C. O., Mun, J. H., Song, Y. H. and Cho,M. J. (1999). Soybean kunitz trypsin inhibitor (SKTI) confers resistance to the brown plant hopper (*Nilaparvata lugens* Stal) in transgenic rice. *Mol. Breed.* 5: 1-9.

Lepore, L. S., Roelvink, P. R. and Granados R. R. (1996). Enhancin, the granulosis virus protein that facilitate nucleopolyhedrovirus (NPV) infections, is a metalloprotease. *J. Invertebr. Pathol.* **68:** 131-140.

Liang, X. Y., Zhu, Y. X., Mi, J. J. and Chen, Z. L. (1994). Production of virus resistant and insect tolerant transgenic tobacco plants. *Plant Cell Rep.* **14**: 141-144

Liu, Y. B., Tabashnik, B. E., Meyer, S. K. and Crickmore, N. (2001). Cross-resistance and stability of resistance to *Bacillus thuringiensis* toxin *Cry1C* in diamond back moth. Appl. Envt. *Microbiol.* **67(7):** 3216-3219

Loc, N. T., Tinjuangjun, P., Gatehouse, A. M., Christou, P. and Gatehouse, J. A. (2002). Linear transgene constructs lacking vector backbone sequences generate transgenic rice plants which accumulate higher levels of proteins conferring insect resistance. *Mol. Breed.* **9:** 231-244.

Luo, M., Zhaoyu, W., Huapeng, L., Kuai, F., Yinpeng, C. and Zheng-Fu, X. (2009). Overexpression of a weed (*Solanum americanum*) proteinase inhibitor in transgenic tobacco results in increased glandular trichome density and enhanced resistance to *Helicoverpa armigera* and *Spodoptera litura. Int. J Mol. Sci.* **10:** 1896-1910.

Macedo, M. L., Castro, M. M. and Freire, M. G. (2004). Mechanisms of the insecticidal action of TEL (*Taliis esculenta* lectin) against *Callosobruchus maculates*(Coleoptera: Bruchidae). *Arch. Insect Biochem. Physiol.* **56(2):** 84-96.

Macedo, M. L., Damico, D. C., Grac, M., Freire M., Toyama, M. H., Marangoni, S. and Novello, J. (2003). Purification and characterization of an N-acetylglucosamine binding lectin from *Koelreuteria paniculata* seeds and its effect on the larval development of *Callosobruchus maculates* (Coleoptera: Bruchidae) and *Anagasta kuehniella*(Lepidoptera: Pyralidae*). J. Agric. Food Chem.* **51:** 2980-2986

Madkour, M., El-Din ,T. N. and Anis, E. (2000). Managing natural and engineered resistance in potato to potato tuber moth. *ASPB Ann. Techn. Rep.* A33-A39

Manikandan, R., Balakrishnan, N., Sudhakar,D. and Udayasuriyan, V. (2016). Transgenic rice plants expressing synthetic*cry2AX1* gene exhibits resistance to rice leaffolder (*Cnaphalocrosis medinalis*). *Biotech.* **6:** 10- 12.

Maqbool, S. B., Husnain, T., Riazuddin, S., Masson, L. and Christou, P. (1998). Effective controlof yellow stem borer and rice leaf folder in transgenic rice indica varieties Basmati 370 and M7 using novel δ-endotoxin *cry2A Bacillus thuringiensis* gene. *Mol. Breed.* **4:** 501-507.

Maqbool, S. B., Riazuddin, S., Loc, N. T., Gatehouse, A. M., Gatehouse, J. A. and Christou, P. (2001). Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. *Mol. Breed.* **7:** 85-93

Marchetti, S., Delledonne, M., Fogher, C., Chiaba, C., Chiesa, F., Savazzini, F. and Giordano, A. (2000). Soybean Kunitz, C-II and PI-IV inhibitor genes confer different levels of insect resistance to tobacco and potato transgenic plants. Theor. *Appl. Genet.* **101**: 519-526.

Markwick, N. P., Christeller, J. T., Docherty,L. C. and Lilley, C. M. (2001). Insecticidal activity of avidin and streptavidin against four species of pest Lepidoptera. Entomol. *Exp. Appl.* **98**: 59-66.

Markwick, N. P., Docherty, L. C., Phung,M. M., Lester, M. T., Murray, C., Yao J. L., Mitra,D. S., Cohen, D., Beuning, L. L., Kutty-Amma, S. and Christeller, J. T. (2003). Transgenic tobacco and apple plants expressing biotin-binding proteins are resistant to two cosmopolitan insect pests, potato tuber moth and light brown apple moth, respectively. *Trans. Res.* **12:** 671-681.

Marsaro, J. A., Lazzari, S. M., Fugueira, E. Z. and Hirooka E. Y. (2005). Amylase inhibitors in corn hybrids as a resistance factor to *Sitophilus zeamais*(Coleoptera: Curculionidae). *Neotrop. Entomol.* **34(3):** 443-450

Mehlo, L., Gahakwa ,D., Trung Nghia, P., Loc,N. T., Capell, T., Gatehouse,J. A., Gatehouse, A. M. and Christou,P. (2005). An alternative strategy forsustainable pest resistance in genetically enhanced crops. *PNAS.* **102**: 7812-7816.

Melander, M., Ahman, I., Kamnert, I. and Stromdahl, A. C. (2003). pea lectin expressed transgenically in oil seed rape reduces growth rate of pollen beetle larvae. *Trans. Res.* **12**: 555-567.

Moar, W. J. (2003). Breathing new life into insect-resistant plants. *Nat. Biotech.* **21:** 1152-1154.

Mochizuki, A., Nishizawa, Y., Onodera, H., Tabei, Y., Toki, S., Habu, Y., Ugaki, M. and Ohashi, Y. (1999). Transgenic rice plants expressing a trypsin inhibitor are resistant against rice stem borers, *Chilo suppressalis*. Entomol. *Exp. Appl.* **93**: 173-178.

Morgan, T. D., Oppert, B., Czapla, T. H. and Kramer, K. J. (1993). Avidin and streptavidin as insecticidal and growth inhibiting dietary proteins. *Entomol. Exp. Appl.* **69:** 97-108.

Morton, R. L., Schroeder, H. E., Bateman, K. S., Chrispeels, M. J., Armstrong, E. and Higgins, T. J. (2000). Bean α-amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *PNAS*. **97:** 3820-3825.

Mota, A. C., DaMatta, R. A., Filho, M. L., Silva C. P., Xavier-Filho, J. (2003). Cowpea (*Vigna unguiculata*) vicilins bind to the peritrophic membrane of larval sugarcane borer (*Diatraea saccharalis*). *J. Insect Physiol.* **49:** 873-880.

Muzaffar, A., Kiani, S., Khan, M. A., Rao, A. Q., Ali, A., Awan, M. F., Iqbal, A., Nasir,I. A., Shahid, A. A. and Husnain, T. (2015). Chloroplast localization of Cry1Ac and Cry2A protein – an alternative way of insect control in cotton. *Biol. Res.* **48:** 14.

Nagadhara, D., Ramesh, S., Pasalu, I. C., Rao,Y. K., Krishnaiah, N. V., Sarma, N. P., Bown, D. P., Gatehouse, J. A., Reddy,V. D. and Rao, K. V. (2003). Transgenic indica rice resistant to sap-sucking insects. *Plant Biotechnol. J.* **1(3):** 231– 240.

Nagadhara, D., Ramesh, S., Pasalu, I. C., Rao Y. K., Sarma, N. P. and Reddy, V. D. (2004). Transgenic rice plant expressing the snowdrop lectin gene (GNA) exhibit high level resistance to the white backed plant hopper (*Sogatella furcifera*). *Theor. Appl. Genet.* **109(7):** 1399-1405.

Oliveira, A. S., Filho, J. X. and Sales, M. P. (2003). Cystene proteinases and cystatins. *Braz. Arch. Biol. Technol.* **46(1):** 91-104.

Ortiz, R. (1998). Critical role of plant biotechnology for genetic improvement of food crops: perspectives for the next millennium. *Electron. J. Biotechnol*. 12: 13-16.

Perlak, F. J., Deaton, R. W., Armstrong, T. A., Fuchs, R. L., Greenplate, J. T., Fischhoff,D. A. (1990). Insect resistant cotton plants. Biotechnol. **8(10):** 939-943.

Perlak, F. J., Fuchs, R. L., Dean, D. A., McPherson, S. L and Fischhoff, D. A. (1991). Modification of the coding sequence enhances plant expression of insect control protein genes. *PNAS*. **88:** 3324-3328.

Perlak,F. J., Stone, T. B., Muskopf, Y. M., Petersen, L. J., Parker, G. B., Mc Pherson,S. A., Wyman, J., Love, S., Reed, G., Biever, D., and Fischhoff, D. A. (1993**).** Genetically improved potatoes: protection from damage by Colorado potato beetles. *Plant Mol. Biol.* **22:** 313-321.

Phillips, M. A., and Croteau R. B., (1999). Resin-based defenses in conifers. *Trends Plant Sci.* **4:** 184-190.

Pitkin, J. W., Krasomil, O. K., Baum,J. A., Donovan, W. P., Gao, A. G., Harrison,L. A., Casagrande, L. A., Biest,N. A., Cliton, W. P., Ilagan, O., Walters,M. R., Curtis, J. M., Simpson, R. D. and Roberts, J. K. (2005). Novel insecticidal proteins secreted by *Bacillus thuringiensis*. In: 38th Annual Meeting of the Society for Invertebrate

Pathology, August 7-11, Anchorage, Alaska, USA.

Pompermayer, P., Lopes, A. R., Terra, W. R., Parra, J. R., Falco, M. C. and Filho, M. C. (2001). Effects of soybean proteinase I nhibitor on development, survival and reproductive potential of the sugar cane borer, *Diatrea saccharalis*. *Entomol. Exp. Appl.* **99:** 79-85.

Powell, K. S. (2001). Antimetabolic effectsof plant lectins towards nymphal stages of the plant hoppers *Tarophagus proserpina* and *Nilaparvata lugens*. *Entomol. Exp. Appl.* **99:** 71-77.

Purcell, J. P., Greenplate, J. T., Jennings, M. G., Ryerse, J. S., Pershing, J. C., Sims, S. R., Prinsen, M. J., Corbin, D. R., Tran, M. andSammons, R. D. (1993). Cholesterol oxidase: a potent insecticidal protein active against boll weevil larvae. *Biochem. Biophys. Res. Commun*. **196:** 1406-1413.

Purcell, J. P., Isaac, B. G., Tran, M., Sammons,R. D., Gillespie, J. E., Greenplate, J. T., Solsten, R. T., Prinsen, M. J., Pershing,J. C. and Stonard, R. J. (1994) Two enzyme classes active in green peach aphid bioassays. *J. Econ. Entomol.* **87:** 15-19.

Puspito, A., Rao, A. Q., Hafeez, M. N., Iqbal,M. S., Bajwa, K. S., Ali Q., Rashid, B., Abbas, M. A., Latif A., Shahid, A. A., Nasir, I. A. and Husnain, T. (2015). Transformation and evaluation of Cry1Ac + Cry2A and GTGene in *Gossypium hirsutum* L. *Front. Plant Sci.* **6**: 943-946.

Ramesh, S., Nagadhara, D., Reddy, V. D. and Rao, K. V. (2004). Production of transgenic indica rice resistant yellow stem borer and sap-sucking insects, using super-binary vectors of *Agrobacterium tumefaciens*. *Plant Sci.* **166:** 1077-1085

Rao, K. V., Rathore, K. S., Hodges, T. K., Fu X., Stoger, E., Sudhakar, D., Williams, S., Christou, P., Bharathi, M., Bown, D., Powell, K. S., Spence, J., Gatehouse,A. M. and Gatehouse J. A. (1998). Expression of snowdrop lectin(GNA) in transgenic rice plants confers resistance to rice brown plant hopper. *Plant J.* **15(4):** 469-477.

Reddy, G. V. and Guerrero, A. (2004). Interactions. of insect pheromones and plant semiochemicals. *Trends Plant Sci.* **9:** 253-261.

Rivero, R. M., Ruiz, J. M., Garcya, P. C., Lopez-Lefebre, L. R., Sanchez E. and Romero L. (2001). Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci.* **160:** 315-321

Romeis, J., Meissle, M. and Bigler, F. (2006). Transgenic crops expressing *Bacillus thuringiensis*toxins and biological control. *Nat. Biotechnol.* **24:** 63-71.

Ryan, C. A. (1990). Proteinase inhibitors in Plants: genes for improving defenses against insects and pathogens. *Ann. Rev. Phytopathol.* **28:** 425-449.

Saadati, F. and Bandani, A. R. (2011). Effects of serine protease inhibitors on growth and development and digestive serine proteinases of the Sunn pest, Eurygaster integriceps. J. Insect Sci. **11:** 72 – 76.

Santos, R. C., Monnerat, R. G., Grossi, S. M., Cordeiro, C. M., Gomes, A. C. and Gander, E. S. (2002). Cholesterol oxidaseinterference on the emergence and viability of cotton boll weevil larvae. *Pesq. Agropec. Bras.* **37(11):** 1525-1530.

Sanyal, I., Singh, A. K., Kaushik, M. and Amla,D. V. (2005). *Agrobacterium*-mediated transformation of chickpea (*Cicer arietinum* L.) with *Bacillus thuringiensis cry*1Ac gene for resistance against pod borer insect *Helicoverpa armigera*. *Plant Sci.* **168:** 1135-1146.

Sarmah, B. K., Moore, A., Tate, W., Molvig,L., Morton, R. L., Rees, D. P.,

Chiaiese, P., Chrispeels, M. J., Tabe,L. M. and Higgins, T. J. (2004). Transgenic chickpea seeds expressing high levels of a bean alpha amylase inhibitor. *Mol. Breed.* **14:** 73-82.

Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree *Azadirachta indica*. *Ann. Rev. Entomol*. **35:** 271-297.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D. R. and Dean, D. H. (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. *Mol. Biol. Rev.* **62(3):** 775-806.

Schroeder, H. E., Gollasch, S., Moore, A., Tabe,L. M., Craig, S., Hardie, D. C., Chrispeels, M. J., Spencer, D. and Higgin, T. J. (1995). Bean alpha amylase inhibitor confers resistance to the pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum*). *Plant Physiol.* **107:** 1233-1239.

Schuler, T. H., Denholm, I., Clark, S. J., Stewart,C. N. and Poppy, G. M. (2004). Effects of Bt plants on the development and survival of the parasitoid *Cotesia plutellae*(Hymenoptera: Bracoidae) in susceptible and Bt-resistant larvae of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Insect Physiol.* **50:** 435-443.

Sears, M. K., Hellmich, R. L., Stanley-Horn,D. E., Oberhauser, K. S., Pleasants,J. M., Mattilda, H. R., Siegfried, B. D. and Dively, G. P. (2001). Impact of *Bt* corn pollen on monarch butterfly populations: A risk assessment. PNAS. **98(21):** 11937-11942.

Setamou, M., Bernal, J. S., Legaspi, J. C., Mirkov, T. E. and Legapsi, B. C. (2002). Evaluation of lectin-expressing transgenic sugarcane against stalk borers (Lepidoptera: Pyralidae): effects on life history parameters. *J. Econ. Entomol.* **95(2):** 469-477.

Sharma, H. C., Sharma, K. K., Seetharama N. and Ortiz R. (2000). Prospects for using transgenic resistance to insects in crop improvement. *J. Biol.* **3:** 1-20.

Sharma, K. K. and Ortiz R. (2000). Program for the application of genetic transformation for crop improvement in the semi-arid tropics. In Vitro Cell. Dev. *Biol. Plant*. **36:** 83-92.

Shelton, A. M., Zhao, J. Z. and Roush, R. T. (2002). Economic, ecological food safety and social consequences of the deployment of Bt transgenic plants. *Ann. Rev. Entomol.* **47:** 845-881.

Shen, Z., Corbin, D. R., Greenplate, J. T., Grebenok, R. J., Galbraith, D. W. and Purcell, J. P. (1997). Studies on the modeof action of cholesterol oxidase on insect mid gut membranes. *Arch. Insect Biochem. Physiol.* **34(4):** 429-442.

Singh, P. K., Kumar, M., Chaturvedi, C. P., Yadav, D. and Tuli, R. (2004). Development of a hybrid δ-endotoxin and its expression in tobacco and cotton for control of a polyphagous pest *Spodoptera litura*. *Trans. Res*. **13:** 397-410.

Singh, S. R., and Emden, H. F. (1979). Insect pests of grain legumes. *Ann. Rev. Entomol.* **24:** 255-278.

Singsit, C., Adang, M. J., Lynch, R. E., Anderson, W. F., Wang, A., Cardineau, G. and Ozias, A. P. (1997). Expression of a *Bacillusthuringiensis cry1A*(c) gene in transgenic plants and its efficacy against lesser cornstalk borer. *Trans. Res.* **6**: 169-176.

Srinivasan, A., Giri, A. P., Harsulkar, A. M., Gatehouse, J. A. and Gupta, V. S. (2005). A kunitz trypsin inhibitor from chickpea (*Cicer arietinum* L.) that exerts anti-metabolic effect on pod borer (*Helicoverpa* *armigera*) larvae. *Plant Mol. Biol.* **57: 359-**374.

Steele, C. L., Lewinsohn, E. and Croteau, R. (1995). Induced oleoresin biosynthesis in grand fir as a defense against bark beetles. *PNAS.* **92**: 4164-4168.

Stewart, C. N., Adang, M. J., All, J. N., Boerma H. R., Cardineau, G., Tucker, D. and Parrot, W. A. (1996b). Genetic transformation, recovery and characterization of fertile soybean transgenic for a synthetic *Bacillus thuringiensis* Cry 1 Ac gene. *Plant Physiol.* **112:** 121-129.

Stewart, C. N., Adang, M. J., All, J. N., Raymer,P., Ramachandran, S. and Parrot,W. A. (1996). Insect control and dosage effects in transgenic canola containing a synthetic *Bacillus thuringiensis Cry1 Ac*gene. *Plant Physiol.* **112:** 115-120.

Stoger, E., Williams, S., Christou, P., Down,R. E. and Gatehouse, J. A. (1999). Expression of a lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenaeMol. Breed.* **5:** 65-73.

Strizhov, N., Keller, M., Mathur, J., Koncz-Kalman, Z., Bosch, D., Prudovsky, E., Schell, J., Sneh, B., Koncz, C. and Zilberstein, A. (1996). A synthetic *cryIC* gene, encoding a *Bacillus thuringiensis* δ-endotoxin, confers *Spodoptera* resistance in alfalfa and tobacco. *PNAS.* **93**: 15012-15017.

Strong, R. D. and Larsson, S. (1992). *The importance of herbivore population density in multitropic interactions in natural and agricultural ecosystems* In: Proceedings of the 8th International Symposium on Insect-Plant relationships. Menken SBJ, Visser JH and Harrewijn P (Eds) Kluwer Acad. Publish, Dordrecht. pp. 5-13.

Suavion, N., Charles,H., Febvay, G. and Rahbe,, Y. (2004). Effects of jack bean lectin (ConA) on the feeding behaviour and kinetics of intoxication of the pea aphid, *Acyrthosiphon pisum. Entomol. Exp. Appl.* **110:** 31-44.

Surekha, C., Beena, M. R., Arundhati, A., Singh,P. K., Tuli, R., Dutta G. A. and Kirti P. B. (2005). *Agrobacterium*mediated genetic transformation of pigeon pea (*Cajanus cajan* (L.) Millsp.)using embryonal segments and development of transgenic plants for resistance against *Spodoptera*. *Plant Sci.* **169:** 1074-1080.

Tabashnik, B. E., Carriere, Y., Dennehy, T. J., Morin, S., Sisterson, M. S., Housh,R. T., Shelton, A. M. and Zhao, J. Z. (2003). Insect resistnace to transgenic Bt crops: lessons from the laboratory and field. *J. Econ. Entomol.* **96(4):** 1031-1038.

Tabashnik., B. E., Dennehy, T. J., Sims, M. A., Larkin, K., Head, G. P., Moar, W. J. and Carriere, Y. (2002). Control of resistant pink bollworm by transgenic cotton with *Bacillus thuringiensis* toxin cry2Ab. *Appl. Environ. Microbiol.* **68:** 3790-3794.

Tamhane, V. A., Chougule, N. P., Giri, A. P., Dixit, A. R., Sainani, M. N. and Gupta,V. S. (2005). In vivo and in vitro effect of*Capsicum annum* proteinase inhibitors on *Helicoverpa armigera* gut proteinase. *Biochem. Biophysica. Acta.* **1722:** 156-167.

Tang, K., Hu, Q., Sun, X., Wan, B., Qi, H. and Lu,X. (2001). Development of transgenicpure lines with enhanced resistance to rice brown plant hopper. In Vitro Cell. *Dev. Biol. Plant.* **37(3):** 334-340.

Thomas, J. C., Adams, D. G., Keppenne, V. D., Wasmann, C. C., Brown, J. K., Kanot,M. R. and Bohnert, H. D. (1995). Protease inhibitors of *Manduca sexta*expressed in transgenic cotton. *Plant Cell Rep.* **14:** 758-762

Thomas, J. C., Wasmann, C. C., Echt, C., Dunn,R. L., Bohnert, H. J. and McCoy, T. J. (1994). Introduction and expression of an insect proteinase inhibitor in alfalfa *Medicago sativa* L. Plant Cell Rep. **14:** 31-36.

Turlings, T. C., Jeanbourquin, P. M., Held, M. and Degen T. (2005). Evaluating the induced-odour emission of a Bt maize and its attractiveness to parasitic wasps. *Trans. Res.* **14:** 807-816

Ussuf, K. K., Laxmi, N. H. and Mitra, R. (2001). Proteinase inhibitors: Plant derived genes of insecticidal protein for developing insect-resistant transgenic plants. *Curr. Sci.* **80(7):** 847-853.

Vaeck, M., Reynaerts, S., Hofte, H., Jansens,S., De Beuckeleer, M., Deaf, C., Zabeau,M., Van Montagu, M. and Leemans, J. (1987). Transgenic plants protected from insect attack. *Nature* **328:** 33-37.

Vajhala, C. S., Vijaya, K. S., Hariprasad. R. N., Sateesh. K. P., Sashavantha. R. Vudem, V. R. Khareedu. (2013). Development of Transgenic cotton lines expressing *Allium sativum*Agglutinin (ASAL) for enhanced resistance against major sap-sucking pests. *PLoS ONE.* **8(9):** 72-82.

Valencia. A., Bustillo. A. E., Ossa. G. E., Chrispeels. M. J. (2000). α-amylase of the coffee berry borer (*Hypothenemus hampei*) and their inhibition by two plant amylase inhibitors. Insect Biochem. Mol. Biol. **30:**207-213.

Valueva. T. A. and Mosolov. V. V. (2004). Role of proteolytic enzymes in plant defense against phytopathogenic microorganisms. *Biochem.* **69(11):**1305-1309.

Van der. Westhuizen. A. J., Qian. X. M. and Botha. A. M. (1998). Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *J. Biol.* **12:** 14 - 18.

Vaughn. T., Cavato. T., Brar. G., Coombe. T., DeGooyer. T., Ford. S., Groth. M., Howe. A., Johnson. S., Kolacz. K., Pilcher. C., Purcell. J., Romano. C., English. L. and Pershing. J. (2005). A method of controlling corn root worm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. *Crop Sci.* **45:** 931-938.

Viswakarma, N., Bhattacharya, R. C., Chakrabarty, R., Dargan, S., Bhat,S. R., Kirti, P. B., Shastri, N. V. and Chopra,V. L (2004). Insect resistance of broccoli('Pusa Broccoli KTS-1') expressing a synthetic *CryIA(b)* gene. *J. Hort. Sci. Biotech.* **79:** 182-188.

Wachtel, T. B., Bernal, J. S. and Bradleigh, V. S. (2003). Impacts of transgenic sugarcane expressing GNA lectin on parasitism of Mexican rice borer by *Parallorhogas pyralophagus* (Marsh) (Hymenoptera: Braconidae). *Envt. Entomol.* **32(4):** 866-872.

Walker, D., Boerma, H. R., All, J. and Parrott,W. (2002). Combining *cry1Ac*with QTLalleles from PI 229358 to improve soybean resistance to lepidopteran pests. *Mol. Breed.* **9:** 43-51.

Wang, E., Hall, J. T. and Wagner, G. J. (2004). Transgenic *Nicotiana tabacum* L. with enhanced trichome exudates cembratrieneols has reduced aphid infestation in the field. *Mol. Breed.* **13:** 49-57.

Wang, J. and Constabel, C. P. (2004). Polyphenol oxidase overexpression in transgenic*Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). *Planta.* **220:** 87-96.

Wang, J., Chan, Z., Du, J., Sun, Y. and Liang, A. (2005). novel insect resistance in *Brassica napus* developed by transformation of chitinase and scorpion toxin genes. *Plant Cell Rep.* **24:** 549-555.

Wang, P. and Granados, R. R. (1997). Molecular cloning and sequencing of a novel invertebrate intestinal mucin cDNA. *J. Biol. Chem.* **272:** 16663-16669.

Wang, P., Hammer, D. A. and Granados, R. R. (1994). Interaction of *Trichoplusia ni*granulosis virus-encoded enhancing with the mid gut epithelium and peritrophic membrane of four lepidopteran insects. *J. Gen. Virol.* **75:** 1961-1967.

Wang, Z., Zhang, K., Sun, X., Tang, K. and Zhang, J. (2005). Enhancement of resistance to aphids by introducing the snowdrop lectin gene GNA into maize plants. *J. Biosci.* **30(5):** 627-638.

Wang, Z. Y., Sun, X. F., Tang, K. X. and Zhang, J. R. (2005). Enhanced resistance of snowdrop lectin (*Galanthus nivalis* L. agglutinin)- expressing maize to Asian corn borer (*Ostrinia furnacalis* Guenee). *J. Integr. Plant Biol.* **47:** 873 – 880.

Wei –Xiang, W., Qing-fu, Y., Hang, M., Xue-jun,D. and Wen-ming, J. (2004). Bt-transgenic rice straw affects the culturable microbiota and dehydrogenase and phosphate activites in a flooded paddy soil. *Soil Biol. Biochem.* **36:** 289-295.

Weng, L. X., Deng, H. H., Xu, J. L., Li, Q., Wang,L. H., jiang, Z. D., Zhang, H. B., Li, Q. W. and Zhang, L. H. (2006). Regeneration ofsugarcane elite breeding lines and engineering of strong stem borer resistance. *Pest manag. Sci***. 62:** 178-187

Wilson, T. G. and Cryan, J. R. (1997). Lufenuron, a chitin-synthesis inhibitor, interrupts development of *Drosophila melanogaster*. *J. Exp. Zool.* **278:** 37-44.

Wraight, C. L., Zangerl, A. R., Carroll, M. J. and Berenbaum, M. R. (2000). Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. Poc. *Nat. Acad. Sci.* **97:** 7700-7703.

Wu, J., Luo, X., Guo, H., Xiao, J. and Tian, Y. (2006). Transgenic cotton expressing *Amaranthus codatus* agglutinin confers enhanced resistance to aphids. *Plant Breed.* **125:** 390-394.

Wu, J., Luo, X., Wang, Z., Tian, Y., Liang, A. and Sun, Y. (2008). Transgenic cottonexpressing synthesized scorpion insect toxin *AaHIT* gene confers enhanced resistance to cotton bollworm (*Heliothis armigera*) larvae. *Biotech. Lett.* **30(3):** 547-554.

Xu, D., Xue, Q., McElroy, D., Mawal, Y., Hilder,A. V. and Wu, R. (1996). Constitutive expression of a cowpea trypsin inhibitor gene *CpTi*, in transgenic rice plants confers resistance to two major rice insect pests. *Mol. Breed.* **2(2):** 167-173.

Yao, J., Pang, Y., Qi, H., Wan, B., Zhao, X., Kong,W., Sun, X. and Tang, K. (2003). Transgenic tobacco expressing *Pinellia ternate*agglutinin confers enhanced resistance to aphids. *Trans. Res.* **12:** 715-722.

Yao, J. H., Zhao, X. Y., Liao, Z. H., Lin, J., Chen,Z. H., Chen, F., Song, J., Sun, X. F. and Tang, K. X. (2003). Cloning and characterization of a novel lectin gene from *Pinellia ehavio*. Cell Res. **13(4):** 301-308.

Yarasi, B., Sadumpati, V., Immanni, C. P., Vudem, D. R. and Khareedu, V. R. (2008). Transgenic rice expressing *Allium sativum* leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. BMC. *Plant Biol.* **8:** 102.

Yoshimura, S., Komatsu, M., Kaku, K., Hori,M., Ogawa, T., Muramoto, K., Kazama,T., Ito, Y., and Toriyama, K. (2012). Production of transgenic rice plants expressing *Dioscorea batatus* tuber lectin 1 to confer reeistance against brown hopper. *Plant Biotechnol.* **29:** 501-504.

Yu, C. G., Mullins, M. A., Warren, G. W., Koziel,M. G., Estruch, J. (1997). the*Bacillus thuringiensis* vegetative insecticidal protein

Vip3A lyses midgut epithelium cells of susceptible insects. Appl. Environ. *Microb.* **63(2):** 532-536.

Zambre, M., Goossens, A., Cardona, C., Van Montagu, M., Terryn, N. and Angenon,G. (2005). A reproducibletransformation system for cultivated *Phaseolus acutifolius*(tepary bean) and its use to assess the role of arcelins in resistance to the Mexican bean weevil. *Theor. Appl. Genet .* **110:** 914-924.

Zhang, Y. J., Zhao, H. Y., Wu, K. M., Zhao, K. J., Peng, Y. F. and Guo, Y. Y. (2004). Expression of Cry1Ac protein in cry1Ac/CpTI transgenic rice and its resistance in different developmental stages to *Chilo suppressalis*. *Chin. J. Agricult. Biotechnol.* **1(3):**149-153.

Zhao, J. Z., Cao, J., Li Y., Collins, H. L., Rous, R. T, Earle, E. D., Shelton, A. M. (2003). Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nat. Biotech.,* acetylglucosamine-specific lectin gene fr *Griffonia simplicifolia*(Leguminosae). *Plant Physiol.* **110:**195-202.

Zhu, K., Huesing, J. E., Shade, R. E., Bressan,R. A., Hasegawa, P. M. and Murdock,L. L. (1996). "An insecticidal N-acetylglucosamine-specific lectin gene from *Griffonia simplicifolia* (Leguminosae)." *Plant physiology*.**110**:195-202.