Potential of Transgenic Grain Legumes for Pest Management and Sustainable Crop Production

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Abstract

Host-plant resistance to insects, in general, is controlled by quantitative traits, and as a result, the progress in developing high-yielding insect-resistant cultivars has been quite slow. With the advent of recombinant DNA technology, it has become possible to clone and insert genes into crop plants to confer resistance to insect pests. Insecticidal genes from the bacterium, *Bacillus thuringiensis* (*Bt*), which are mainly effective against the lepidopteran insects, have been inserted into several grain legumes such as - chickpea, pigeonpea, cowpea, soybean, groundnut, faba bean, lentil, field pea, greengram, and blackgram. However, there is a need to discover and deploy genes that are effective against the hemipterans and dipterans that are important pests of grain legumes. Although insect-resistant transgenic plants have been developed in several grain legumes, these have not been deployed for pest management because of the concerns raised about food safety and their possible effects on the non-target organisms. To ensure a sustainable deployment of transgenic insect-resistant grain legumes, it is important that they are compatible with other control methods, including the biological control agents that are important for natural regulation of pest populations. Toxin genes from *Bt* and non-*Bt* proteins are considered to be environmentally benign and their use will reduce the hazards associated with the use of synthetic insecticides. However, it is important to follow the bio-safety regulations, and assure the general public about the biosafety of food derived from transgenic grain legumes, and their biosafety to the non-target organisms in the environment for sustainable crop production.

Introduction

More than 30% of the crop yields are lost due to pests, diseases and weeds despite spending heavily on chemical pesticides (Manjunath 2007). Over dependence on pesticides to minimize the losses due to insect pests, diseases, and weeds has resulted in

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adverse effect on the beneficial organisms, leaving harmful residues in the food, and in environmental pollution. A large number of insects have shown resistance to insecticides belonging to different groups, and over 700 insect species have developed resistance to insecticides. The cotton whitefly, *Bemisia tabaci* (Gennadius), tobacco caterpillar, *Spodoptera litura* (Fabricius), green peach/potato aphid, *Myzus persicae* (Sulzer), cotton aphid, *Aphis gossypii* (Glover), and diamond back moths, *Plutella xylostella* (Linnaeus) exhibit various levels of resistance to insecticides in several crops. *Helicoverpa armigera* (Hubner) has developed resistance to several groups of insecticides resulting in widespread failure of pest control operations, and application of higher dosages and more number of pesticide applications (Armes et al. 1996; Kranthi et al. 2002).

Grain legumes such as chickpea (*Cicer arietinum* L.), pigeonpea [*Cajanus cajan* (L.) Millsp.], cowpea (*Vigna unguiculata* Walp.), field pea (*Pisum sativum* L.), groundnut (*Arachis hypogaea* L.), lentil (*Lens culinaris* Medic.), greengram [*Vigna radiata* (L.) Wilczek], blackgram [*Vigna mungo* (L.) Hepper], common bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), and grass pea (*Lathyrus sativus* L.) are the principal source of dietary protein among vegetarians, and are an integral part of daily diet in several forms worldwide. Grain legumes are cultivated on 23 million hectares, accounting for over 18% of the total arable area, but only 8% of the total grain production. The global pulse production in 2004 was over 60.45 million tons over an area of 71.44 million ha, and average productivity of 846 kg ha-^{1} (FAO 2004). In India, the total pulse production in 2004 was 14.94 million tons on an area of 23.44 million ha, with an average productivity of 637 kg ha^{-1}. Worldwide, chickpea and pigeonpea are the two major food legumes, cultivated on an area of 10.38 and 4.57 million ha, total production being 8.57 and 3.29 million tons, with an average productivity of 826 and 720 kg ha^{-1}, respectively. In addition to being a source of dietary proteins and income to resource poor farmers, food legumes play an important role in sustainable crop production. They are an important component of cropping systems to maintain soil health because of their ability to fix atmospheric nitrogen, extract water and nutrients from the deeper layers of the soil, and add organic matter into the soil through leaf drop. However, food legumes are mainly grown under rainfed conditions and the productivity levels are low, mainly because of severe losses due to insect pests and diseases.

Insect pests of grain legumes

Grain legumes, being a rich source of proteins, are damaged by a large number of insect pests, both under field conditions and in storage (Clement et al. 2000). Amongst the many insect pests damaging food legumes, the pod borers, *H. armigera* and *H. punctigera* (Wallengren) are the most devastating pests of chickpea and pigeonpea in Asia, Africa, and Australia. They also damage other food legumes to varying degrees in these regions. The spotted pod borer, *Maruca vitrata* (Geyer), is a major pest of cowpea and pigeonpea, but also damages other food legumes, except chickpea and lentil (Sharma et al. 1999). The pod fly, *Melanagromyza obtusa* Malloch and pod wasp, *Tanaostigmodes cajaninae*

La Sale causes extensive damage to pigeonpea in India. The leaf miner, *Liriomyza cicerina* (Rondani) is an important pest of chickpea in West Asia and North Africa (Weigand et al. 1994), and has also been reported from North India (Naresh and Malik 1986). The spiny pod borer, *Etiella zinckenella* Triet. is a major pest of pigeonpea, field pea, and lentil. The aphid, *Aphis craccivora* Koch infests all the food legumes, but is a major pest of cowpea, field pea, faba bean, and *Phaseolus* beans, while *Aphis fabae* (Scop.) is a major pest of faba bean and *Phaseolus* beans. The pea aphid, *Acyrthocyphon pisum* (Harris) is a major pest of field pea worldwide. The cotton whitefly, *B. tabaci* infests all the crops, except chickpea, but is an important pest of *Phaseolus* spp., blackgram, and greengram. The defoliators, *S. litura* in Asia and *S. exigua* Hubner in Asia and North America, are occasional pests. The Bihar hairy caterpillar, *Spilosoma obliqua* Walker is a major pest of greengram and blackgram in North India, while the red hairy caterpillars, *Amsacta* spp. damage the rainy season pulses in South-central India. Leafhoppers, *Empoasca* spp. infest most of the food legumes, but cause economic damage in blackgram, greengram, and *Phaseolus* beans. Pod sucking bugs (*Clavigralla tomentosicollis* Stal., *C. gibbosa* Spin., *Nezara viridula* L. and *Bagrada hilaris* Burm.) are occasional pests, but extensive damage has been recorded in cowpea by *C. tomentosicollis* in Africa, *C. gibbosa* in pigeonpea in India, and *C. scutellaris* (Westwood) is common in Asia and Africa. The redlegged earth mite, *Halotydeus destructor* Tucker is a seedling pest of field peas in Australia (Thackray et al. 1997; Liu and Ridsdill-Smith 2001). The pea and bean weevil, *Sitona lineatus* L. is a pest of field pea in the U.S. Pacific Northwest, while *S. crinitus* Herbst. is a pest of pea and other legumes in Asia. The thrips *Megaleurothrips dorsalis* Karny and *Caliothrips indicus* Bag. cause extensive flower damage in food legumes. The bruchids, *Collasobruchus chinensis* L. and *C. maculatus* Fab. cause huge losses in storage in all the food legumes worldwide. The pea weevil, *Bruchus pisorum* L. is a major pest of field pea in most production areas (Clement et al. 1999).

Extent of losses

Insect pests in India cause an average of 30% loss in pulses valued at $815 million, which at times can be 100% (Dhaliwal and Arora 1994). In Africa, insect pests can be responsible for extensive damage (up to 100%) in cowpea, the major food legume of this continent (Singh and Jackai 1985), while in the US, the avoidable losses have been estimated at 40 to 45% (Javaid et al. 2005). In Pakistan, nearly 10% of the chickpea grain is lost due to bruchids in storage (Aslam 2004), and at times, there may be complete loss of grain in storage. *Helicoverpa armigera* – the single largest yield reducing factor in food legumes, causes an estimated loss of US$ 317 million in pigeonpea, and $328 million in chickpea (ICRISAT 1992). Globally, it causes an estimated loss of over $2 billion annually, despite over $1 billion worth of insecticides used to control this pest (Sharma 2005). In general, the estimates of yield losses vary from 5 to 10% in the temperate regions and 50 to 100% in the tropics (van Emden et al. 1988). The avoidable losses in food legumes at current production levels of 60.45 million tonnes would be nearly 18.14 million tons (at an average loss of 30%), valued at nearly US$ 10 billion. Insect pest management by chemicals has brought about a considerable protection to crop yields over the past five decades. Unfortunately, extensive and indiscriminate usage of chemical pesticides has resulted in environmental degradation, adverse effects on human health and other organisms, eradication of beneficial insects and development of pesticide resistant insects. As a result, chemical control of insect pests is under increasing pressure. There has been a wide publicity of Integrated Pest Management (IPM) approaches since 1960s, in which the host plant resistance to insect pests is the key component (Smith and Van den Bosch 1967). Despite the efforts made over the past four decades to breed varieties with resistance to insects, the progress has been less than satisfactory in many cases.

Potential of transgenic grain legumes for pest management

Considerable progress has been made over the past two decades in handling and introduction of novel genes into crop plants to impart resistance to biotic and abiotic stresses, improve nutrition, and increase crop yields. Genetic engineering of crops offer exiting opportunities to develop plants with new traits such as resistance to insect pests, diseases, drought, heat, cold, salinity, etc. Despite significant advances over the past decade, the development of efficient transformation methods can take many years of painstaking research (Sharma et al. 2005c). The major components for the development of transgenic plants include the development of reliable tissue culture regeneration and transformation systems. Grain legumes generally have been regarded as recalcitrant to transformation because of poor regeneration ability (especially via callus), and also because *in vitro* regeneration is genotype specific and there is also the problem of compatible gene delivery system (Obembe 2008). However, the recent advances in transformation of grain legumes such as chickpeas (Sharma et al. 2006a), pigeonpeas (Sharma et al. 2006b), cowpeas (Obembe 2008; lentils (Gulati et al. 2002) and phaseolus (De Clercq et al. 2002) have sparked new hope that the menace of insect pests can be dramatically reduced by introducing resistance genes into the grain legumes.

So far, one of the best strategies for reducing insect pest induced losses in crops is the introduction of *Bt* genes into plants. The non-*Bt* genes, i.e., Protease inhibitors, plant lectins, ribosome inactivating proteins, amylase inhibitors, chitinases, secondary plant metabolites, avidins, cholesterol oxidase are the other potential genes for engineering insect resistance in plants (Liener and Kakade 1969; Green and Ryan 1972; Ryan 1990; Gatehouse et al. 1993; Chrispeels et al. 1998; Gatehouse and Gatehouse 1998), and are useful for developing insect-resistant transgenic plants. Genes from the bacterium, *Bacillus thuringiensis* (*Bt*) have been deployed successfully for pest control through transgenic crops on a commercial scale (Hilder and Boulter 1999; Sharma et al. 2004). Transgenic cotton and maize cultivars with resistance to lepidopteran insects have been released for cultivation in several countries (James 2005), while transgenic chickpea and pigeonpea with resistance to *H. armigera* are currently under various stages of development (Sharma et al. 2005b). Crop area under transgenic crops is increasing at a fast rate, and has reached to 114.3 million ha, of which transgenic crops with resistance to insect pests constituted more than 40 million ha during 2007 (James 2008). These *Bt* and non-*Bt* proteins are considered to be environmentally benign and their deployment will reduce the hazards associated with the use of synthetic insecticides for pest management. However, transgenic plants should be deployed such that they compliment other methods of insect control, including biological control agents. This paper provides an overview of the progress made in developing insect-resistant transgenic plants with different toxin genes; assess their role in integrated pest management, and the environmental concerns.

Bacillus thuringiensis

Bacillus thuringiensis (Bt) is one of more than 20 species of soil growing *Bacilli. Bacillus thuringiensis* (Bt) is a gram positive, aerobic and an endospore-forming bacterium recognized by its parasporal body (known as crystal) that is proteinaceous in nature and possesses insecticidal properties. Insecticidal activity of the bacterium is due to three proteins viz., crystal (cry) cytolytic (cyt) and vegetative insecticidal protein (vip) produced during the bacterial infection on the larvae. These proteins are accumulated in the crystalline inclusion bodies produced by the bacterium on sporulation (Cry proteins, Cyt proteins) or expressed during bacterial growth (Vip proteins). Genes encoding these proteins are expressed in plants to protect them from insect damage. More than 150 different Cry toxins have been cloned and tested for their toxicity on various insect species till date, however, about 40% of the currently identified *Bt* toxins are not active on insects, due to various reasons like low solubility in the insect gut environment, lack of binding to BBMV in the larval midgut, presence of protease cleavage sites. At least 10 genes encoding different *Bt* toxins (Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba, Cry1Ca, Cry1H, Cry2Aa, Cry3A, Cry6A, and Cry9C) have been expressed in different crop plants for resistance to different groups of insect pests (Schuler et al. 1998). It is important to develop an appropriate strategy to maximize the contribution of insect-resistant genetically modified crops in pest management.

There have been several reports on using genetic transformation technology for engineering resistance to insect pests in grain legumes, including the early indications reported on inhibition & development of and feeding by *H. armigera* in chickpeas transformed with *cry1Ac* gene (Kar et al. 1997). Sanyal et al. (2005) developed transgenic chickpea plants with *cry1Ac* gene under the control of *CaMV35S* promotor and *nptII* as a selection marker. The transgenic plants showed protein expression levels of 14.5 to 23.5 ng mg^{-1} of extractable protein. Chickpea plants with Cry1Ac expression levels of >10 ng mg^{-1} showed >80% mortality of the neonate *H. armigera* larvae (Sharma et al. 2005b). Pigeonpea plants transformed with *cry1Ab, cry1Ac,* and soybean trypsin inhibitor (*SBTI*) genes have been developed at ICRISAT, and are being tested against *H. armigera*

(Sharma et al. 2005b; Sharma et al. 2006a, b). Pigeonpea plants transformed with *cryIE-C* gene from *Bt* under the control of *CaMV35S* promotor and *nptII* as a selection marker have shown resistance to the larvae of tobacco caterpillar, *S. litura* (Surekha et al. 2005). A codon-modified *cry1Ac* gene has been introduced into groundnut by using micro-projectile bombardment (Singsit et al. 1997). The immunoassay of plants selected with hygromycin has shown the expression of Cry1Ac protein up to 0.16% of the total soluble protein. Complete mortality or up to 66% reduction in larval weight has been recorded in the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). There was a negative correlation between larval survival and larval weight of the lesser cornstalk borer with the amount of *Bt* protein. Parrot et al. (1994) reported the successful expression of a truncated synthetic *cry1Ab* gene from *B. thuringiensis* subsp. *kurstaki* HD-1 in soybean for the first time, however, the protein expression was undetectable in T_1 plants and showed a similar level of resistance to velvetbean caterpillar, *Anticarsia gemmatalis* (Hubner) to that of a conventially bred resistant line (Beach and Todd 1987, 1988). Subsequent attempts to obtain improved expression of a *Bt* toxin gene in *Bt*-transgenic soybean plants (Jack-*Bt*) showed 3 to 5 times less defoliation by corn earworm, *Helicoverpa zea* (Boddie), 8 to 9 times less dmage from velvetbean caterpillar, *A. gemmatalis*, and 4-times greater resistance to soybean looper, *Pseudoplusia includens* (Walker) as compared to untransformed Jack plants in detached leaf feeding and cage bioassays (Stewart et al. 1996; Walker et al. 2000).

Vegetative insecticidal proteins (Vip)

In addition to Cry proteins, several strains of *Bt* are known to produce insecticidal proteins during the vegetative phase of growth. These proteins are called vegetative insecticidal proteins (Vip). The first *vip* genes described were *vip*3Aa1 and *vip*3Aa2 isolated from *Bt* strain AB88 and AB424 (Koziel et al. 1996). Vips possess toxicity of the same magnitude as that of *Bt* δ-endotoxins against the susceptible insects. They induce gut paralysis, followed by complete lysis of the gut epithelium cells, resulting in larval mortality (Yu et al. 1997). The Vips have shown a broad insecticidal spectrum, including activity toward a wide variety of lepidopteran and coleopteran pests (Koziel et al. 1996; Estruch et al. 1996; Warren 1997; Selvapandiyan et al. 2001; Bhalla et al. 2005). They represent second generation of insecticidal toxins that can be used to target *Bt* δ-endotoxins-resistant insect pests (Estruch et al. 1997).

Enzyme inhibitors

The enzyme inhibitors act on key insect gut digestive enzymes such as α-amylase and proteinases. Several kinds of α-amylase and proteinase inhibitors present in seeds and vegetative organs in plants influence food utilization by the phytophagous insects (Ryan

1990; Chrispeels et al. 1998; Gatehouse and Gatehouse 1998). The usefulness of some of these biochemical compounds for developing insect-resistant transgenic plants has been discussed in the following section.

Protease inhibitors

Protease inhibitors (PIs) of plants are involved in a number of functions, including the control of endogenous proteolytic enzymes, and the plant defense against insect attack (Thomas et al. 1994; Lawrence and Koundal 2002). Since protease inhibitors are primary gene products, they are excellent candidates for engineering insect resistance into plants. Disruption of amino acid metabolism by inhibition of protein digestion has been one of the targets for use in insect control (Johnson et al. 1989). Genes encoding inhibitors specific for serine-proteases are the main digestive proteases in most lepidopteran insects (Boulter 1993). Deployment of protease inhibitors for insect control requires a detailed analysis of particular insect-plant interaction. The ability of some insect species to compensate for protease inhibition by switching on to an alternative proteolytic activity or over-producing the existing proteases may limit the application of protease inhibitors in such species (Jongsma et al. 1995). Adaptive mechanisms elevate the levels of other classes of proteinases to compensate for the trypsin activity inhibited by dietary proteinase inhibitors. Soybean Kunitz type trypsin inhibitor (*SBTI*) and soybean Bowman-Birk type trypsin-chymotrypsin inhibitor (*SBBI*) reduced the larval weight of *H. armigera*, and such effects were greater for *SBTI* than *SBBI* (Johnston et al. 1993; Shukla et al. 2005). Larvae feeding on diet containing 0.234 mM *SBTI* also reduced the trypsin like enzyme activity in the gut of *H. armigera*. The diversity in proteinase activity in *H. armigera* gut and the flexibility in their expression during developmental stages depending upon the diet provides a basis for selection of proper PIs for use in transgenic plants (Patankar et al. 2001). *Helicoverpa armigera* fed on chickpea showed more than 2.5- to 3-fold proteinase activity than those fed on pigeonpea and cotton. Since the first successful example of genetic engineering of plants for insect resistance using genes of plant origin, cowpea protease inhibitor (CpTi) (Hilder et al. 1987), there has been a considerable progress in deploying PI-transgenic plants for controlling various insect pests.

Alpha amylase inhibitors

Six different types of ∝-amylase inhibitors: lectin-like, knottin-like, cereal-type, Kunitz-like, gamma-purothionin-like, and thaumatin-like could be used in pest control (Franco et al. 2002). These inhibitors show remarkable structural diversity leading to different modes of inhibition and different specificity profiles against diverse ∝-amylases. Specificity of inhibition is an important issue as the introduced inhibitor neither should affect the plant's own ∝-amylases nor the nutritional value of the crop. The ∝- amylase inhibitors are attractive candidates for the control of seed weevils as these are highly dependent on starch as an energy source. The enzyme plays a key role in carbohydrate metabolism of microorganisms, plants, and animals. Several insects, especially those similar to weevils that feed on starchy seeds during larval and/or adult stages, depend on their α-amylases for survival. Amylase inhibitors from pigeonpea inhibit 22% amylase activity resulting in adverse effects on growth, development, and survival of *H. armigera* larvae (Giri and Kachole 1998). Enhanced levels of resistance to the bruchids have also been obtained in seeds of transgenic adzuki beans with ∝-amylase gene (Ishimoto et al. 1996). The α-amylase inhibitors from *P. vulgaris* seeds used in developing transgenic pea against bruchid beetles (Shade et al. 1994), effectively reduced the development of *C. maculatus* (Ishimoto and Chrispeels 1996; Ishimoto et al. 1996), and gave a boost to the development of amylase inhibitor transgenic crops. The α-amylase inhibitor-1 inhibits pea bruchid, *B. pisorum* α-amylase enzyme by 80% resulting in larval mortality, while α-amylase inhibitor-2 inhibits the enzyme by 40% and delays the larval development, and have also been found effective in protecting peas from weevil damage under field conditions (Morton et al. 2000).

Enzymes

Insect moulting enzyme, chitinase, which degrades chitin to low molecular weight soluble and insoluble oligosaccharides, is a metabolic target of selective pest control (Chit Kramer and Muthukrishnan 1997). The cDNA and genomic clones for the chitinase from the hornworm, *Manduca sexta* (L.) have been isolated, characterized, and deployed in producing chitinase-transgenic tobacco plants, and these chitinase expressed transgenic plants have shown resistance to several lepidopteran insects (Ding et al. 1998). Accumulation of bean chitinase (*BCH*) increased as the potato plant developed, with maximum levels recorded in mature plants, which have also increased the efficacy of the toxin from *Bt* (Down et al. 2001). Chitinase also potentiates the efficacy of the toxin from *B. thuringiensis*.

Plant lectins

Plant lectins are a heterogeneous group of sugar binding proteins, which have a protective function against a range of organisms. The plant lectins such as snowdrop lectin (*Galanthus nivalis* agglutinin; GNA) and avidin, have been introduced into a number of crop plants for resistance against coleopteran, lepidopteran, and homopteran insect pests (Kramer et al. 2000; Markwick et al. 2001; Malone et al. 2002). Plant lectins are particularly effective against the sap sucking Hemiptera (Hilder et al. 1995; Shukle and Murdock 1983; Czapla and Lang 1990). Lectins from *Canavalia ensiformis* (L.) DC (*Concanavalin A*), *Amaranthus caudatus* L. *Lens culinaris* Medik., and *Galanthus nivalis* L. induced significant mortality in *A. pisum* (Rahbe et al. 1995). Snowdrop, garlic, and chickpea lectins have shown adverse effects on survival, growth, and development of *H. armigera* (Shukla et al. 2005; Sharma et al. 2005b). Many mannose-binding lectins were toxic to *A. pisum*. *Concanavalin A* has also been tested against *A. gossypii*, *Aulacorthum solani* Kalten., *Macrosiphum euphorbiae* (Thomas), *Macrosiphum albifrons* Essig, and

M. persicae at concentrations between 10 to 1500 μg ml^{-1}, showing variable efficacy among species. Although, plant lectins have shown biological activity against a range of insects, consideration should be given with regard to their deployment in transgenic plants because of their known toxicity to mammals and humans.

Viruses, neurotoxions, and insect hormones

Neurotoxins: Spiders and scorpions produce powerful neurotoxins that have been expressed in transgenic organisms (Barton and Miller 1991). Jiang et al. (1995) constructed the gene expressing the venom of an Australian spider, *Androctonus australis* Hector by annealing partially complementary single-stranded oligonucleotide using codons preferred in plants. This gene was then introduced into a plasmid for expression in plants. Transgenic plants of tobacco have been obtained containing an insecticidal spider peptide gene, and some of these plants have shown resistance to *H. armigera* (Jiang et al. 1996). The role of neurotoxins from insects and spiders needs to be studied in greater detail before they are deployed in other organisms and plants because of their possible toxicity to mammals.

Viruses: Enhancin genes from *Trichoplusia ni* (Hubner) or *H. armigera* baculoviruses introduced into tobacco plants resulted in slower development and increased larval mortality on some transgenic lines (Cao et al. 2002). The majority of the transgenic plants had little or no inhibitory effect. A recombinant *Autographa californica* (Speyer) nucleopolyhedrovirus (AcNPV) with the enhancin gene from *T. ni* granulovirus, *AcEnh26* expressed in tobacco plants showed a 10-fold increase in AcNPV infection (Hayakawa et al. 2000). Transgenic tobacco transformed with the *T. ni* granulovirus enhancin gene has also been shown to enhance baculovirus infection in the larvae (Hayakawa et al. 2004). Long-term feeding of lyophilized transgenic tobacco material with the enhancin gene resulted in adverse effects on the larvae of *Mythimna separata* (Walk.) and *S. exigua*, suggesting that baculovirus enhancin gene products have potential for use in pest management in grain legumes.

Neuropeptides and peptidic hormones: Neuropeptides and small peptidic hormones are another interesting class of molecules that can be deployed as possible insecticides through transgenic plants (Tortiglione et al. 1999; Altstein et al. 2000). These molecules regulate several physiological processes in insects and are active at very low concentrations. There are several examples of neuropeptides encoded by a single gene coding for multiple copies of one or more peptides. Backbone cyclic (*BBC*) neuropeptide based antagonists (*NBA*) has been applied to insect pyrokinin/pheromone biosynthesis activating neuropeptide (*PBAN*) family. It has led to the discovery of potent antagonists and metabolically stable peptidomimetitic antagonists devoid of agonistic activity, which *in vivo* inhibited *PBAN*-mediated activities in moths (Altstein et al. 2000). Transgenic tobacco plants have been produced expressing a precursor of a regulatory peptide from *Aedes aegypti* (L.) (Trypsin Modulating and Oostatic Factor, *Aea-TMOF*), which interferes with the development of tobacco budworm, *H. virescens* larvae (Tortiglione et al. 2002). The potential for *GNA* to act as a carrier protein to deliver an insect neuropeptide from *M. sexta,* allatostatin (*Manse-AS*) to the haemolymph of lepidopteran larvae has been examined by expressing a *GNA/Manse-AS* fusion protein (*FP*) in *Escherichia coli* and feeding purified *FP* to larvae of the tomato moth, *L. oleracea* (Fitches et al. 2002). The *FP* administered at 1.5 or 0.5% of dietary proteins strongly inhibited feeding and growth of fifth-instar larvae. *GNA/Manse-AS* and similar fusion proteins offer a novel and effective strategy for delivering insect neuropeptides by oral administration, which could be used in conjunction with expression in transgenic plants for pest management (Fitches et al. 2002). Gamma-aminobutyrate (*GABA*), which accumulates in plants in response to biotic and abiotic stresses via activation of glutamate decarboxylase, acts as an inhibitory neurotransmitter in insect pests (Shelp et al. 2003). Ingested *GABA* disrupts nerve functioning and causes damage to oblique-banded leaf roller, *Choristoneura rosaceana* (Harris) and tobacco budworm, *H. virescens.* Feeding by tobacco budworm and oblique-banded leaf roller larvae stimulated *GABA* accumulation in soybean and tobacco. Elevated levels of endogenous *GABA* in genetically engineered tobacco deterred feeding by tobacco budworm larvae and infestation by the northern root-knot nematode, *Meloidogyne hapla* Chitwood. Genetically engineered plants over expressing glutamate decarboxylase and having high *GABA*-producing have potential for use in integrated pest management (Shelp et al. 2003).

Strategies for efficient expression of Bt genes

While the quality and quantity of transgene expression is of great importance in generating transgenic *Bt* plants, high-dose expression also reduces the risk of development of resistance. However, *cry* genes do contain sequences that limit their expression in transgenic plants. Perlak et al. (1991) had earlier demonstrated that modifications made in the wild-type *cry* genes could increase the levels of insect control proteins 100-fold. Although, there have also been various reports of transgenic grain legumes (see above) carrying these truncated versions (synthetic genes) of *cry* genes designed for high expression in plants, there has been a limited success in generating plants with insecticidal proteins, with high expression levels and also the corresponding efficacy have not been sufficient to qualify as high dose against *H. armigera.* This clearly indicates that although the expression cassettes carrying *cry* genes used for transformation of these pulse crops have been optimized for dicot systems (eg. cotton), it’s not necessary to be successful for all other dicots including legumes. Hence, the underlying difference in the performance of an expression cassette has to be organism/family specific and related to differences in transcript regulation and the stability of transcripts and proteins.

Hence at ICRISAT there have been efforts made towards synthesizing Bt gene by codon optimization adjusted to legume plant usage and eliminating possible polyadenylation signals. Most changes are made to the third codon thereby minimizing changes in the amino acid sequence and increasing the expression of *Bt* toxin by 10 to 100-fold (Perlak et al. 1991).

In addition, evolving levels of insect resistance to *Bt* can be dramatically reduced through the genetic engineering of chloroplasts in plants or by inserting more than one gene in the same plant. These organelles are inherited maternally and therefore, pollen-based dispersal is not possible and hence seems to be environmental friendly by preventing the spread of chloroplast based genes into the environment. Svab and Maliga (1993) expressed *cry1A* gene in tobacco chloroplasts using chloroplast transformation vectors and particle bombardment. Transgenic tobacco leaves expressing the *Cry2Aa2* protoxin in chloroplasts when fed to susceptible, *Cry1A*-resistant (20,000 to 40,000-fold resistant) and Cry2Aa2-resistant (330- to 393-fold resistant) insects (*H. virescens*, *H. zea* and S. *exigua*), 100% mortality was observed in all insect species and strains (Kota et al. 1999).

Choice of promoters

An important aspect of transgenic technology is the regulated expression of transgenes. Tissue specificity of transgene expression is also an important consideration while deciding on the choice of the promoter in order to achieve a better control of the expression of these pest resistance genes in crops. For expression of the *Bt* gene in the higher plants, a recognizable promoter and a terminator sequence must bracket the *Bt* gene. The modified versions of the insecticidal *cry* genes thus require presence of suitable promoters and a polyadenalation sequence for their efficient expression in the plants. Popular constitutive promoters include cauliflower mosaic virus (CaMV35S) and ubiquitin are powerful in driving the gene expression but the gene is expressed in all tissues and at all stages. Although, a number of heterologous promoters such as the constitutive promoters have been shown to regulate the expression of genes controlling them in transgenic plants, they are not the most efficient means of expression. Hence, tissue specific expression *viz*., flower specific expression and green tissue-specific expression of codon optimized *cry1Ab*, *cry1Ac and cry2A* genes needs to be carried out to combat the *H. armigera* more efficiently in grain legumes. The use of tissue-specific promoters and wound-inducible promoters (Vaeck et al. 1987), will minimize the superfluous expression of transgenes. Tissue specific promoters can limit the expression to target tissues for example, Rubisco small subunit in transgenic soybean for limiting the gene expression to green tissues (Miklos et al. 2007). Cotton cultivar Coker 312 was first transformed by using modified *cry1A(c)* gene under the control of CaMV35S promoter containing a duplicated enhancer region. The transformed plants showed total protection against *S. exigua* and *H. zea*. The maximum level of toxin protein was 0.1% of the total soluble protein. By placing the *cry1A(c)* gene under the control of *Arabidopsis thaliana* small subunit promoter with its chloroplast transit peptide sequence, Wong et al. (1992) produced tobacco plants with 10 to 20 fold increase in Cry1A(c) mRNA and protein compared to gene constructs with CaMV35S promoter with duplicated enhancer region. This increased the *Bt* toxin production to nearly 1% of the total leaf protein. Fujimoto et al. (1993) used a similar approach to transform rice plants as discussed above for maize. The transformed plants had nearly 0.05% toxin of the total soluble leaf protein, and showed resistance to the rice leaf folder*, Cnaphalocrosis medinalis* (Guenee) and yellow stem borer, *Chilo suppressalis* (Walker). Thus, the strength of the promoter and the possibility of using developmental-stage-or tissue-specific promoters have a potential to tailor the plants to better respond to biotic constraints such as insect pests in grain legumes.

Potential benefits of insect-resistant transgenic crops

To date *Bt* toxins are the only insecticidal proteins expressed in commercial genetically modified crops. The insect-resistant transgenic crops have benefitted growers through increased crop yield, lower input costs, and easier agronomic management. These factors are likely to have substantial impact on the livelihoods of farmers in both industrial and developing countries. Insect-resistant corn and cotton, herbicide tolerant soybean, canola, corn and cotton, and virus-resistant papaya and squash adopted by US growers increased crop yields by 1.4 billion kg, saved US$1.2 billion by lowering the cost of production, and reduced pesticide use by 46 million pounds (Gianessi et al. 2002). The *Bt* cotton released for commercial cultivation in India has shown that the technology substantially reduces pest damage and pesticide application, and increases cottonseed yield (Qaim and Zilberman 2003; Sharma and Pampapathy 2006). Cultivation of *Bt* cotton in India has resulted in 29% increase in crop yield due to effective control of bollworms, 60% reduction in chemical sprays, and 78% increase in net profit as compared to the non-transgenic cottons (Brookes and Barfoot 2005). The yield gains are much greater than those reported for other countries where genetically modified crops were used mostly to replace enhanced chemical pest control. In China, *Bt* cotton growers have reduced pesticide spraying for cotton bollworm control from 20 to 6 times per year, and reduced 28% cost of cultivation over the non-transgenic cotton (Huang et al. 2002). Similar benefits of *Bt* cotton have also been obtained in Australia, Mexico, Argentina, South Africa, and Indonesia. Adoption of transgenic crops offers the additional advantage of controlling insect pests that have become resistant to commonly used insecticides. Deployment of transgenic insect-resistant crops will lead to:

- Reduction in insecticide sprays.
- Reduced exposure of farm labor to insecticides.
- Reduction in harmful effects of insecticides on non-target organisms.

- Increased activity of natural enemies.
- Reduced amounts of pesticide residues in food and food products.
- A safe environment to live.

However, there is considerable public debate on the issue, and several claims to the contrary have also been published in the public media. In many developing countries, small-scale farmers suffer pest-related yield losses because of technical and economic constraints. Pest-resistant genetically modified crops expressing different *Bt* and non-*Bt* genes can contribute to increased yields, agricultural growth, and decreased pesticide pollution in the environment in such situations.

Biosafety of transgenic food

There is a need for the new technologies to be tested rigorously for potential allergenic, toxic, and antimetabolic effects in a transparent manner. The *Bt* proteins are rapidly degraded by the stomach juices of vertebrates. Most *Bt* toxins are specific to insects as they are activated in the alkaline medium of the insect gut. The levels of nutrients and the antinutrients gossypol, cyclopropenoid fatty acids, and aflatoxins were similar in the seed from the *Bt*-transgenic and nontransgenic cotton genotypes (Berberich et al. 1996). Processing removed >97% of the active proteins in the transgenic cottonseed, and very little amounts of *Bt* toxins remain in plant parts to be consumed by human beings or dairy cattle (Sims and Berberich 1996). There are no specific receptors for *Bt* protein in the gastrointestinal tract of mammals, including man (Kuiper and Noteborn 1994). Histopathological effects have been observed in the gut mucosa, but no systemic adverse effects have been observed in mice and rabbits following oral administration. Transgenic crops pose no additional risk to human and animal health. However, a number of aspects concerning the safety assessment of genetically modified crops would require further study. There were no differences in the survival and body weight of broilers reared on meshed or pelleted diets prepared with *Bt* transgenic maize and similar diets prepared using control maize (Brake and Vlachos 1998). Several protein families that contribute to the defense mechanisms of food plants are allergens or putative allergens, and some of these proteins confer resistance to insect pests. These include α-amylase and trypsin inhibitors, lectins, and pathogenesis-related proteins (Franck Oberaspach and Keller 1997). Several of these secondary plant substances are toxic to mammals, including humans, and should not be deployed in transgenic crops for insect resistance.

One of the major concerns of transgenic crops is their effect on the non-target arthropods including natural enemies of crop pests. A number of studies have revealed direct toxic effects of purified serine-type protease inhibitors to adult honeybees when fed at high concentrations under laboratory conditions such as Bowman-Birk inhibitor, basic pancreatic inhibitor, Pot-I and Pot-II, and soybean trypsin inhibitor (Malone et al. 1998; Burgess et al. 1996; Pham-Delègue et al. 2000). In contrast, ingestion of high doses of purified Cystatin or OC-I from rice, belonging to the cystein-type inhibitors did not cause any effect on bee survival (Girard et, al., 1998). Direct exposure of GNA and avidin at high concentrations or through transgenic plants have been reported to be effective against pest arthropods belonging to the orders of Coleoptera, Lepidoptera, Acaridae, Orthoptera and Neuroptera (Levinson et al. 1992; Kramer et al. 2000; Markwick et al. 2001; Malone et al. 2002), however, GNA- or avidin-expressing GM plants might pose a risk to the predatory coccinellids and chrysopids which need to be addressed before the release of these transgenic plants (Lawo and Romeis 2008; Dhillon et al. 2008). No adverse effects of Cry1Ab or Cry1Ac *Bt* toxins were observed on *Cheilomenes sexmaculatus* (F.) when the larvae were reared on *A. craccivora* fed on different concentrations in the artificial diet, however, a direct exposure to *Bt* toxins expressed in transgenic plants or predation on *H. armigera* on *Bt*-transgenic pulses will have little effect on the activity and abundance of the ladybirds (Dhillon and Sharma 2009). Although *H. armigera* larvae fed on *Bt* proteins in artificial diet or *Bt*-transgenic cotton showed some adverse effects on *Campoletis chlorideae* (Uchida), these effects were host nutritional quality mediated and early mortality of the host larvae (Sharma et al. 2007, 2008a), the reduction in *C. chlorideae* population on *Bt*-susceptible *H. armigera* larvae on the *Bt*-transgenic crops would be density-dependent, and the parasitoid might have some level of survival on the *Bt*-resistant host insects to help manage the populations of *Bt*-resistant *H. armigera* in the transgenic crops and on other host insects in different agroecosystems (Dhillon and Sharma 2007). Numbers of non-target arthropods such as aphids [*Metopolophium dirhodum* (Walker.), *Rhopalosiphum padi* (L.), and *Sitobion avenae* (F.)], bug, *Orius insidiosus* (Say), syrphid, *Syrphus corollae* (Fab.), ladybird beetle, *Coccinella septempunctata* L., lacewing, *Chrysoperla carnea* (Stephens), thrips, and hymenopteran parasitoids did not differ between the *Bt* and non-*Bt* maize, suggesting no adverse effects of *Bt* maize on non-target arthropod communities (Bourguet et al. 2002). The diversity of the arthropod community in *Bt* cotton fields is greater than that in conventional cotton, suggesting that *Bt* cotton is highly favorable for integrated management of cotton pests (Wu et al. 2003). The numbers of leafhoppers, *A. biguttula biguttula*; grey weevil, *Myllocerus* sp.; red cotton bug, *Dysdercus keonigii* F., and aphids, *A. gossypii* have been found to be similar between the *Bt* transgenic and nontransgenic cotton, and there were no apparent effects of transgenic cotton on the relative abundance of predatory spiders, coccinellids and the chrysopids (Sharma and Pampapathy 2006; Sharma et al. 2007). In addition, the scientific research aimed at risk analysis, prediction, and prevention, combined with adequate monitoring and stewardship, must continue so that negative ecological impact from GM crops is kept to a minimum (Sharma et al. 2008b).

Conclusions

Incorporation of insecticidal genes in grain legumes will have a tremendous effect on pest management. Genetically engineered grain legumes for insect resistance, could potentially benefit a diversity of legume crops. Emphasis should be placed on combining exotic genes with conventional host plant resistance, and also with traits conferring resistance to other insect pests and diseases of importance in a crop in the target region. While several crops with commercial viability have been transformed in the developed world, very little has been done to use this technology to develop insect-resistant cultivars to increase food production in the harsh environments of the tropics. Substantial investments are therefore needed to develop, field test, and commercialize new transgenic varieties expressing insecticidal proteins, or proteins providing resistance to insect pests revolutionizing agriculture especially in arid and semi-arid regions of the world. There is a need to follow the biosafety regulations and make this technology available to farmers at a reasonable price, who cannot afford the high cost of seeds and chemical pesticides.

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