Plant Insecticidal Proteins and their Potential for Developing Transgenics Resistant to Insect Pests

K R Koundal* and P Rajendran
National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110 012, India

Insects cause heavy damage to cultivated crop plants. Production of proteinaceous inhibitors that interfere with the digestive biochemistry of insect pests is one of the naturally occurring defence mechanisms in plants. These proteins include lectins, arcelins and inhibitors of alpha amylases and proteases of various larval pests. Use of plant genes encoding effective inhibitors of major digestive enzymes, such as protease and α-amylase inhibitors of the target pest species is emerging as viable approach for the production of pest resistant transgenic crop plants. Therefore, it is important to characterize proteins and their genes from our indigenous crops in order to strengthen and broaden our gene bank for pest control manipulations. The availability of diverse insecticidal proteins and their genes from different plant species will make it easier to use one or more genes in combination to develop resistant crop plants. Eventually, insect resistant transgenic plants will certainly prove more economic than any conventional control strategy if long-term benefit of transgenic crops especially factors such as environmental damage and human health risks are considered.

Introduction

Pest infestation leading to massive crop damage is the most serious limiting factor in crop productivity. In the course of evolution, many classes of proteins and secondary metabolic substances have been expressed in plants as effective counter measures against herbivory and the application of these natural plant defence traits as candidates for genetic engineering for crop pest control is now a reality. Plant secondary metabolites include a plethora of biochemical entities that are synthesized through complex pathways and therefore, the attempts to explore their biochemistry and physiology have always remained challenging. Broadly, they belong to totally unrelated classes of compounds like alkaloids, flavonoids, foliar enzymes (polyphenol oxidases & peroxidases), foliar phenolic acid esters (rutin & chlorogenic acid), non-protein amino acids, peptide hormones, pyrethrins, steroids, and terpenoids. Yet another group, but more important from the application point of view belongs to plant defence proteins that are more specific against insect pests (Maqbool et al, 2001). In most plants, they offer horizontal resistance against insect attack (Fabrick et al, 2002). This review is exclusively focussed on the potential of these inhibitors or their encoding genes in pest control manipulations. Attempts are made to depict the structural functional relationships of these inhibitors that direct their application to develop transgenics resistant to various insect pests.

Plant Proteins with Insecticidal Activity

Production of proteinaceous inhibitors that interfere with the digestive biochemistry of insect pests is one of the naturally occurring defence mechanisms in plants. This mechanism is manifested in the form of accumulation of one or several defence proteins such as lectins, arcelins and inhibitors of alpha-amylases and protease of larval pests. As insect larvae develop, they secrete into their guts a variety of enzymes that break down carbohydrates and proteins present in the ingested food. The larvae then feed on the released components from the plant tissue rapidly causing crop damage and yield loss. Plants respond to this invasion by synthesizing proteins that can inhibit the action of these enzymes. If a food source contains a supply of digestible protein or carbohydrate, but also an inhibi-
tor that acts as a biological check, the larval enzymes cannot digest the food and the larva will not grow and develop through its normal cycle (Boulter, 1993; Fabrick et al., 2002).

The potential for using this natural host plant resistance in pest control across the plant genetic barriers has increased with the development of gene transfer techniques. Though exogenous natural pesticidal agents such as Bt toxin are also effective in deterring plant predators, in spite of their current successes, they may create environmental safety and consumer health debates in future in addition to a multitude of ethical concerns (Gatehouse et al., 1998). Further, there is growing evidence that genes derived from plants could be excellent alternatives to the toxins from Bacillus thuringiensis. There are now several examples of protease inhibitors of plant origin conferring insect resistance when expressed in transgenic plants including tobacco, rice, cotton, strawberry, poplar and peas (Ussuf et al., 2001). Plant derived genes target different sites in insects than the synthetic chemicals and may be deployed in combination with the bacterial insecticidal genes for complete insect control. However, this approach can be exploited effectively if encoding genes for proteins with appreciable inhibitory properties on the target pest's enzymes are available for genetic manipulations. Thus, it is imperative to evaluate as many plant sources as possible for identifying the presence of proteins with ideal insecticidal properties (Sparvoli et al., 2001). It is equally important to characterize these proteins and their encoding genes to strengthen and broaden the resistance gene pool. Among them, alpha-amylase inhibitors, protease inhibitors and lectins have undergone extensive investigations particularly in the last two decades and the research on these proteins and their encoding genes has proved to be rewarding. In addition, some related but less studied proteins like arcelins and vicilins have also invoked research attention in recent years.

(i) Alpha-amylase Inhibitors

Alpha-amylase a ubiquitous enzyme, catalyses the endolytic cleavage of 1→4 linked glucose polymers, mainly starch to give hydrolytic products with the α-configuration. This digestive enzyme has been of interest to plant biologists for many years because of its agricultural and industrial importance. In plants, α-amylase inhibitors are stored in seeds and tubers towards the end of the life cycle in copious amounts. They are also synthesized in plants almost at the same time and usually in the same organs that store the starch and amylases. Their role is mainly defence through inhibition of a variety of alpha-amylases from diverse sources by forming irreversible complexes with them. Chrzaszez & Janicki (1934) were the first to report the presence of α-AIs in buckwheat. Thereafter, α-AI has been isolated from many plants, mainly cereals and legumes. α-AI inhibitors are primarily the products of single gene, and are small, low molecular weight (in the 14-8 kDa range) monomeric proteins, α-AIs from bean contain two or more subunits (Ho & Whitaker, 1993) and α-AIs in wheat, barley and rye are tetrameric in nature (García et al., 1996; Sanchez-Monge et al., 1996). Several α-AI proteins that have been isolated from wheat kernels strongly inhibit alpha-amylases from insects with null or weak effect on mammalian amylases (Feng et al., 1996). These properties make them attractive candidates for plant genetic manipulations for imparting resistance to insect pests. Incorporation of increasing amounts of α-AI into the diet of the pea weevil (Bruchus pisorum) was directly correlated with delayed larval development time (Ishimoto & Kitamura et al., 1992). The resistance of common bean (Phaseolus vulgaris) seeds to bruchids like cowpea weevil and adzuki bean weevil has largely been attributed to the presence of α-AIs (Huesing et al., 1991). The level of α-AIs in their seeds has been found sufficient to inhibit the development of the larvae of those bruchid species that normally do not feed on common bean.

Though the cereal and leguminous α-amylase inhibitors share a similar mode of action, studies on their structure and evolutionary relationships have clearly shown that they belong to entirely different classes of proteins that might have evolved independently in monocotyledon and dicotyledon in a convergent manner. The cereal trypsin/ α-AI proteins are members of a super family of seed proteins with limited sequence homology. Homologous relationships of the cereal α-AIs were established with the 2S storage proteins from castor bean and the Kazal secretary trypsin inhibitor from bovine pancreas (Odani et al., 1983), thus showing that this protein super family is distributed beyond the plant kingdom. The CM proteins from wheat, barley and rye were eventually found to be members of the trypsin/α-AI family (Shewry et al., 1984) and the suBrilisin/endogenous
α-AIs from cereals were found to be homologous to the soybean trypsin inhibitor (Maeda, 1986).

On the other hand, the common bean α-amylase inhibitor belongs to a family of vacuolar glycoproteins that comprises phytohemagglutinins (PHA), arcelins and α-AIs (Moreno & Chrispeels, 1989; Chrispeels & Raikhel, 1991). PHA is the true lectin whereas arcelins may retain a weak carbohydrate binding activity and α-AIs is completely devoid of such activity. Their genes are tightly linked, behave as a single Mendelian locus, and have likely arisen by duplication and divergence of an ancestral gene (Nodari et al., 1993). Three dimensional models of α-AIs, arcelins and lectins have shown that arcelins and α-AIs are truncated lectins (Rouge et al., 1993). In the course of evolution, the loss of one or two loops located in the region of monosaccharide binding site in lectin led to the development of arcelins and α-AIs, respectively. This might be the reason why arcelins exhibit a weak agglutinating activity, while α-AIs cannot agglutinate erythrocytes. Further, an α-Al-like protein, YBP 22, isolated from the tuberous roots of the Mexican yam bean [Pachyrhizus erosus (Linn.) Urban] showed sequence homology with several known protease inhibitors, and a polyclonal antibody raised against the protein cross-reacted with soybean trypsin inhibitor (Gomes et al., 1997). The cDNA clones of lectin related seed proteins isolated from Lima bean showed 93.7% nucleotide identity and encoded an arcelin-like and an α-Al-like protein respectively (Sparvoli et al., 1998).

(ii) Protease Inhibitors (PIS)

Read and Haas (1938) reported the presence particularly in legume seeds of proteases and their biochemistry as protein molecules was confirmed by Kunitz who isolated and crystallized the trypsin inhibitor from soybean (Kunitz, 1945). Protease inhibitors is the largest class of proteins that have undergone extensive investigations and consequently their structure, properties, function and metabolism have been well documented. They are almost ubiquitous in plants and those present in the Leguminosae, Gramineae and Solanaceae have been studied and characterized in greater detail (Richardson, 1977; Connors et al., 2002). Although, some of them may play a role in endogenous protein metabolism, most of the protease inhibitors that have been characterized from plants do not inhibit endogenous plant proteases, but have specificities for animal or microbial enzymes (Las-kowski & Kato, 1980; Laskowski et al., 1988). In vitro feeding trials using artificial diets containing the inhibitors have confirmed the protective role for protease inhibitors against several crop pests. The effects of Pis on susceptible insects are generally seen as an increase in mortality, decrease in growth rate and prolongation of developmental period of the larvae. These detrimental effects are accomplished by blocking insect midgut proteinases thus impairing protein digestion, which inhibits or at least delays (in the case of weak inhibitors) the release of peptides and amino acids from dietary protein. The presence of inhibitor thus leads to the loss of nutrients particularly sulphur containing amino acids, and thereby weak and stunted growth and ultimate death (Gatehouse et al., 1992).

A systematic classification of Pis is not viable due to their diversity in terms of source, structure, specificities and size. However, these are mainly grouped in the four specificity groups, viz. serine, cysteine, metallo and aspartic protease inhibitors (inhibiting serine, cysteine, metallo and aspartyl proteases, respectively) (Garcia-Olmedo et al., 1987). Among them, the potato inhibitor I and II families, the Bowman-Birk inhibitor (BBI) family, and the soybean trypsin inhibitor (Kunitz) family are very important due to their inhibitory potential on gut enzymes of major crop pests.

(iii) Potato Inhibitor 1 and 11 Families

Among the Pis, the wound-inducible inhibitors from potato and tomato represent a unique group with insecticidal properties due to several interesting features of these proteins and their encoding genes. They comprise a non-homologous gene family in which members have been identified mainly from the solanaceous plants. Among them, potato inhibitor I and II and tomato protease inhibitor I and II have been well characterized (Plunkett et al., 1982). The unique and most striking feature of their encoding genes is the presence of introns, two each in inhibitor I genes and one in the gene encoding potato inhibitor II. In fact, they are the only protease inhibitor genes reported so far to contain introns. In potato alone, a mixture of ten or more isoinhibitors of protease inhibitor I and at least three forms of inhibitor II have been identified. In addition, homologues of the inhibitor have been found in some non solanaceous plants like alfalfa, broad bean, clover, cowpea, cucumber, French bean, grape, squash, strawberry, barley and buckwheat (Lorene et al., 2001; Ye et al., 2001). In leaves of to-
tomato and potato, they are expressed constitutively at low levels during plant growth and development. In response to wounding by insects or other mechanical damage, their concentration increases dramatically even in the unwounded leaves of the same plant, and within a few hours of injury, their levels often exceed 10% of total soluble proteins. In potato tubers, they accumulate throughout the course of tuber development, and represent a substantial fraction of the soluble protein. Thus, unlike other plant protease inhibitor genes, these genes are regulated environmentally as well as developmentally, and their expression is believed to be under a complex control involving several cis and transacting factors making them excellent models for study of plant gene regulation (Keil et al., 1986; Kouzuma et al., 2001).

All these proteins belong to the serine protease (endopeptidases) inhibitor group and are relatively small in size. Potato inhibitor I is a pentameric protein of Mr 41000 and is made up of monomers of Mr 8100 with single reactive site to inhibit chymotrypsin where as the corresponding inhibitor species in tomato, which is also a pentamer, with subunits of Mr 7800 each. Their cysteine content is low and they either have one disulfide bridge or none and the presence of cysteine seems to be not essential for their activity. In other inhibitor families, disulfide bridges stabilize the three dimensional structure and are believed to be essential for their activity. In the inhibitor II family, both in tomato and potato, the mature protein is a dimer of about Mr 25000 made up of two promoters of identical size, with five disulfide bonds and two reactive sites per monomer, which inhibit chymotrypsin and trypsin respectively. Wound induction of these inhibitor proteins is triggered by a small (18-mer) peptide hormone, systemin or Protease Inducing Factor (PIIF), released into the vascular system in response to wounding which in turn is transported rapidly to other tissues of the plant where it initiates the transcription of the PI genes. In addition, various factors including fungal elicitors, bacterial infection, sucrose, abscisic acid, and jasmonic acid have direct or indirect role in the increased synthesis of these inhibitors in potato. Nuclear runoff assays have shown that the accumulation of inhibitors I and II in tomato is transcriptionally regulated (Graham et al., 1986). Once synthesized, the inhibitor I and II proteins are sequestered into the central vacuole, which ensures their way long half lives. Analysis of the genomic clone encoding the potato protease inhibitor II has revealed the presence of sequences characteristic of typical plant signal peptides located immediately downstream of the only intron present in the gene (Keil et al., 1986). In fact, signal peptides of molecular weight around 2000-3000 daltons have been detected in newly translated inhibitor proteins. Moreover, induction of the inhibitor synthesis was possible in transgenic tomatoes carrying the pro-systemin gene.

(iv) Bowman-Birk Inhibitor (BBI) Family

The trypsin subclass of serine protease inhibitors from legume seeds exhibit insecticidal effects against several crop pests belonging to the orders of Lepidoptera, Coleoptera and Orthoptera (Shukle & Murdock, 1983). Many of these inhibitors are products of multigene families with varying specificities towards different proteases (Ryan, 1990). These inhibitors are cysteine-rich with a molecular mass of 8-20 kDa (Chen et al., 1997).

The Bowman-Birk inhibitor (BBI) and its related family of isoinhibitors comprise a closely related group of serine Pis. The protein was first identified and isolated from soybean seeds by Bowman (1946) and further characterized by Birk and associates (Birk et al., 1963), hence the name Bowman-Birk inhibitor (BBI). Later on, this class of inhibitors was isolated from a large number of legumes and several other plants including monocot. They contain higher amounts of sulphur amino acids and have a molecular mass less than 10,000 kDa. These proteins are classified as double-headed serine protease inhibitors due to the presence of two reactive site domains within the same polypeptide, one each for trypsin (Lys-Ser) and chymotrypsin (Leu-Ser) molecules. These are products of small gene analogues without introns (Hilder et al., 1987; Boulter et al., 1989). The BBIs from seeds are double headed proteins of 8kDa, whereas the 8kDa inhibitors from monocotyledonous seeds are single headed. However, 16 kDa double-headed inhibitors have also been reported to be present in them. Comparison of the primary structure of the 8 kDa the BBI domains have revealed that a crucial disulfide which connects the amino and carboxy termini of the active site loop, while present in the double-headed inhibitors from dicotyledons is lost monocotyledons leading to the loss of a reactive site and making them single-headed. Thus, the gene duplication event leading to a 16-kDa double-headed inhibitor in monocotyledons might have occurred, probably after the divergence of
monocotyledons and dicotyledons and also after the loss of a second reactive site in monocotyledons.

The cowpea trypsin inhibitor constitutes a somewhat larger gene family of four major iso-inhibitors, although the exact number of active genes is not known. Three of the iso-inhibitors are specific for trypsin at each active site and fourth is a trypsin chymotrypsin bifunctional inhibitor. Feeding trials on artificial diets have shown that the CpTI had inhibitory effects against a wide range of insects including Lepidoptera like Heliothis spp. (tobacco budworm and corn silkworm), Spodoptera (armyworms), Manduca sexta, Coleoptera (beetles) including Callosobruchus maculatus, Diabrotica sp. (corn rootworms), Anthonomous grandis (cotton boll weevil) and a range of other insects. This broad range is typical of many plant based protection mechanism (Park et al, 2001).

The trypsin inhibitor extracted from cowpea was a more effective anti-metabolite than those extracted from a number of other legumes (Boulter, 1993). Comparison of the content of anti-metabolites in cowpea varieties has revealed that the seeds of the wild cowpea (Vigna vexillata) contained higher amounts of trypsin inhibitor and chymotrypsin inhibitor and less of lectins compared to the cultivated accessions (Marconi et al, 1993). The high resistance to bruchid and the high TI and CTI contents of V. vexillata suggested that though there may not be any direct correlation between bruchid resistance and TI content, the protease inhibitors promote or at least strongly influence the plant’s defence system.

The cowpea protease inhibitor protein is comprised of readily indentifiable core region covering the invariant cysteine residues and active serine centres that are bound to highly variable amino and carboxy terminal regions. The specificity and binding properties of CpTI have been documented as attributes related to the first and second domains comprising the core region of the inhibitor protein. It is likely that isoforms with striking variations in inhibition and binding properties may be products of crucial mutations in regions adjoining the binding sites in one or both the domains (Laskowski et al, 1988). An added advantage from the biosafety point of view is that the cowpea trypsin inhibitor is very sensitive to pepsin digestion, which is unlikely with many trypsin inhibitors. Since the ingested food is initially exposed to pepsin in the mammalian digestive system, the CpTI may be completely digested by pepsin rendering it safe for consumption.

The PI genomic clone (pLK8) isolated recently in our laboratory is from a genomic DNA library of cowpea, cv 130 possessed an ORF which on translation gave a protein with 167 amino acids (Lawrence et al, 2001). The molecular weight of this protein was calculated to be 18.5 kDa; in which 18 amino acids, strongly basic, 20 strongly acidic, 40 hydrophobic and 62 polar, with an isoelectric point of 6.5. The protein had a N terminal signal sequence of 69 amino acids which is important in its translocation as has been reported in many proteinase inhibitors such as soybean (Hammond et al, 1984), cowpea, (Hilder et al, 1989) pea, (Domoney et al, 1995), maize (Rohrmeir et al, 1993), and alfalfa (McGurl et al, 1995). The cowpea protease inhibitor had the signal peptide cleavage site between 69-70 amino acid residues which is comparable to the alfalfa trypsin inhibitor (ATI) having a signal peptide of 44 or 55 amino acid residues which may target the ATI to the ER and then eventually deposit it in the vacuole of the cell (McGurl et al, 1995).

(v) Lectins

Lectins are defined as proteins possessing at least one non-catalytic domain that reversibly binds to specific carbohydrate(s). They are a heterogeneous group of proteins differing from each other with respect to their molecular structure, carbohydrate-binding specificity and biological activities. In most plant species, they are found, at least in low amounts, in storage tissues of seeds, stems or bulbs (Esteban et al, 2002). There are four major sub-groups of plant lectins: the legume lectins (arachis, soybean, lentil, kidney bean, pea, and faba bean), the monocotyledons mannose binding lectins (onion, leek, taro & garlic), the chitin binding lectins (barley, rice, wheat, rye, tomato & potato) and the type 2 ribosome inactivating proteins (castor bean & elderberry). However, heterologous lectins outside these groups have been reported from banana, jackfruit and pumpkin. Among them, those from leguminous plants form a large family of homologous proteins with strong similarity at the level of their amino acid sequences and tertiary structures, but with highly variable carbohydrate specificities and quaternary structures.

Over two decades ago, it was recognized that lectins possessed insecticidal properties. Lectins exert their inhibitory effect by binding to glycoproteins embedded within the peritrophic matrix lining the insect midgut and in turn disrupting digestive processes and
nutrient assimilation. Ingestion of lectin results in both binding of the lectin to membrane proteins in the insect gut epithelium, and transport to the haemolymph, and may result in systemic effects on insect development. Wheat germ agglutinin was the most effective among 17 plant lectins screened for their inhibitory effect on larval development of \textit{C. maculatus} (Murdoch \textit{et al}, 1990). While, the E-type lectins (erythrocyte-binding type) from common bean retarded the larval growth of \textit{C. maculatus} (Gatehouse \textit{et al}, 1984), the lectin from kidney bean did not thoroughly inhibit the larval growth of the pest (Ishimoto \textit{et al}, 1996).

The mannose-specific lectin (GNA) derived from the snowdrop, \textit{Galanthus nivalis}, has been found to be toxic to major sap-feeding insect pests of agronomically important crops like rice (against \textit{Nilaparvata lugens} & \textit{Nephotettix virescens}), and pea (against \textit{Callosobruchus maculatus}), and has been expressed in at least nine food crops including oil seed rape, potato, rice, and tomato (Sauvion \textit{et al}, 1996; Gatehouse \textit{et al}, 1996; Zhang \textit{et al}, 2000). The range of toxicity of GNA has recently been extended to the Lepidopteran, \textit{Lacanobia oleracea} (tomato moth), where it was observed that the mean larval biomass was reduced by approximately 50\% when reared upon transgenic potato (Fitches \textit{et al}, 1997). In transgenic tobacco expressing the pea lectin (P-lec) gene, the leaf damage caused by the larvae of \textit{Heliothis virescens} was greatly reduced (Boulter, 1990; Ye \\& Ng, 2001). The snowdrop lectin gene has also been successfully expressed in potato to give protection against aphids (Hilder \textit{et al}, 1995), lepidopteron pests, and in rice against the rice brown plant hopper (\textit{Nilaparvata lugens}).

### Potential of Insecticidal Proteins for Developing Transgenics Resistant to Insect Pests

In general, transgenic plants developed using protein inhibitors of insect digestive enzymes with a view to control crop pests are designed not to kill the insects that feed, but to retard their development. And presumably, this is the fundamental difference between this strategy and the chemical pest control or use of \textit{Bt} toxins that are aimed at complete control through pest mortality. Thus, perceived effects of the inhibitors on a pest population are usually much less dramatic than in the case with synthetic chemical pesticides. Complete control of insects cannot be expected in any realistic trial, tending rather to increase mortality to a limited extent but to retard insect growth and development significantly. However, in an integrated pest management programme, crop protection is accomplished through the concerted effects of several complementing control measures. Moreover, the inhibitory effect of PIs could improve the efficiency of defence proteins like \textit{Bt} toxins or the plants' own defence proteins by preventing their degradation by the target pest proteases (Jongsma \\& Bolter, 1997). Therefore, even in situations where transgene expression does not keep the pest population below the threshold for intervention, it should allow a much wider window within which intervention can be successfully employed.

The first ever transgenic plants were produced by Hilder \textit{et al} (1987) using cowpea trypsin inhibitor cDNA clone. The transgenic plants were resistant against herbivorous insects such as \textit{collosobruchus maculatus}, \textit{Heliothis spodoptera} and \textit{Diabrotica sp} and \textit{Triobolium sp}. Johnson \textit{et al} (1989) transformed tobacco plants with gene coding tomato and potato inhibitor proteins and the transgenic plants found resistant to \textit{M. sexta}.

Sane \textit{et al} (1997) amplified the cowpea genomic DNA by polymerase chain reaction and cloned the fragment in a plant expression vector coupled with CaMV 35S promoter and NOS terminator and used for tobacco transformation. The transgenic tobacco plants were tested against \textit{Spodoptera litura} and were found resistant. Recently, a protease inhibitor gene isolated from a native variety of cowpea in laboratory was used to transform pigeon pea through Agrobacterium \textit{tumefaciens}-mediated genetic transformation (Lawrence \textit{et al}, 2001). The gene was driven by CaMV 35S promoter containing kanamycin resistance as plant selection marker. Molecular analysis of the \textit{in vitro} cultured transgenic pigeon pea plants confirmed the integration and stable expression of the protease inhibitor gene (Lawrence \\& Koundal, 2001). The first generation of these transgenic pigeon pea plants is being maintained in glass houses for insect feeding experiments and preliminary insect bioassays showed encouraging results against pod borer (\textit{Helicoverpa armigera}). Duan \textit{et al} (1996) produced transgenic plant of rice by using wound inducible expression of potato protease inhibitor II. Bioassay of fifth generation transgenic rice plants showed increased resistance to major rice pest, pink stem borer. Similarly, lectin genes have isolated and transformed into crop plants. Transgenic plants expressing snowdrop lectin...
(Gatehouse et al, 1997), pea lectin (Boulter et al, 1990) are found resistant to sap sucking insects. Recently, we have isolated and characterized a lectin cDNA clone from cowpea (Datta et al, 2000). The gene was introduced into Brassica using an expression cassette. Comprising the CaMV S35 promoter, GUS and NOS terminator. Other molecular analysis of transgenics and in vitro feeding trails for testing their efficacy against pea aphids showed significant inhibitory effort on the growth and development (unpublished). However, efforts are being made to isolate and characterize lectin from other legumes for identifying the novel genes that affect the insect pests but have no effect on non-target organisms but present no risks in human health.

When α-AI was expressed at high levels in transgenic peas, weevil larval development was completely blocked and the effects of weevil damage on overall seed yield was significantly reduced (Schroeder et al, 1995). Larval development of several species of Callosobruchus beetles, a pest of adzuki bean, was completely inhibited when fed transgenic pea seeds expressing the alpha-amylase inhibitor from common bean. At the same time, Zabrotes subfasciatus, a natural bruchid pest of common bean, could freely feed on the transgenic plant (Ishimoto et al, 1996). Recently, an international collaboration between Maarten Chrispeels, University of California and T J Higgins, CSIRO, Australia conducted the first field trials with transgenic pea plants carrying α-AI genes from Phaseolus vulgaris. The genes encoding two α-AI isoforms, alpha AI-1 isolated from the cultivated common bean (P. vulgaris) and alpha AI-2 gene from a wild accession, were introduced into pea (Pisum sativum) cultivars by using an Agrobacterium system (Morton et al, 2000). These transgenic plants expressed high levels of α-AI in their seeds. Significant mortality of the first and second instar grubs was found with the α-AI-1 lines resulting in less weevil emergence than the control plants. Emergence was delayed in the α-AI-2 lines but further analysis showed that the αAI-2 had no effect on overall mortality. Initial safety studies on mammals showed that 300 or 700 g of peas Kg⁻¹ of feed had no impact on the nutritional value of transgenic peas fed to rats (Puzstai et al, 1999) have also demonstrated that the α-AIs cause minimum mammalian toxicity in comparison with the other plant inhibitor proteins. The toxic effects of alpha-amylase inhibitors vary with the species of insect amylase. The variation in sensitivity towards the inhibitor may be attributed to either the presence of inhibitor-insensitive amylases or to the secretion of proteases that can degrade the inhibitor in the larval midgut (Bown et al, 1997; Gatehouse et al, 1997). The availability of such genes from different plant species make it easy to use one or more genes in combination to develop transgenics for durable resistance against insect pests. The transgenic technology may not replace the use of chemical pesticides in future but effectively complement it.

Constraints of Transgenic Crops

There are several constrains and apprehensions regarding genetically modified GM food crops. These include: toxicity, allergenicity, carcinogenicity, use of antibiotic resistant genes and nutritional value. Decision regarding safety should be based on the nature of the product rather than other method by which it has modified. It is more important to bear in mind that many of the crop plants used today, contain natural toxins and allergens health hazards from food and how to reduce them is an issue. Every effort should be made to avoid the introduction of known allergens into food crops and foods have to be clearly labelled worldover. The concerns have been raised that the widespread use of antibiotics resistance and genes as selective markers in plant could increase the antibiotic resistance in human pathogens. Plant breeders should continue to remove all such markers from GM plants and utilize alternative markers for the selection of new varieties.

Most of the environmental concerns about GM technology in plants have derived from the possibilities of gene flow to close relatives of transgenic plants creating super weeds or causing gene pollution among other crops. There is a need for thorough risk assessment of likely causes of concern, at an early stage in the development of transgenic plants varieties and evaluate these risks in subsequent field tests and releases. Internal agricultural community of every nation should support serious efforts to establish a system to adequately assess and also avoid human health and environment risks. India is among a few countries where regulation and guidelines on research and monitoring of transgenic plants and their food safety assessment have been are developed by the Department of Biotechnology, Government of India. However, certain amount of risk is inherent in every new technology and a careful risk benefit analysis is necessary for making balance decision.
In spite of all the ideological dispute and ensuing democratic disagreements on the acceptability of genetically modified plants, genetic engineering for pest control has now been realized as an environmentally benign, flexible technology that can be safely fitted in any integrated pest management protocol. Furthermore, it allows the possibility of developing entirely new biological insecticides that retain the advantages of classical biological control agents, but have fewer of their drawbacks. In reality, insect resistant transgenic plants will certainly prove to be more economic than any conventional control strategy if the long-term benefits of transgenic crops especially factors such as environmental damage and health risks are considered.

Future Strategies

The use of insect resistant transgenic plants is a viable means of producing crops with significantly enhanced level of resistance. Several transgenic plants expressing plant-borne inhibitor proteins have been developed in the last decade. Various approaches that are being proposed and tried by different research groups include:

(i) Gene Combinations/Packaging/Pyramiding

The protective efficacy, spectrum of activity and the durability of resistance offered by the introduced genes can be greatly enhanced through careful design of packages of different genes that contain components which would act on quite different target insects. Protease inhibitors may have a major role in such gene pyramiding approaches. Apart from their inherent insecticidal property, they would protect other introduced gene products from premature digestion in the insect gut and improve the overall performance through their mutually complementing or synergistic effects. The first demonstration of this approach has been the introduction of both cowpea trypsin inhibitor and pea lectin in transgenic tobacco plants where the two gene products had an additive effect on tobacco budworm caterpillars (Boulter et al., 1990). It may be a useful approach to combine genes that encode proteinase inhibitors among themselves or along with suitable lectin, α-AI and/or Bt genes so that multiple pest resistance may be achieved in a single event in agronomically important crop plants. Cross breeding of primary transformants carrying the desirable gene combinations would also prove useful in terms of enhanced insect resistance (Schuler et al., 1998).

(ii) Protein Engineering

In-depth exploration of protein structure and function may allow researchers to use protein engineering as a strong tool for designing novel chimeric proteins for insect control. These chimeras are constructed by tailoring together the sequences that encode discrete domains of the protein intended to act on defined targets. In vitro mutagenesis can be exploited for creating very effective chimeric genes carrying desirable domains with defined activity spectrum. The long-term goal of protein engineering would be the construction of modular protein that will target specific pests without any harmful effects on the beneficial organisms. In principle, any domain from any protein can be used in this modular system to construct proteins with a given set of attributes. Although still in its infancy, protein engineering will allow us to design proteins for use against the most insect pests.

(iii) Single-chain Antibodies

This approach makes use of engineering antibodies or antibody fragments specific to the target pest’s essential protein and expressing it in the crop plant so that both specificity and efficacy of action can be incorporated in a single event (Hilder & Boulter, 1999). Besides, it has an additional advantage of avoiding action on the non-target organisms, particularly predators.

(iv) Phage Display

The technique combines in vitro mutagenesis, rapidity of molecular cloning, specificity of protein-protein interactions, and precision of molecular screening techniques. After isolation and cloning of an ideal inhibitor gene, a large collection of its variants are prepared in the form of a library by altering its sequence at every possible positions in the regions critical for its action. In fact, the gene can be modified for all the coding frames with every codon for each of the 20 possible amino acids so that the resultant changes in specificity, binding and other attributes in each of the modified product (protein) can be examined. The cloned genes are then expressed on the surface of phage particles and the displayed proteins are screened for the variant inhibitor protein, which exhibits the maximum affinity (binding) for the target protease enzyme. Thus, such technique envisages the screening of millions of cloned proteins with the desired one being physically separated from others based upon its affinity to the target larval enzyme.
(v) Directed Protein Evolution

This technique, a variant to phage display may turn to be very useful in screening of large population of inhibitor variants for their specificity with greater accuracy. Here, protein and peptide libraries are used to select interactive proteins (ligands) without prior information concerning their sequence or structure. It can be used to construct a phage display library of mutant proteins or peptides as fusion proteins on the surface of the phage. The library can then be used for screening any enzyme for its binding specificity so that the inhibitor with maximum affinity towards the enzyme can be easily located. The possible outcome of the aforesaid developments would be the availability of the technology to overtake the insect’s molecular potential for resistance development through more programmed, eco-friendly and efficient plant genetic engineering strategies.

Conclusion

The continuous use of pesticides for crop protection had resulted in damaging impact on biological ecosystems, bio-magnification effects through pesticide residue deposition in food and feed pollution. The use of target specific compounds with low persistence and the exploitation of intrinsic plant resistance mechanisms are suggested as safer alternative strategies for effective insect pests management. Thus, insect resistant GM plants will curtail the use of those hazardous pesticides by engineering genes that encode natural biodegradable proteins with no harmful effect to animals and human beings. It is important to remember that biotechnology tools complement and extend the traditional methods used to enhance agricultural productivity and to develop new production systems. The progress of agriculture in developing, non-industrialized countries where the economic activity still provides 60-80% employment and 50% of national income will be possible. The application of plant biotechnology will be no doubt, more meaningful in the poor and non-industrialized parts of the world where socio-economic development will be possible only through sensible adoption of advanced agricultural practices that help for enhanced food, feed and fibre production and an overall improvement in living standard. Despite, the opposition from the anti-GM movements all over the world, undoubtedly, will find its more and immediate application in insect pest control of major crops. The availability of diverse insecticidal genes from different plant species makes it a possibility to use one or more genes in combination whose products are targeted at different biochemical and physiological processes. The transgenic crops developed for insect resistance should be compatible with other components of integrated pest management programmes for pest resistance to be durable and effective. It is envisaged that it will have a major impact on agricultural systems both in the developed and developing countries in the near future.

References


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