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Putatively Expressed Proteins in GM Crops for Insect Pest Resistance

Review Article

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Abstract

The growing demands to produce more food and fibre to feed increasing population and also for reducing chemical inputs in agriculture to overcome adverse affects impetus to the development of alternative forms of insect-pest control strategies. Biological entomotoxic molecules provide an attractive alternative candidate to the use of synthetic pesticides. These molecules are naturally occurring in the organisms and proved to be less toxic to non target pests.

Key words: Biological molecules; Transgenic plants; Insect pests.

Introduction

Challenges facing global agriculture

Agriculture is an essential component of societal well-being and it occupies 40% of the land surface, consumes 70% of global water resources and manages biodiversity at genetic, species and ecosystem levels. According to data provided by World Bank, the world population is currently about 7 billion on October 31, 2011, increase in the population holds serious implications on global food security, because of these the available necessary biotic and abiotic (nonliving) production factors are shrinking. Agriculture in the 21st century faces multiple challenges; it has to produce more food and fibre to feed a growing population. It is estimated that the planet's demand for food and feed crops will almost double by 2050 [1], this can be achieved largely through higher yields per unit of land and novel practices of crop intensification [2]. With an increasing global population and overall purchasing power, the global per capita food required was 2800 kcal/day [3]. The impact of climate change is likely to reduce agriculture production, thus reducing food availability [4] and also most studies concluded that climate changes also influences the eruptive dynamics of pest insects [5]. Moreover, pest population generally become more abundant as temperature increases, through a number of inter-related processes, including increased rates of population development, growth, migration and overwintering [6]. However agriculture production is greatly affected by biotic stress by variety of pests including insects, nematodes, virus, bacterial and fungal induced diseases and weeds, which resulting in losses as high as 41%. Among these, insect pest menace is the major factor that destabilizes crop productivity in agricultural ecosystems by more than 10,000 species worldwide [7,8]. Amongst different orders of agriculture insect pests; Dipterans, Lepidopetarn and Homeopteran are major insect pests which cause severe damage to economically important agricultural crops. Some of the important agriculture pests in India are presented in Table 1.

Crop loss due to these harmful insect pests can be substantial prevented or reduced by crop protection measures by using pesticides. Pesticide is any substance or mixture of substances used to destroy, suppress or alter the life cycle of insect pest. A pesticide can be a synthetically produced (Chemical control agents) or substance naturally derived (Biological control agents).

Synthetic pesticides are not a modern invention, and it's proved

Table 1: Important agriculture crops damaged by chewing and sucking insect pests

Crop	Insect pests	Insect Order	Actual production of crop (million tons)	Approximate crop damage by insects (million tons)
Rice	*Nilaparvata lugens* *Scirpophaga incertulas*	Hemiptera Lepidoptera	96.7	32.2
Cotton	*Helicoverpa armigera* *Spodoptera litura* *Aphis gossypii*	Lepidoptera Lepidoptera Hemiptera	44.03	18.9
Sugar cane	*Scirpophaga novella* *Chilo infuscatellus*	Lepidoptera	348.2	87.1
Wheat	*Sitobion avenae* *Rhopalosiphum padi* *Helicoverpa armigera*	Hemiptera Lepidoptera	78.6	4.1
Ground nut	*Stomopterix nertaria* *Spodoptera litura*	Lepidoptera Lepidoptera	9.2	1.6
Mustard	*Lipaphys erysimi*	Hemiptera	5.8	1.5

to be effective and most widely used in the world. Insect pest management by synthetic chemicals obviously has brought about considerable protection to crop yields over the past five decades. It is estimated that, 5.2 billion pounds of pesticides used worldwide yearly to control insect's pest [9]. Chemical pesticides have played a role in increasing agricultural output throughout the globe. Unfortunately, extensive and, very often, indiscriminate usage of chemical pesticides has resulted in environmental degradation, adverse effects on human health and other organisms, eradication of beneficial insects and development of pesticide-resistant insects. Alternatives to synthetic pesticides, use of biological pest controls strategies by employing molecular biology techniques and genetic engineering during the past few decades led to the introduction of novel strategies for insect control and pest management. These powerful techniques allow ectopic expression of single or multiple endogenous putative defence proteins that are toxic to crop pests [10,11]. The aim of the present study is to introduce and highlight insecticidal activity of some the important biological molecules from different sources.

Role of Transgenic plants in agriculture

A sub-branch of plant biotechnology, Transgenic technology, aims to transfer genes from one species to another either related or not. The process of introducing a gene into an organism via recombinant DNA technology is known as transformation and recovered plant species are called as transgenic plants or genetically modified (GM) plants. Genetically engineered crops offers user-friendly, environment-friendly and consumer-friendly method of crop development to meet the demands of sustainable agriculture in the 21st century. Transgenic crops offers the prospect of many advantages; not just widening the potential pool of useful genes but also permitting the introduction of a number of different desirable genes at a single event and of reducing the time needed to introgress introduced characters into an elite genetic background [12]. Plant biotechnology can potentially help to higher yields within shorter growing duration, avoiding chemical fertilizers and increased nutrients quality [13]. As discussed, GM technology enables the development of new crop varieties, which have beneficial characteristics for farming; this could be resistance to drought, pests or diseases.

Principle and ectopic expression of entomotoxic biological molecules

Bt protein (Bacillus thuringiensis)

Bacillus thuringiensis (Bt), a well-known gram-positive bacterium, produces δ-endotoxins, or insecticidal crystal proteins (Cry proteins) during the sporulation phase [14]. The crystals, upon ingestion by the insect larva, are get solubilized in the alkaline midgut into individual protoxins. These protoxins are acted upon by midgut proteases which cleave them into two halves, the N-terminal half is responsible for the production of the toxic Bt protein. This crystal protein was exploited in the plant transgenic research to overcome crop damage by insect pests. The Belgian company Plant Genetic Systems was the first company to develop a genetically engineered plant with insect tolerance by expressing a cry gene from Bt in tobacco in 1987. The tobacco plants engineered with cry1Aa genes and the cry1Ab toxins were found to be resistant to the larvae of Manduca sexta (tobacco hornworm) [15]. Subsequently, many crop plants which include cotton, rice, maize, peanut, soybean, canola, tomato, potato and cabbage were transformed with various modified cry genes [16]. The commercialization of Bt-crops started in 1996 with the introduction of bollworm-resistant cotton ('Bollgard') in USA. Introduction of Bt endotoxin genes into crops became immensely popular, and it is estimated that Bt crops are cultivated in 66 m hectares area worldwide [17]. However, these transgenic crops expressing Bt endotoxin have been effective in controlling chewing pests but are less effective in providing protection against sap-sucking pests [18]. Despite the great commercial successes of Bt crops, there are growing concerns about the ability of insect to develop resistance to the Bt endotoxins [17,19, 20]. In contrast to Bt crops, alternative sources of potential insecticidal gene products need to be explored. To date, several different classes of proteins including lectins, ribosome-inactivating proteins, protease inhibitors and α-amylase inhibitors and many more have been shown to be insecticidal effects towards a range of economically important insect pests by direct assay or by expression in transgenic plants [21,22].

Vegetative Bt protein

A novel class of proteins called vegetative insecticidal proteins (Vips) produced by Bt during its vegetative stages of growth have been identified [23]. Although B. thuringiensis δ-endotoxins are effective insecticidal proteins, there are several agronomically important insects that are less sensitive to their action. The 88 kDa vegetative insecticidal protein has a putative bacillar secretory signal at the N-terminal which is not processed during its secretion. Vegetative insecticidal proteins (Vip) are effective against lepidopteran insect larvae black cutworm (Agrotis ipsilon), fall armyworm (Spodoptera frugiperda), beet armyworm (Spodoptera exigua), tobacco budworm (Heliothis virescens), and corn earworm (Helicoverpa zea). However, Vip does not show any homology with the known crystalline insecticidal proteins. This structural dissimilarity is indicative of a possible divergent insecticidal mechanism than the other known Bt-

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toxins. These observed structural divergences of Vip with Bt toxins make them an ideal candidate for deployment in insect management programs together with the other category of Bt-toxins.

Proteinase inhibitors

The protease inhibitor (PIs) proteins are natural antagonist's proteins and they are quite common in all life forms [24]. In plants they play defensive mechanisms against phytophagous insects and microorganisms. Most PIs interact with their target proteases by contact with the active (catalytic) site of the protease, resulting in the formation of a stable protease-inhibitor complex that is incapable of enzymatic activity [25]. Proteinases inhibitors are classified according to their catalytic mechanisms into four classes (1) serine proteinases, with a serine and histidine; (2) cysteine proteinases, with a cysteine; (3) aspartic proteinases, with an aspartate group and (4) metalloproteinases, with a metallic ion (Zn^{+2}, Ca^{+2} or Mn^{+2}) [26]. In plants, proteinase inhibitors have different role, such as storage proteins, as regulators of endogenous proteolytic activity [27], as participants in many developmental processes, including programmed cell death and as components associated with the resistance of plants against insects and pathogens [28]. They may be synthesized constitutively during normal development or may be induced in response to insect and pathogen attacks [27].

Transgenic plants expressing PI genes began when Hilder et al. [29] transformed tobacco plants with the trypsin inhibitor gene (CpTI) of Cowpea which showed reduced growth and mortality in larvae of Heliothis virescens (bollworm). In the 1990s Gatehouse et al. [30] transformed tobacco plants with the trypsin inhibitor gene of soya (Kunitz family) (SBTI), which showed a high inhibitory effect on the larvae of H. virescens. Many PIs were expressed in transgenic plants which conferred the protection against Chrysodeixis eriosoma (green measuring worm), Sesamia inferens (pink stem borer), Spodoptera litura (tobacco budworm), Nilaparvata lugens (brown planthopper), Sitotroga cerealella (Angoumoid grain moth), Tribolium castaneum (brown flower beetle) [30-32]. Considering all the evidence which has appeared in the text, the effect that the PIs have on insects is evident; however, negative effects have also been shown on beneficial insects [33]. Later it was realized that the insects overcome enzyme inhibitory property (PIs) by altering their enzyme specificity by mutations, thus dampening the hopes of using enzyme inhibitors for transgenic crops [10].

α-Amylase inhibitors

α-Amylases (α-1, 4-glucan-4-glucanohydrolases) are widespread hydrolytic enzymes found in microorganisms, animals and plants. They catalyze the initial hydrolyses of α-1,4-linked sugar polymers, such as starch and glycogen into shorter oligosaccharides, an important step towards transforming sugar polymers into single units that can be assimilated by the organism. Higher plants and animals produce a large number of different protein inhibitors of α-amylases in order to regulate the activity of these enzymes [34]. However these α-AIs are used to generate transgenic plants that are resistant against insect pest [35]. The expression of the α-Al gene encoding protein in plant system, such as pea (Pisum sativum L.) and azuki bean (Vigna anguralis L.) showed promising effect against bruchid beetle pests (Coleoptera: Bruchidae) [36]. Rye α-amylase inhibitor expressed in transgenic tobacco seeds (Nicotiana tabacum) caused 74% mortality in Anthonomus grandis first instar larvae when transgenic seed flour mixture used in artificial diet [37]. However these inhibitors used as plant resistance factors are effective against Coleoptera order and they cannot be used to control different orders of agriculture insect pests [38-40].

Arcelins

Post harvest loss due to insect pests largely affects the overall food grain production and consumption and it is estimated to be 13%. Arcelins are antinutritional insecticidal seed storage proteins, found in the wild bean Phaseolus vulgaris, which have been shown to prevent infestation by post harvest insect pests such as bruchid beetles [41]. Amino acid sequence comparison shows that arcelins belong to the bean lectin-like family which includes the two types of phytohemagglutin subunits (PHA-L and PHA-E) and α-amylase inhibitors [42]. Although the members of this protein family display similar tertiary structures, they differ in their biochemical properties, glycosylation patterns, quaternary structure and sugar binding specificities [43]. Insecticidal properties of arcelins variants toward bruchid pests Z. subfasciatus has been reported [44], which is known to be one of the most important pests of stored beans.

Ribosome-inactivating proteins

Ribosome-inactivating proteins (RIPs) are a group of plant proteins that are capable of specifically and irreversibly inactivating eukaryotic ribosome's and inhibits protein translation, which plays an important role in plant defense and hence can be exploited in plant protection [45]. Over a hundred RIPs have been isolated from various plants and bacteria with varying degrees of toxicity. RIPs are subdivided on the basis of their molecular structure into three distinct groups. Type I RIPs are monomeric proteins of approximately 30 kDa which possess RNA N-glycosidase enzymatic activity. Type II is a heterodimer consisting of an A-chain with RNA N-glycosidase activity associated to one or several B-chain(s) of approximately 35 kD, functionally equivalent to the Type I polypeptide [46] linked to a B subunit. The B-subunit is a lectin-like peptide that has strong affinity for sugar moieties displayed on the surface of cells and helps to promote translocation through the plasma membrane [47]. While Type III is synthesized as inactive precursors (Pro RIPs) that require proteolytic processing events to form an active RIP [48]. Ricin, Abrin and Modeccin are well known examples of RIPs, which irreversibly inactivate ribosomes by removing a specific adenine from a highly conserved tetra-nucleotide loop present in the large ribosomal subunit [49]. Some of these RIPs, such as ricin (type-II RIPs) have high toxicity affect against a variety of insects, although these effects are variable on different insect orders [50]. More recently, Shahidi-Noghabi et al. [51] demonstrated that expression of Sambucus niger agglutinin (SNA-I, type-II RIPs) from elderberry bark in transgenic tobacco has a deleterious effect on two important insect pests, the tobacco aphid Myzus nicotianae and the beet armyworm Spodoptera exigua. Although the biochemical properties of the RIPs are well studied, but their exact mechanisms of action at the tissue level of RIPs-ingested insects are not well understood [51-53]. In view of these argued apprehensions on the sustainability of Bt crops, and other

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narrow range of biological pest control molecules, alternative sources of potential insecticidal gene products need to be explored which shows wide range of insect control. Lectins from different sources and classes are an attractive alternative candidate in transgenic-based pest control strategies [45].

Lectins

Lectins are a class of proteins of non-immune origin that possess at least one non-catalytic domain that specifically and reversibly bind to mono- or oligosaccharide [54]. A typical lectin is multivalent; and because of its specific carbohydrate binding property it is able to agglutinate cells. Lectins are extensively distributed in nature and several hundred of these molecules have been isolated so far from plants, microorganisms, fungi and vertebrates, including mammals [55]. Because of their unique ability to bind to certain specific membrane glycoproteins, some of lectin exerts proliferative, antiproliferative, immunomodulatory effects. Such properties exhibited by lectins made them useful tools in diverse areas like cancer diagnosis and therapeutics, virology, structural biology, separation technology, bacterial typing and insect toxicology [56,57].

Lectins with insecticidal property

Several biological functions of lectins have been reported [58] among them anti-insect activities have received particular attention in the pest management strategies [59,60]. Lectins basically bind to glycosylated proteins, as the glycosylation of protein is a key post translational event. In organisms there are two types of glycosylation pattern occur such as, N-linked and O-linked based on the linkage of carbohydrate moiety to the protein backbone. N-glycans are linked to Asn residues of protein backbone via N-acetyl glucosamine, whereas O-glycans linked to hydroxyl group of serine or threonine residues via N-acetyl galactosamine. N- glycans and O- glycans are profusely found in insects [61,62], because of lectins unique ability to bind to certain specific membrane glycoproteins in the insects made them valuable tool in the pest management science. There are substantial evidences that, lectins bind to gut surface glycans of gut epithelial cells or bind to secretory gut proteins causing physiological imbalance, resulting in immunomodulatory effect such as apoptosis [60,63,64]. However, the molecular mechanism of lectin induced toxicity in insects has not been understood in detail [60,64].

Many lectins are highly toxic for phytophagous insects; the use of lectins in transgenic plants has yielded positive results on insect's pest belonging to different orders such as Lepidoptera, Coleoptera, Diptera and Hemiptera. Among lectins, Plant originated N-glycan specific lectins received greater attention due to their toxic effects against broad range of economically important insect pests [62]. The first N-glycan specific lectin from plant origin, Galanthus nivalis agglutinin (GNA) has showed toxic effects against hemipterans and other economically important insect pests. GNA has been successfully engineered into a variety of crops including sugarcane, rice, wheat, potatoes or tobacco to give them a higher resistance against different order insect pest. Followed by GNA many plant lectins such as, Wheat germ agglutinin (WGA), Pisum Sativum Agglutinin (PSA), Phaseolus vulgaris Agglutinin (PHA) and Allium sativum lectin (ASAL) were successfully expressed in important agriculture crops and they have been shown to exert deleterious effects on a range of important pest insects [60,64]. Unlike the N- linked mannose binding lectins, very little is known about O-glycan specific lectin. Recently O-glycan (Gal/GalNAc) specific lectins are reported for their toxic effects on insect pests. Among plant lectin, Amaranthus caudatus is the only reported O-linked glycan specific lectin employed in transgenic plants shown to be effective against insects [65,66]. Compared to plant and animal lectins, very little information is available on lectins from fungal origin [67, 68]. In recent years, mushroom and other fungal lectins of different carbohydrate specificity have got much attention in agriculture field as a bio-insecticide. All these studies shows that lectins from different origins, potential to be exploited in crop protection against various insect pests. Thus the use of lectins from different origin have proved to be more efficient ways to control chewing and sap-sucking insect pests on agriculturally important crops.

Conclusion

From the literature available, it has been shown that, the use of transgenic insect resistant crops reduced chemical pesticides and its secondary effects on living organisms. Thus, the combination of biological proteins with different modes of action, as well as the correct application of IPM has the potential to improve resistance against insects over the long-term. Nevertheless, the potential direct and indirect effects of transgenic plants expressing these biological molecules on beneficial insects and higher animal's needs to be investigated comprehensively before it could be used for agricultural application.

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