Transgenic plants for insect resistance







Introduction

- Insect pests are the major scourge of agriculture down the ages.
- Agronomically important crops and their high-yielding genotypes are highly susceptible to insect pests.
- ➤ Previously chemically synthesised pesticides were used which are harmful to biological organisms and environment.
- The effective alternatives against harmful chemical pesticides are genetically engineered crops, resistant to insect pests that can be integrated in agricultural ecosystems.
- Insecticidal crystal proteins found in a soil bacterium, *Bacillus thuringiensis* (Bt) was introduced into the market on 1996.

Transgenic plants

- Insect-resistance transgenes can be introduced into plants to increase the level of insect resistance.
- The first transgenic insect resistant plant was developed in 1987 in tomato and tobacco plants.
- ➤ Bt genes were then transferred into variety of plants such as cotton, rice, maize with Lepidoptera as the main targets.
- In contrast to most plant genes, these bacterial genes are extremely rich in A+T nucleotides, thus extensive modifications, which include removal of AT-rich regions from the coding sequence and use of modified constitutive or tissue-specific promoters, are carried out.

Insecticidal proteins having potential application in insect pest management.

Insecticidal protein	Source	
Insecticidal crystal proteins	Bacillus thuringiensis	
Vegetative insecticidal proteins	Bacillus thuringiensis	
Protease inhibitors	Plants and animals	
α-amylase inhibitors	Plants	
Lectins	Plants	
Cholesterol oxidase	Streptomyces sps.,	
Chitinases	Plants and insects	
Tryptophan decarboxylase	Plants	
Isopentenyl transferase	Agrobacterium	
Insecticidal toxin complex	Photorhabdus sps.,	
Peroxidase and lipoxygenase	Plants	

Use of *Bacillus thuringiensis* δ -endotoxins

- ➤ B. thuringiensis is a gram positive bacterium that synthetizes insecticidal crystalline inclusions during sporulation.
- \triangleright This crystalline structure of inclusion is made up of protoxin subunits, called δ -endotoxins.
- The crystalline proteins (Cry proteins) produced by *B. thuringiensis* shows a narrow host range.
- ➤ Based on protein structural homologies and host range, the genes were first classified in different classes cryl, cryll, crylll.
- > Cryl toxins are active against Lepidoptera while Crylll are active against Coleoptera.
- The use of *B. thuringiensis* sprays is still relatively limited, due to their low field persistence.

Improving expression levels of Bt toxins

> Plastid Genome Transformation

- Introduction of unmodified Bt genes into the chloroplast genome results in high levels of toxin accumulation.
- ➤ Various Cry proteins have been expressed in plastids of tobacco and Cry1Ab has been expressed in soybean (Glycine max) plastids.
- ➤ Mutagenesis of Three-Domain Cry Toxins
- > Modification of Bt toxins by site-directed mutagenesis can increase the toxicity toward target pest.
- ➤ Plants expressing multiple toxins Gene stacking technology or Gene pyramiding.

Strategies to avoid Bt resistant insects

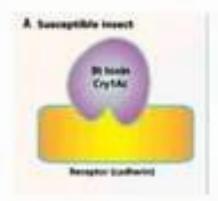
- Use of inducible promoters (that can be turned on only when there is an insect problem)
- Construction of hybrid Bt toxins
- Introducing more than one Bt gene ("stacking")
- Introduction of the Bt gene in combination with another insecticidal gene
- Spraying low levels of insecticide on Bt plants
- Use of spatial refuge strategies

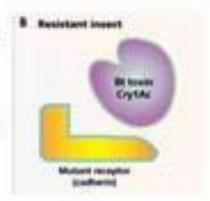
Activity of Cry1AMod toxins, which are able to form toxin oligomers in the absence of receptors, against different resistant populations, including those affected in the ABC transporter and the role of dominant negative mutants as antitoxins, supports the hypothesis that toxin oligomerization is a limiting step in the Cry insecticidal activity.

Knowledge of the action of 3d-Cry toxin and the resistance mechanisms to these toxins will set the basis for a rational design of novel toxins to overcome insect resistance, extending the useful lifespan of Cry toxins in insect control programs.

Mechanisms of Bt toxin resistance

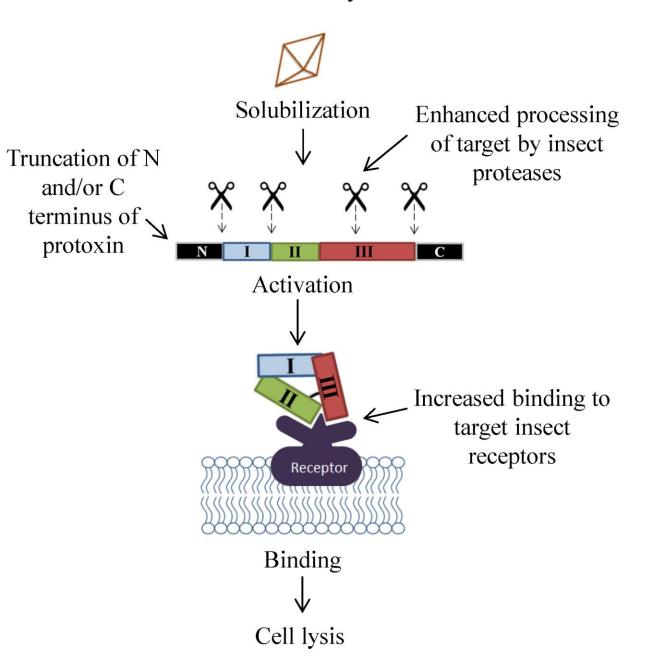
- Bt works by binding to toxin receptor (cadherin), which triggers cleavage of Bt protein
- Bt-resistant insects express mutated cadherin proteins that do not bind toxins.
 - Modified toxins can make resistant cadherinmutated insects susceptible again (Soberon et al, Science, 2007)
- Toxins with independent actions bind to different sites
- Multiple resistance: one toxin can bind to several sites (e.g., insect develops resistance to multiple Bt toxins after repeated exposure to one)





Potential sites for Cry modification

Insects targeted by modified Cry toxins





Cry2A:
Truncated N-terminus
increases efficacy against
Helicoverpa armigera [16]



Cry4Ba:
Changes in loop 3
of Domain II
broadens toxicity against
Culex quinquefasciatus [17]



mCry3A: chymotrypsin/cathepsin cleavage site in Domain I increases efficacy against *Diabrotica virgifera* virgifera [18]

Mechanisms of resistance to Cry toxin action

Alterations in Cry toxin activation

Plodia interpunctella Spodoptera littoralis Ostrinia nubilalis Sequestration of the toxin by glycolipid moieties or esterases

Helicoverpa armigera

Elevated immune response
Helicoverpa armigera
Ephestia kuehniella

Spodoptera exigua

ALP APN Cadherin

ABCC2 transporter

Alteration of Cry toxin receptors

ALP Heliothis virescens Helicoverpa armigera Spodoptera frugiperda

APN Spodoptera exigua

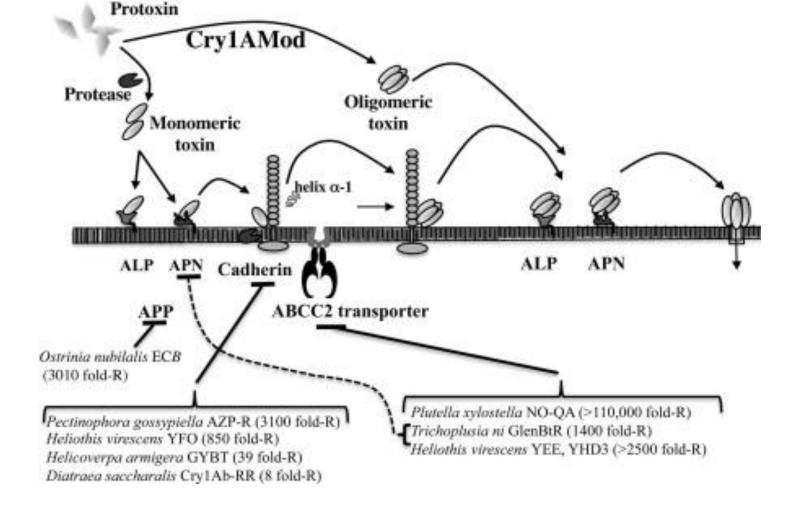
Spodoptera littoralis Helicoverpa armigera

Trichoplusia ni

CAD Heliothis virescens
Helicoverpa armigera
Diatraea saccharalis
Pectinophora gossypiella

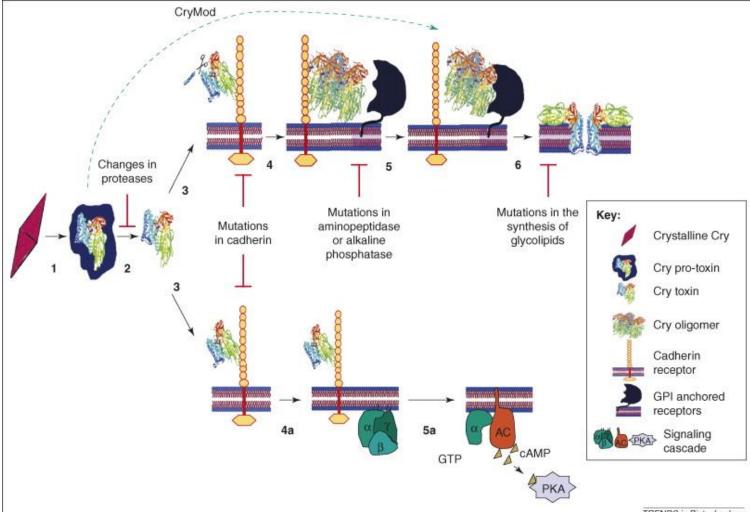
Alteration of ABCC2 transporter

Heliothis virescens Plutella xylostella Trichoplusia ni



Mechanism of action of Cry toxins and identification of resistant mechanism in different insect pests that became resistant to Cry1A toxins.

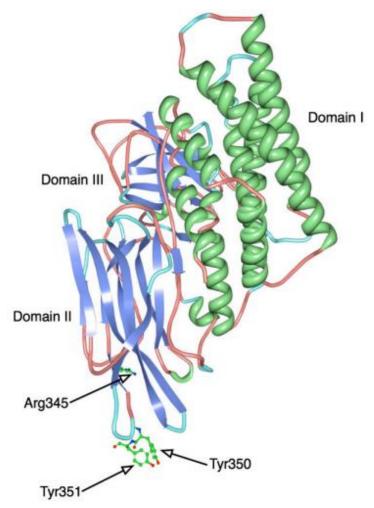
Cry1AMod toxins form oligomeric structures in absence of receptors, the Cry1AMod oligomeric structures are required to bind to APN or ALP in order to insert into the membrane and kill the cell. The Cry1AMod are only able to kill insects that were susceptible to Cry1A toxins, and were unable to kill other insects such as mosquitoes or coleopterans



(6). The signal transduction model (bottom) proposes that the interaction of the Cry toxin with a cadherin receptor triggers an intracellular cascade pathway that is mediated by activation of protein G (4a), which, in a subsequent step (5a), activates adenylyl cyclase. This signal then increases the levels of cyclic adenosine monophosphate, which activates protein kinase A and leads to cell death. See Refs [13,15–20,51–54] for the different mechanisms that have resulted in toxin resistance in several insects. The CryMod toxins, in which helix a-1 is deleted, avoid resistance by bypassing cadherin interaction

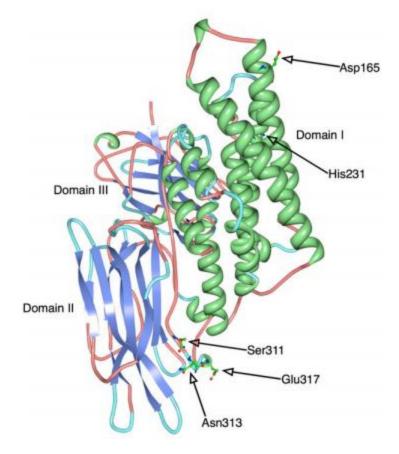
Models of the mode of action of Cry toxins and resulting mechanism for resistance. Two different mechanisms can be distinguished: the pore-formation model (top) and the signal transduction model (bottom), which both include similar initial steps for toxin solubilization in midgut lumen (1), activation by midgut proteases (2), and binding to primary receptor cadherin (3). In the poreformation model (top), step 3 induces the cleavage of helix a-1 and triggers toxin oligomerization (4). The toxin oligomer then binds to a secondary receptor, such as aminopeptidase or alkaline phosphatase, which are anchored by a glycosylphosphatidylinositol anchor in the membrane (5). Finally, the toxin inserts itself into the membrane, thereby forming a pore that kills the insect cells

Engineering specificity in a three-domain Cry toxin; mutagenesis of the toxin-receptor interaction loop in domain II. Three dimensional structure of Cry3A.



- Three dimensional structure of Cry3A (1dlc; RCSB) is shown in ribbon format.
- Domain I (helices) is at top right, and domain II (sheet structure) is at bottom left.
- Domain III (carbohydrate-binding domain; sheet structure) is behind the other domains, central in this view.
- ➤ Residues mutated (Wu et al., 2000) to increase toxicity toward yellow mealworm, Colorado potato beetle, and cottonwood leaf beetle are shown in ball-and stick representation.

Engineering specificity in a three-domain Cry toxin; mutagenesis to improve channel-forming ability. Three-dimensional structure of Cry3Bb.



- Residues mutated to increase toxicity toward corn rootworm are shown in ball-and-stick representation.
- Mutations are made in helices of domain I and in the region linking domains I and II.

Fusion proteins

- A single transnationally fused coding sequence encoding two Cry proteins has been used.
- Expression of the fusion protein in transgenic maize and rice plants gave resistance to larvae of stem borers and leaf armyworm.

Examples of insect-resistant transgenic plants that have been obtained through the transfer of *B. thuringiensis* toxin genes

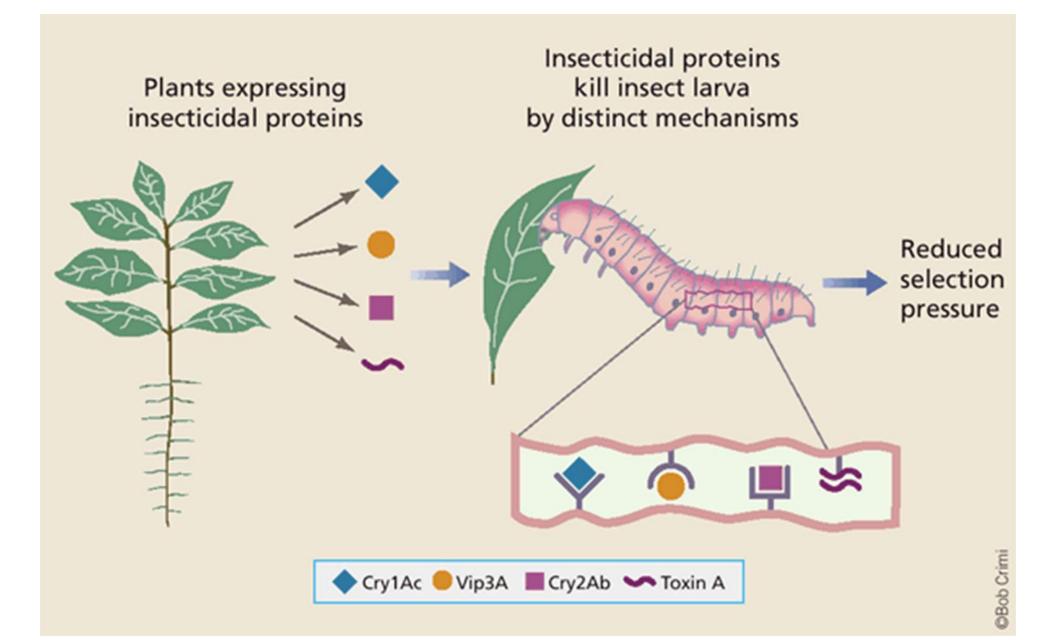
Plant	Gene	Type	Target insect	
Tobacco	cryIA(a)	WT	WT Manduca sexta (L)	
	cryIA(b)	WT	Manduca sexta (L)	
	cryIA(b)&(c)	PM	Manduca sexta (L)	
	cryIA(b)	WT	Manduca sexta (L)	
(Chloroplasts)	cryIA(c)	WT	H. virescens, H. zea, S. exigua (L)	
	cryIC	S	Spodoptera littoralis (L)	
Tomato	cryIA(b)	WT	Heliothis virescens (L)	
Cotton	cryIA(b)&(c)	S	H. zea, H. virescens (L)	
Potato	cryIA(b)	WT	Phthorimaea operculella (L)	
	cryIIIA	S	Leptinotarsa decemlineata (C)	
	cryIIIA	S	Leptinotarsa decemlineata (C)	
Alfalfa	cryIC	S	Spodoptera littoralis (L)	
Canola	cryIA(c)	S	S Plutella xylostella, H. zea (L)	
			Trichoplusia ni, S. exigua (L)	
Soybean	cryIA(c)	S	H. zea, H. virescens (L)	
			Pseudoplusia includens (L)	
Maize	cryIA(b)	S	Ostrinia nubilabis (L)	
Rice	cryIA(b)	S	Chilo suppressalis (L) and	
	•		Cnaphalocrosis medinalis (L)	
Poplar	cryIA(a)	PM	Lymantria dispar (L)	
-	cryIIIA	WT	Chrysomela tremulae (C)	

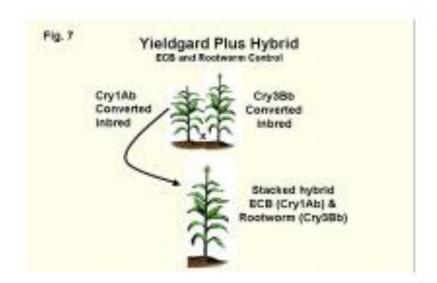
L, Lepidoptera; C, Coleoptera; WT, native gene; PM, partly modified; S, synthetic gene.

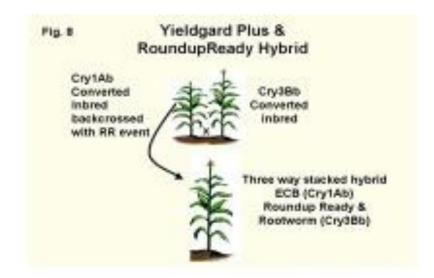
- In plants expressing synthetic genes, the level of expression of proteins is higher than with those expressing bacterial cry genes.
- In most cases, the *B. thuringiensis* toxin coding sequences have been placed under the control of constitutive promoters (CaMV 35S promoter or derivatives thereof).
- Tissue-specific promoters (a maize PEPC promoter expressed in green tissues and a maize pollen specific promoter) or wound-inducible promoters (the pathogenesis-related PR-1a gene or the *A. tumefaciens* mannopine synthase) were also used.

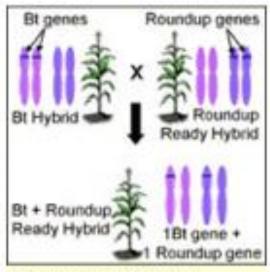
Gene stacking or Pyramiding

- Gene stacking enhances and simplifies pest management for biotech crops as demonstrated by multiple insect resistance based on Bt gene technology. Experience has shown that the resistance conferred by a single Bt gene has the potential to break down as the target insect pest mutates and adapts to defeat the Bt trait.
- To prevent or delay the emergence of resistance to the *Bt* gene, many regulatory agencies require a refuge or an area planted to a non-*Bt* variety alongside the *Bt* crop. Typically a refuge is about 20 percent of the total crop area for a mono-*Bt* trait variety.
- While the refuge strategy lessens the chance for the insect pest to overcome the *Bt* trait, farmers cannot realize the full production benefit of the *Bt* crop.
- The next generation of *Bt* crops with multiple modes of action for insect control were then developed by stacking several classes of Bt genes.
- This gene stacking approach has reduced the potential of resistance breakdown as it is more difficult for the pest to overcome multiple insecticidal proteins. This greater durability of Bt stacks allow a lower refuge area requirement that somehow limits yield.¹









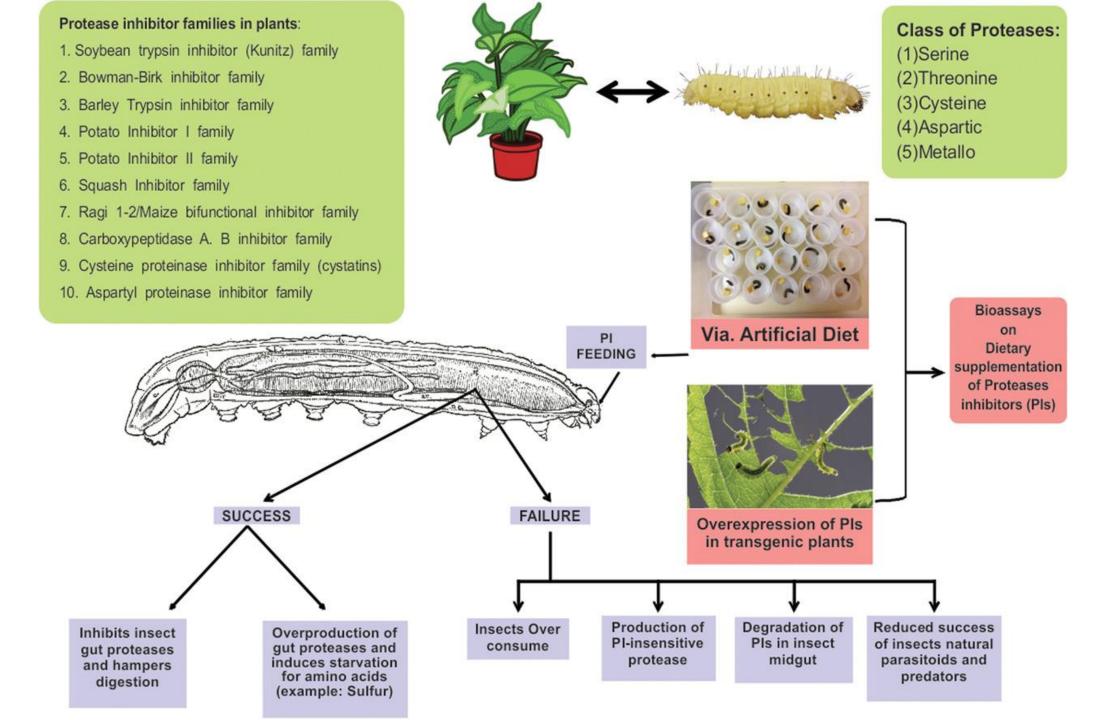
Mating a homozygous
Roundup resistant plant with
a homozygous Bt plant will
result in progeny stacked for
both traits. The progeny will
have one copy of the
Roundup resistance gene
and the Bt gene.

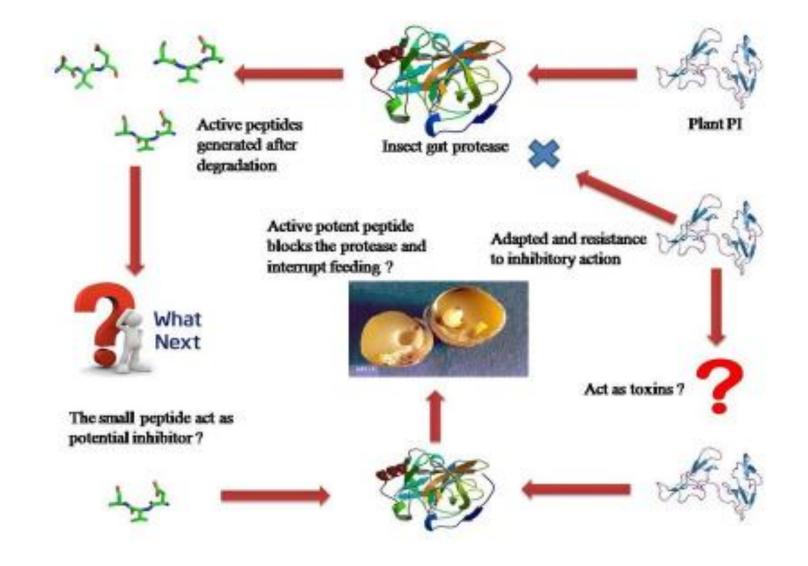
Use of plant derived genes

The synthesis of antimetabolic proteins, which interfere with the digestive processes in insects, is a defence strategy that plants use extensively.

Proteinase inhibitors (PI)

- ➤ PI can inhibit insect larval growth.
- ➤ Divided into 4;
- Inhibiting serine, cysteine, metallo- or aspartyl proteases.
- riangleright serine PIs possess two active sites which inhibit trypsin and chymotrypsin.
- riangleright serine PIs possess two active sites which inhibit trypsin and chymotrypsin.
- Expression of an antisense prosystemin gene in transgenic tomato decreased the ability of the plants to produce PIs and thus reduced resistance towards *Manduca sexta* larvae.



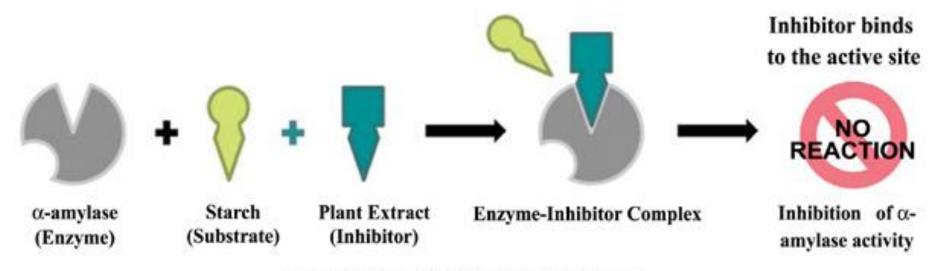


- The first gene to be successfully transferred to another plant species encoded the trypsin/trypsin inhibitor CpTI.
- Insects can adapt to the ingestion of PIs, Lepidoptera and Coleoptera can overexpress existing gut proteases, or induce the production of new types that are insensitive to the introduced PIs and overcome the deleterious effect of PI ingestion.

2. α -Amylase inhibitors

- \triangleright α -AI forms a complex with certain amylases and play a role in plant defense against insects.
- The introduction and expression of the bean a-AI gene under the control of the 5% and 3% regions of the bean phytohemagglutinin gene in pea confers resistance to the bruchid beetles, *Callosobruchus maculatus* and *C. chinensis*.

- \blacktriangleright In nature, Acnathoscelides obtectus and Zabrotes subfasciatus, two other bruchids, can feed on plants producing α -AI because they possess a serine protease able to cleave some kinds of α -AI.
- Thus it is difficult to evaluate the long term interest of the expression of these genes in plants.
- Several kinds of α -amylase and proteinase inhibitors, present in seeds and vegetative organs, act to regulate numbers of phytophagous insects [[9-11]]. α -Amylase inhibitors are attractive candidates for the control of seed weevils as these insects are highly dependent on starch as an energy source.
- Amylase inhibitors are found in the seeds of plants such as cereal grains (wheat, maize, rice, barley) and legumes (kidney beans, cowpea, adzuki beans). Amylase inhibitors inhibit amylases of insects in general and inhibit the growth of insects, and thus serve as defense proteins in both cereal grains and bean seeds.



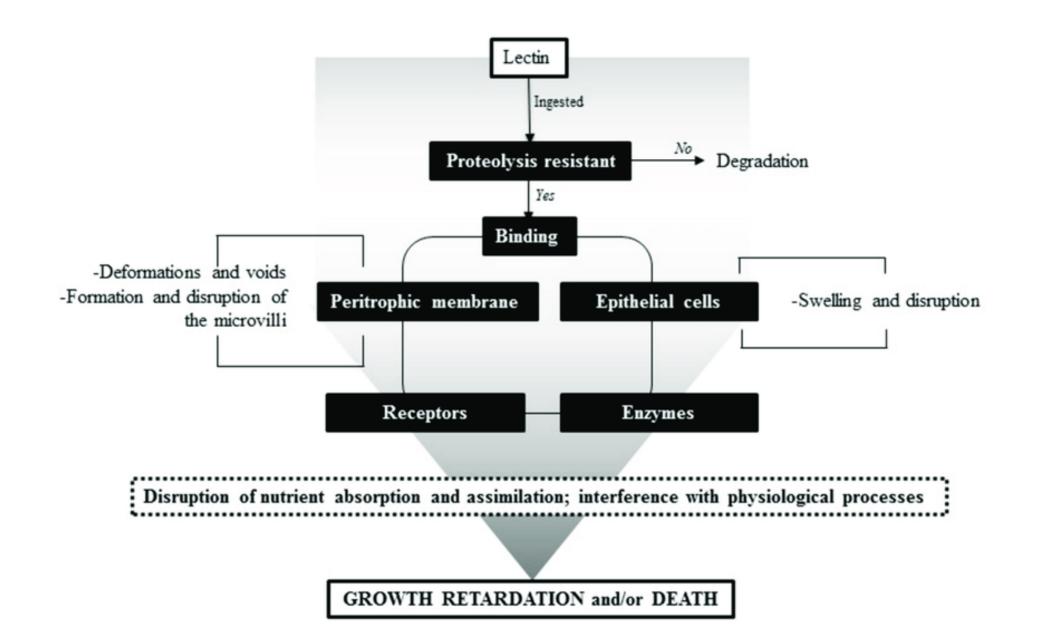
Mechanism of α-amylase inhibition by plant extract.

Examples of plants with enhanced resistance towards insects by expression of enzyme inhibitors

Plant	Gene	Type	Target insect
Tobacco	CpTI	Cowpea serine PI	Manduca sexta (L)
			Heliothis virescens (L)
Tobacco	PPI-II	Potato serine PI	Manduca sexta (L)
	TI-II	Tomato serine PI	Manduca sexta (L)
Tobacco	PPI-II	Potato serine PI	Chrysodeixis eriosoma (L)
Tobacco	spTi-1	Sweet potato PI	Spodoptera litura (L)
Rice	CpTI	Cowpea serine PI	Seramia inferens (L)
Rice	PPI-II	Potato serine PI	Seramia inferens (L)
			Chilo suppressalis (L)
Potato	CpTI	Cowpea PI	Lacanobia oleracea (L)
Poplar	OCI	Rice cysteine PI	Chrysomela tremulae (C)
Pea	α -AI	Bean α-amylase I	Callosobruchus maculatus (C)
Pea	α -AI	Bean α-amylase I	Bruchus pisorum (C)
Azuki bean	α -AI	Bean α-amylase I	Callosobruchus chinensis(C)
		-	C. maculatus, C. analis (C)

3. Lectins

- Lectins are carbohydrate-binding proteins found in many plant tissues, and are abundant in the seeds and storage tissues of some plant species.
- These lectins are toxic to insects, however the exact mechanism of action is not clear.
- Lectins such as those purified from snowdrop or garlic are toxic to insects but not to mammals.
- Tobacco plants expressing a pea lectin were shown to be toxic to the Lepidoptera Heliothis virescens.
- ➤ potato plants expressing the snowdrop lectin (GNA) were toxic to the Lepidoptera Lacanobia oleracea.



Examples of plants with enhanced resistance toward insects by expression of genes of different origins.

Plant	Protein	Type	Insect
N. plumbaginofolia	IPT	Cytokinin synthesis	Manduca sexta
Alfalfa	M. sexta PI	Insect serine PI	Frankliniella spp (thrips)
Tobacco	M. sexta PI	Insect serine PI	Bemisia tabaci (D)
Tobacco	GNA	Snowdrop lectin	Myzus persicae (A) Aulacorthum solani (A)
Potato	GNA	Snowdrop lectin	Lacanobia oleracea (L) Myzus persicae (A)
Potato	BCH	Bean chitinase	Lacanobia oleracea (L)

4. Chitinases and Tryptophan decarboxylase

- Insects contain chitin, not only as an exoskeletal material, but also at the level of the peritrophic membrane.
- The expression of a chitinase of insect origin in transgenic plants seemed to be more effective in causing larval mortality to a beetle, *Oryzaephilis mercator*.
- > Alkaloids are often considered as antifeedant for many insects.
- The production of tryptamine in transgenic tobacco causes a decrease in whitefly (*Bemisia tabaci*) pupae emergence.

5. Genes of other origin

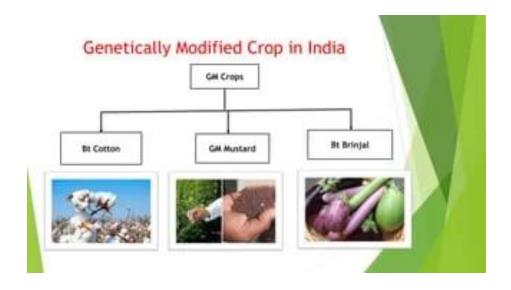
- ➤ Proteinase inhibitors are present in different kingdoms and their activity spectrum could be different from that of plant origin.
- The expression of the insect *Manduca sexta* PI in tobacco was effective against *Bemisia tabacci*, a whitefly.
- A recombinant *Manduca sexta* insect chitinase purified from transgenic tobacco plants was toxic for the merchant grain beetle Oryzaphilus mercator.
- Expression of the bacterial isopentenyl transferase (ipt) gene, is deleterious to *Manduca* sexta (a Lepidoptera) and to *Myzus persicae* (an aphid).

6. New insecticidal genes

- ➤ B. thuringiensis produces a protein, Vip3A, active against lepidopteran insects such as the black cutworn (Agrotis ipsilon), a corn pest.
- Streptomyces cultures secrete a cholesterol oxidase active against the boll weevil (Anthonomus grandis).
- ➤ Photorhabdus luminescens Insecticidal Proteins, produced by P. luminescens, contains a large number of potentially insecticidal components.
- ➤ Bacterial cholesterol oxidase has an insecticidal activity comparable to Bt toxin.

7. Novel approaches: exploiting secondary metabolism

- ➤ Engineering Secondary Metabolism of Plant Defensive Compounds
- Genes encoding two Cyt P450 oxidases and a UDP glycosyltransferase from sorghum (Sorghum bicolor) have been transferred to Arabidopsis.
- **≻**RNAi
- Disrupting gene function by the use of RNAi is a well-established technique in insect genetics based on delivery by injection into insect cells or tissues.

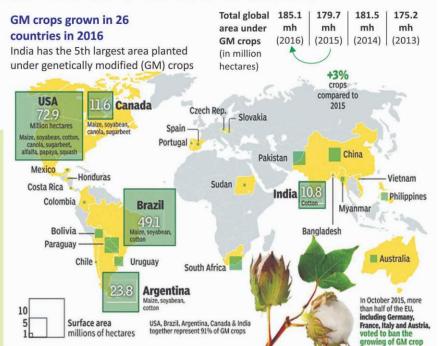


GM FOOD CROPS IN INDIA

GM crop footprint in India is all set to grow once the govt gives its final nod to GM Mustard, a variety grown by a Delhi University institution. It would be a strong push for genetically modified variants of food crop, which have been fiercely opposed by farmer bodies, food experts and activists.

The Backstory

- India slowed down on GM trials after 2010 amid stiff opposition from farmers, activists
- NDA govt changed course on GM field testing. Eight BJP-ruled states have now approved field trials of GM crops, including transgenic rice, cotton, maize (corn), mustard, brinjal and chickpea
- In 2010, the then UPA govt had barred commercial planting of Bt Brinjal and given states the power to veto transgenic-crop field trials, effectively pausing such trials



India's BT Experience

BT Cotton

- Grown in India for over a decade output's up four-fold since commercial cultivation began in 2002
- 95% of 11-12mh under the crop is BT cotton
- But BT cotton, supposed to be immune to pests, crumbled under a whitefly attack in Punjab in 2015
- Over 95% of damaged crop was BT cotton. Damage estimated at Rs 4,500 crore
- The crisis was blamed for over a dozen farmer suicides

BT Brinjal

- Though this was approved for cultivation by India's Genetic Engineering Approval Committee (EGEAC) in Oct 2009, protests saw then environment minister Jairam Ramesh putting an indefinite ban on its cultivation in Feb 2010.
- Brinjal farmers would be dependent on MNCs for seeds from the company that makes them, argued the anti-GM activists
 GM Mustard
- GEAC has recommended

cultivation of GM mustard, taking it closer to becoming India's first GM food crop

- Those opposing GM Mustard are against the genetic modification technology in agriculture over food safety issues
- Anti-GM activists say that claims that the variant, DMH11, has a 30% higher yield are false. There are several naturally grown mustard seed variants and there have been no issues of low productivity, they say
- Regular seeds can be reused, are cheap and widely available. GM seeds can't be reused and must be bought. They contain so-called 'terminator technology', meaning they've been genetically modified such that resulting crops do not produce viable seeds of their own
- When crops failed in the past, farmers could save seeds, replant the following year. Not possible with GM seeds

BT COTTON: MIRED IN CONTROVERSY

Ever since its introduction in India, Bt cotton has faced severe opposition on the basis of its environmental and health implications, though there is no conclusive evidence for the same, and the dominance of Monsanto, which created the Bt cotton seeds

India's cotton yields are expected to be 9% less in 2017-18 than in the previous year because of pink bollworm infestation, though production could be 11% more due to increased acreage

In March 2016 the government decided to cap the price of Bollgard-II seeds at ₹800 per 450 gm pack (it had been selling at ₹830-1,000 in different states) and also the royalty paid to Mahyco Monsanto Biotech (India) at ₹49 per packet, compared to ₹184 earlier, after which Monsanto threatened to exit the country. Months later, Bayer AG announced it would acquire Monsanto

Monsanto withdrew its application seeking approval for a new herbicide-tolerant (HT) Bt cotton seed. But according to a report, 35 lakh packets of HT seeds, worth < 470 crore, have been sold illegally in 2017-18. State governments are looking

Over the last three years, reports have emerged of the pink bollworm becoming immune to Bollgard II. The Maharashtra government estimates 80% of the cotton

into the issue

© BCCL 2023 area in raffertard less link ballworms though the extent of damage is still being studied

Genetically Modified Crops

About

- Genetic modification of plants involves adding a specific stretch of DNA into the plant's genome, giving it new or different characteristics
- Also called Transgenic crops

Global Cultivation

- o Top 5 GM growing countries USA,
- Brazil, Argentina, India and Canada o Major GM Crops - Soybean, maize,

cotton and canola

- Concerns
- Manipulation of GM Seed Cost
 Seeds don't create viable offsprings
- Insect-resistant plants harm non-targeted species too
- Intermixing violates natural plants' intrinsic values

Objective

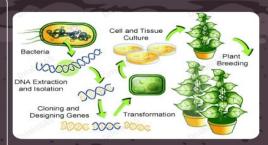
- o Increase yield
- o Increase tolerance to herbicides
- o Improve nutritional value
- Provide resistance to disease/drought

GM Crops in India

- Bt cotton only one GM crop approved, (90% of India's total cotton acreage) (resistance against pink bollworm)
- Ht Bt cotton resistance against glyphosate (herbicide)
- DMH-11 mustard recommended for commercial use (high yield)
- Golden rice probably the best variety of GM rice (Vitamin A)

GM Crop Regulation

- Statutory Provision:
- Rules for Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms (HM) Genetically Engineered Organisms or Cells, 1989 under the Environment Protection Act (1986)
- Statutory Bodies:
- Genetic Engineering Appraisal Committee (GEAC) (under MoEF&CC) administers commercial release of GMC
- Recombinant DNA Advisory Committee (RDAC)
- Institutional Biosafety Committee (IBSC)
- Review Committee on Genetic Manipulation (RCGM)
- State Biotechnology Coordination Committee (SBCC)



Cartagena Protocol on Biosafety (2000)

- It seeks to protect biological diversity from the potential risks posed by Living Modified Organisms resulting from modern biotechnology
- India is a signatory to this protocol



Understanding DMH-11

Genetically modified mustard, after the GEAC approval seems set to be India's first transgenic food crop

Dhara Mustard Hybrid-11 (DMH-11)

DMH-11 works on the principle of removing male fertility in one parent and restoring it in the offspring

WHO DEVELOPED IT?

Scientist, ex-DU vice-chancellor Deepak Pental developed it in 2007. It had been stuck in the regulatory process after initial approval in 2017.

70 Cr cost of the partially govt-funded project

ITS ADVANTAGES: It would bring "better yields, lower costs for farmers", Pental said. It allows for hybridisation of a plant that otherwise self-pollinates (making hybrids next to impossible), leading to high-output hybrids AND CONCERNS: GM
technologies are fiercely
resisted, amid fears they may
compromise food security, lead
to seed monopolies, biosafety hazards
Enalition for a GM-free India called the
clearance "shocking", alleging that the
"regulator colluded with the developer



