Applications:

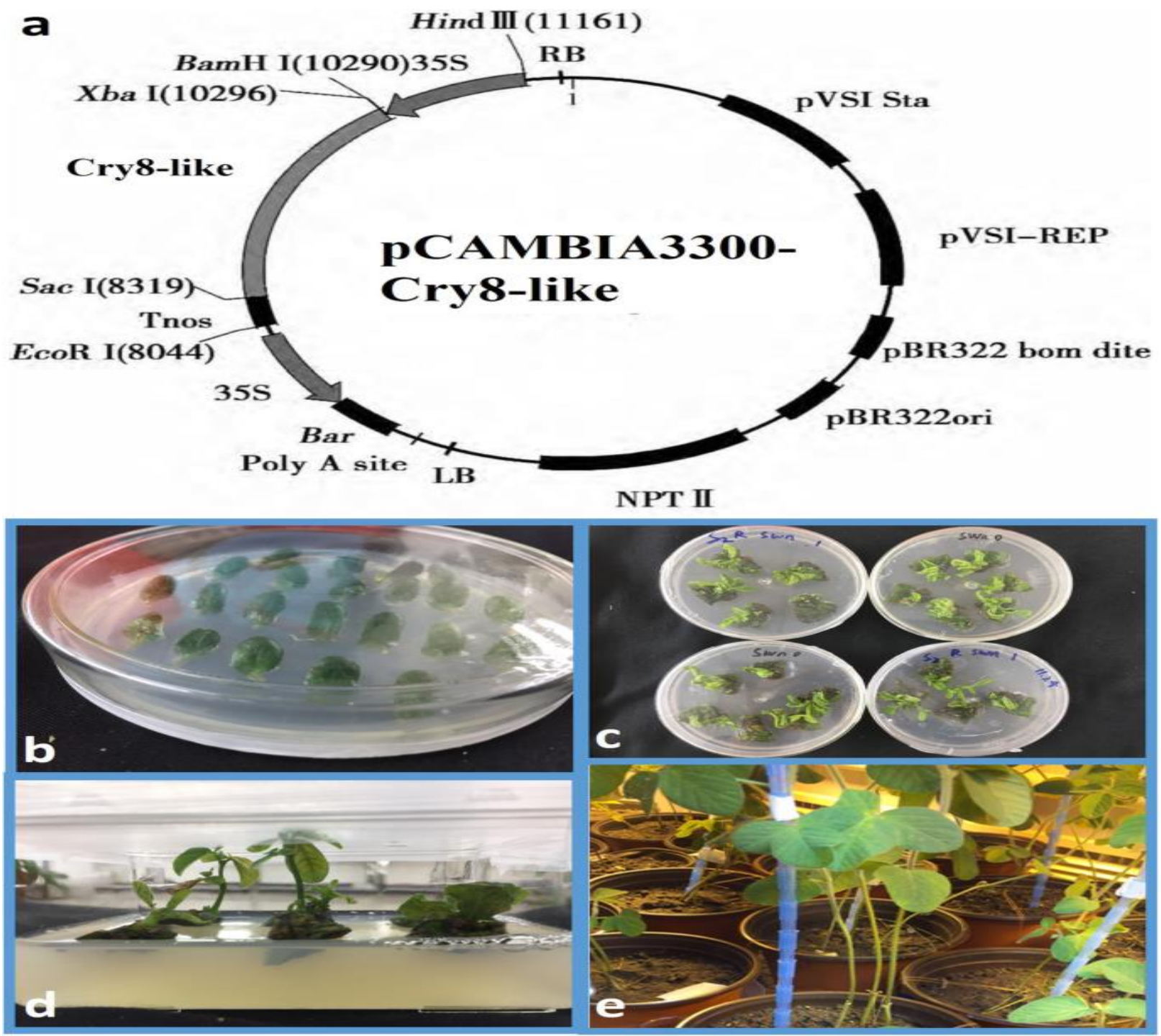
- The applications of Bt can be in two ways
- 1. Bt formulations
- 2. Transgenic plants.

Transgenic plants:

- The area devoted to growing transgenic plants expressing insecticidal Cry proteins derived from *Bacillus thuringiensis* (Bt) is increasing worldwide.
- A major concern with the adoption of Bt crops is their potential impact on nontarget organisms including biological control organisms.
- Regulatory frameworks should advocate a step-wise (tiered) approach to assess possible nontarget effects of Bt crops.

Transgenic plants:

- In 1995, the Environmental Protection Agency (EPA) in USA approved the commercial production and distribution of the *Bt* crops: corn, cotton, potato, and tobacco.
- Currently, the most common *Bt* crops are corn and cotton. The crystal, referred to as Cry toxins, is proteins formed during sporulation of some *Bt* strains and aggregate to form crystals.
- Such Cry toxins are toxic to specific species of insects belongs to orders: Lepidoptera, Coleoptera, Hymenoptera, Diptera, and Nematoda.
- In 2016, the total world area cultivated with genetically modified crops (GM crops) reached about 185 million ha
- There is a worldwide controversy about the safety of *Bt* crops to the environment and mammals. Some researchers support the cultivation of *Bt* crops depending upon the results of their laboratory and field studies on the safety of such crops.
- Others, however, are against *Bt* crops as they may cause risk to human.


a
Hind Ⅲ (11161)
RB
BamH I(10290)35S
Xba I(10296)
pVSI Sta
Cry8-like
pVSI–REP
pCAMBIA3300-
Cry8-like
Sac I(8319)
Tnos
EcoR I(8044)
pBR322 bom dite
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Bar
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LB
NPT Ⅱ
b
c
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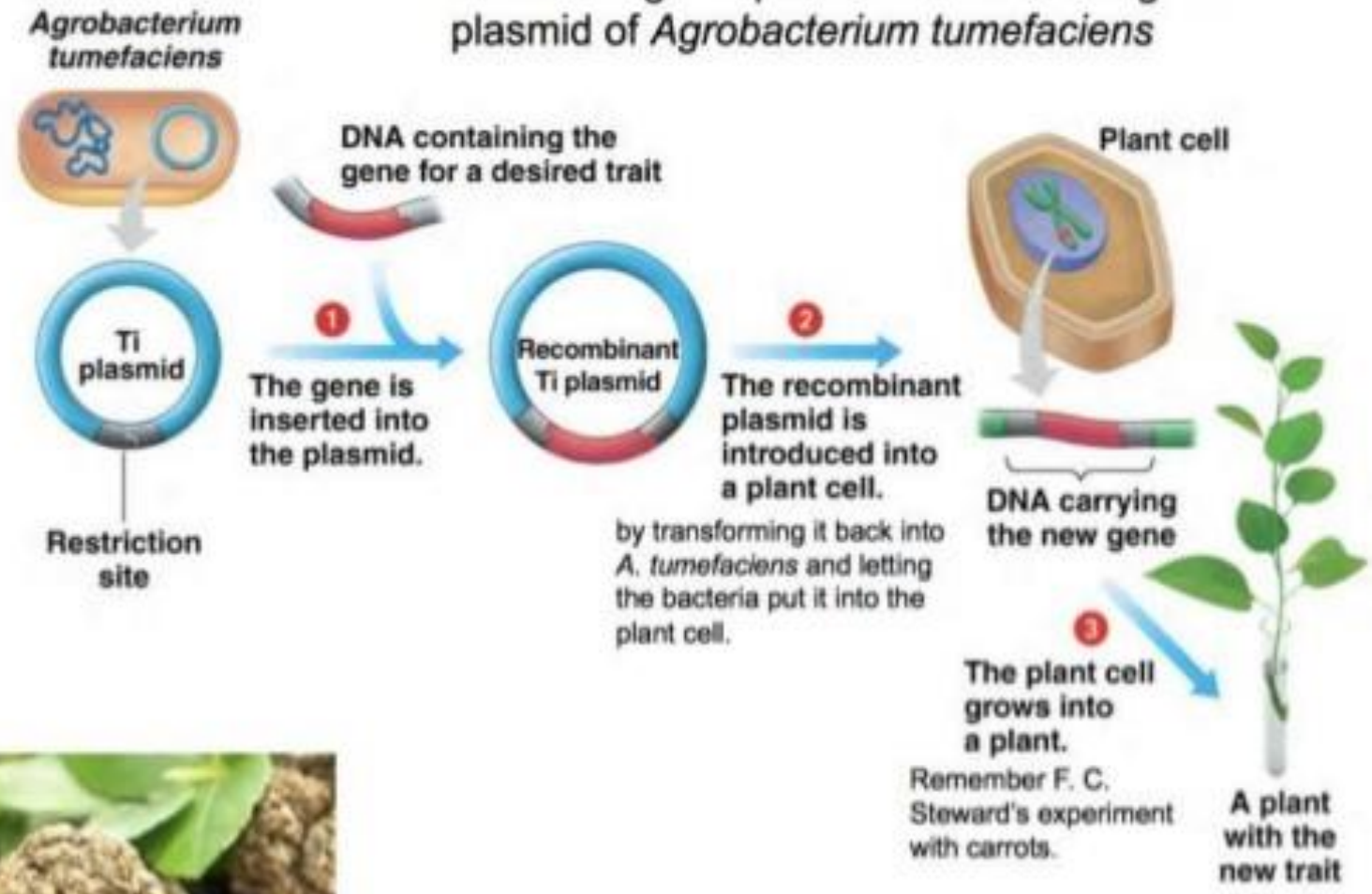
e

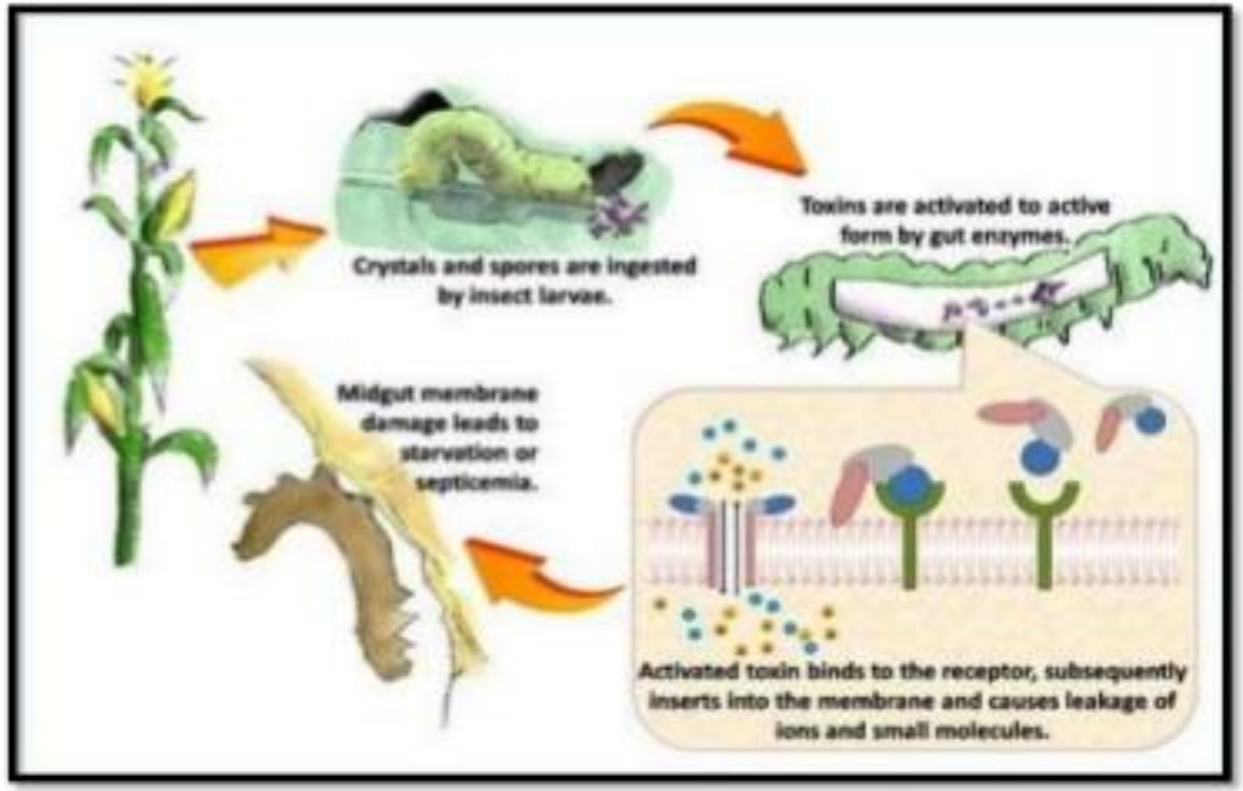
Most transgenic plants are made using the Ti plasmid of *Agrobacterium tumefaciens*

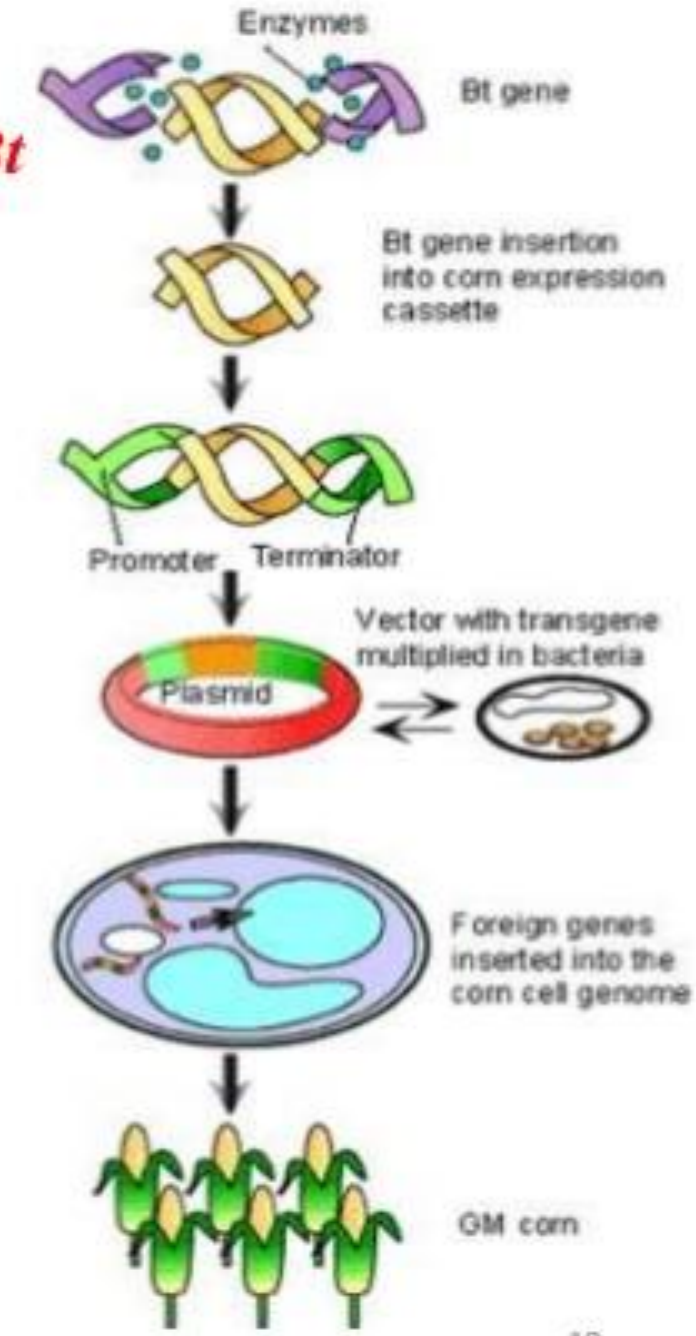


A crown gall tumor. The tumor-inducing genes have been deleted from the Ti plasmid for use as a recombinant DNA vector .

Endotoxin gene from *Bt*

The gene responsible for producing endotoxin is isolated from *Bt* and cloned into plants to develop resistance to insects





Bt crops:

Advantages of Bt Crops

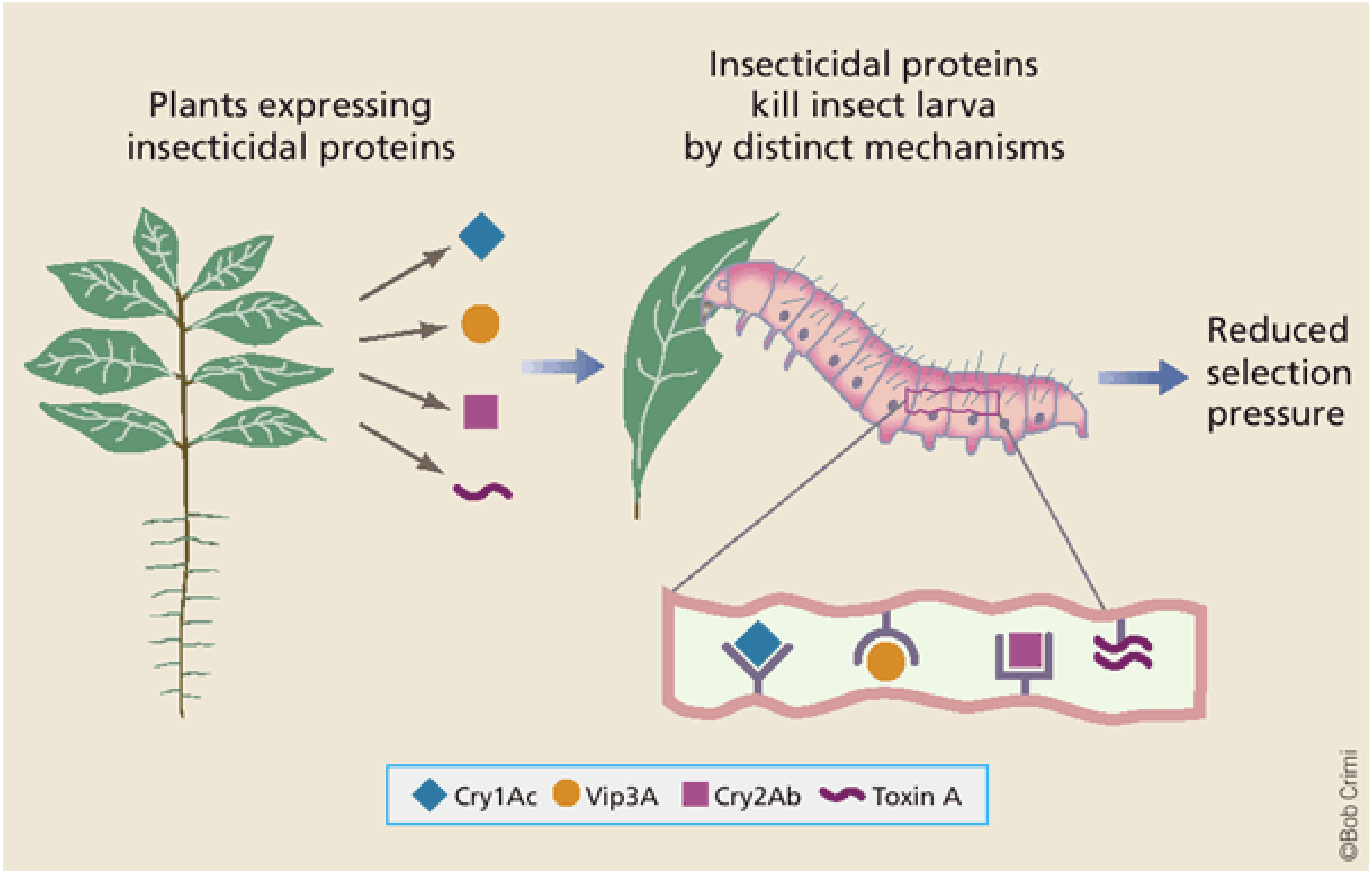
- Following are the major advantages of Bt crops:
- It helps in improving the crop yield, thereby, raising the farmer's income. This results in increased farm production.
- They help in controlling soil pollution as the use of synthetic pesticides is reduced.
- Bt crops help in protecting beneficial insects.
- It can easily feed an increasing population due to increased yields in a short time.
- It leads to the production of disease-free crops owing to the reduction of pesticides.
- It leads to more productivity in a small area of land.

Disadvantages of Bt Crops

- Bt crops have a few disadvantages as well:
- Bt crops are costlier than naturally grown crops.
- It can disrupt the natural process of gene flow.
- The pests might become resistant to the toxins produced by these crops and the crop production might decline.

Can insect pests develop resistance to Bt crops?

- The caterpillar **pest** *Helicoverpa zea* (also known as cotton bollworm and **corn** earworm) has evolved **resistance** to four **Bt** proteins.
- But **insects** that have **developed resistance to Bt** toxins **can** live on undeterred, and that **resistance** is growing.
- We will discuss more in the topic “Microbes in Agrobiotechnology”

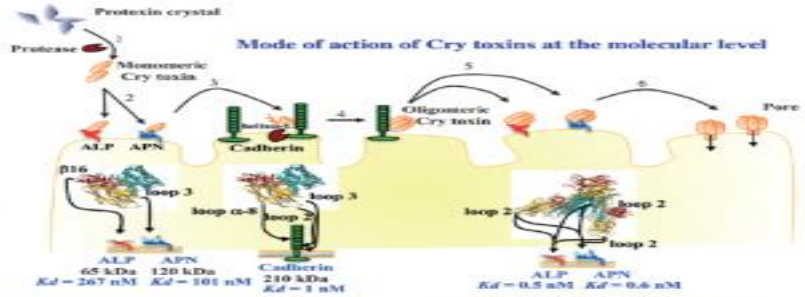

Plants expressing
insecticidal proteins
Insecticidal proteins
kill insect larva
by distinct mechanisms
Reduced
selection
pressure
Cry1Ac
Vip3A
Cry2Ab
Toxin A
©Bob Crimi

Insect Resistance:

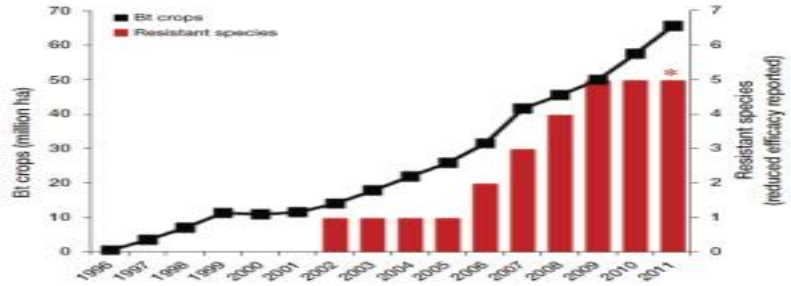
Manageable Obstacle
or
Downfall of GMOs?

Insects Damage Crops

Bt Plants Protect Themselves

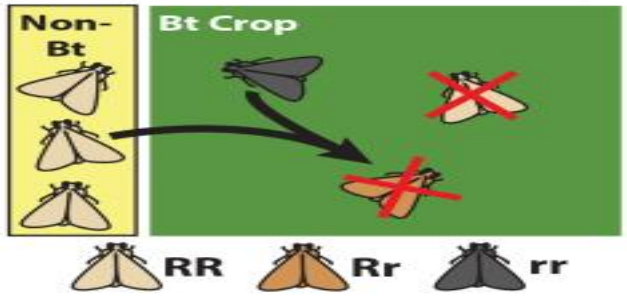


Resistance Happens

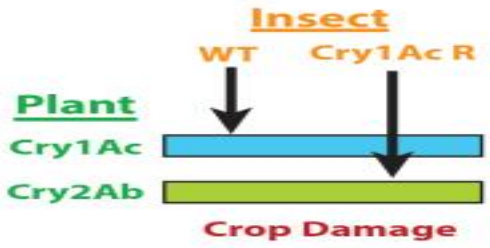


What Can Be Done?

High Dose/Refuge



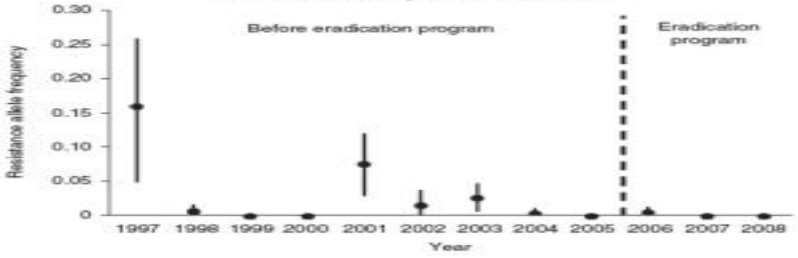
Pyramiding

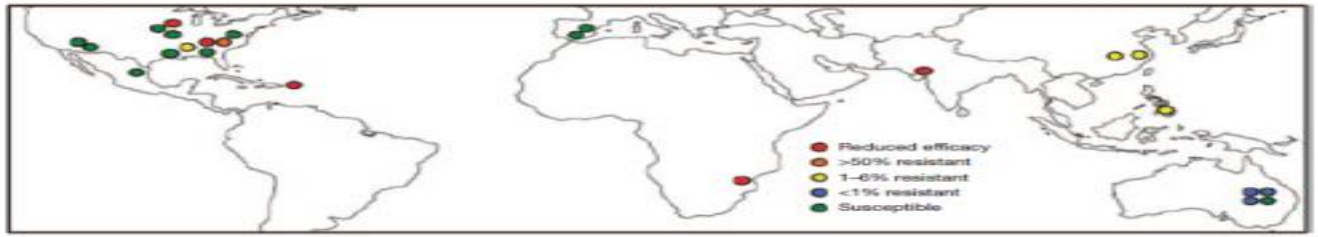


Stacking genes for multiple layers of protection

Combinatorial Control

Combine different approaches for robust pest control





Poor management of Bt technology results in loss of efficacy

Management Matters

Bt formulations:

Instead of Bt Transgenic, Why not to Use Bt Formulation Products

Bt-Formulation ?

History of Bt–bacteria Formulated Products:

In 1901, Japanese Biologist – **Shigetane Ishiwatari**

-Investing The Cause of Sotto Disease (Sudden-Collapse Disease)

- Killing Large Population Of Silkworm

Cause of above Disease was the Bacterium

In 1911, **Ernst Berliner** – **Isolated same** Bacterium which had killed Mediterranean Flour Moth

How Bacterium named as *Bacillus thuringensis* ?

Ishiwatari – Named Bacterium – *Bacillus Sotto*-1901

Ernst Berliner-Name Bacterium *Bacillus thuringensis*-1911

(Germen Town **Thuringia** where Mediterranean Moth was found)

Name *Bacillus thuringensis* Ruled Permanently to Bacterium

Berliner – Reported The existence **of Crystal Protein** within that Bacterium

After Knowing, Spores of Bt Bacterium Contains Crystal Protein

- Which has Insecticidal Property
- Bt- Formulated Products started to prepare and Farmers also started to use it.

Farmers using Bt- Forsmulated Products

But,

These Bt- Biopestcides has following **Disadvantages :**

1) Bt –Products such as sprays are rapidly washed by rain
2) Become ineffective within 24 hours
3) Require to spray continuously,

(Because bt products (crystal protein) degraded under the sun's UV ray's)

4) Failure to penetrate tissues and therefore to reach insects in all parts of the plant.

(Bt sprays are non-systemic insecticides and are therefore ineffective against insects that do not come into direct contact with the crystals, such as sap sucking and piercing insects, against root dwelling pests, or larvae that after hatching rapidly burrow or bore into plant tissues). (McGaughey and Whalon, 1992)

That's why Bt transgenic are only way to solve above problem

Types of Pesticide Formulations

- Liquid Formulations
  Emulsifiable Concentrates
  Solutions
  Liquid Flowables
  Aerosols
- Fumigants
- Adjuvants
- Dry Formulations
  Dusts
  Baits
  Granules or Pellets
  Wettable Powders
  Soluble Powders
  Microencapsulated
  Dry Flowables

Adjuvants

Activators

Wetter/Spreader
- Nonionic surfactants

Sticker
- Oils / Acrylic latex

Humectant
- Glycerin / Polyethylene glycol

Penetrator
- COC / MSO

Utility

Defoamer
- Methopolysiloxane

Water Conditioner
- AMS Acids

Activator	Acidifying agent	Additive
Antifoam/ Defoam	Antifreeze	Attractant
Buffering agent	Binder	Coupler
Chelating agent	Compatibility agent	Colorant/ Dye
Detergent	Deposition agent	Drift control agent
Dispersant	Emulsifier	Evaporation reducer
Foam marker	Humectant	Inert
Neutraliser	Modified seed oil	Preservative
Petroleum oil	Penetrator	Rainfast agent
Spreader sticker	Synergist	Safener
Surfactant	Translocation aid	Thickener
UV protectant	Vegetable oil	Wetting agent

Source: Green (2000)

Adjuvant type	Example
Penetration agents	Petroleum or mineral oils, vegetable oils, organosilicon
Oder masking agent	1-octanal
Dyes	fd&c blue no. 1, fd&c red no. 40
Preservatives	Hexamethylenetetramine, potassium benzoate, sorbic acid
Stabilizer	Diisopropanolamine, hydroxyethylidene diphosphonic acid, silver nitrate
Diluents	Aluminum hydroxide
Surfactants	Anionic: alkylbenzene sulfonates, sodium laureth sulfate, soap Cationic: dioctadecyldimethylammonium chloride Amphoteric: cocamidopropyl betaine, cocamidopropyl hydroxysultaine Non-ionic: alkoxylated alcohol, ethoxylated alcohol, nonylphenol ethoxylate, tallow amine ethoxylate, alkyl polyoxyethylene ether
Emulsifiers	Alkanoic and alkenoic acids, monoesters and diesters of α-hydro-ω-hydroxypoly (oxyethylene), glyceryl monostearate, sodium metasilicate
Propellant	1,1-Difluoroethane, butane, propane
Solvents	*N*-methyl-2-pyrrolidone, polychloromethanes, chlorinated volatile organic compounds, xylene, isopropanol
Antifoaming agent	silicones (e.g., dimethylpolysiloxane), fatty acids
Carriers	Biochar, cyanobacteria, clay minerals, siliceous minerals, zinc-layered hydroxide, polymeric materials such as chitosan, lignin, and poly(ethylene) glycol

This non-exhaustive list presents compounds grouped by category that are classically used as adjuvants in commercial pesticide formulations. Some of these molecules can have dual roles. For instance, surfactants (wetters) are also used as plant penetration agents. Compiled from Ref. (13–16).

Bt formulations

company	Product name	formulation	Dosage
Eupnoea technisol Pvt. Ltd, New Delhi	BACIL-EU	WP	1.5-2g/L
Prathibha biotech, Hyderabad	*Bacillus thuringiensis var kurstaki*	WP, liquid formulation	Seed treatment 10 g/kg seed 4-5 ml/kg seed 500-750g/acre 200ml/ha
Som phytopharma (India) Ltd, Hyderabad	Lipel	WP	
Junna life sciences Pvt Ltd, Hyderabad	*Bacillus thuringiensis var. israelensis*	WP	

Formulation	Application
Emulsions	Agriculture and forestry
Encapsulations	Agriculture and forestry
Wettable powders	Gardens and agriculture
Granules	Agriculture and forestry
Powders	Forestry
Briquettes	Aquatic systems

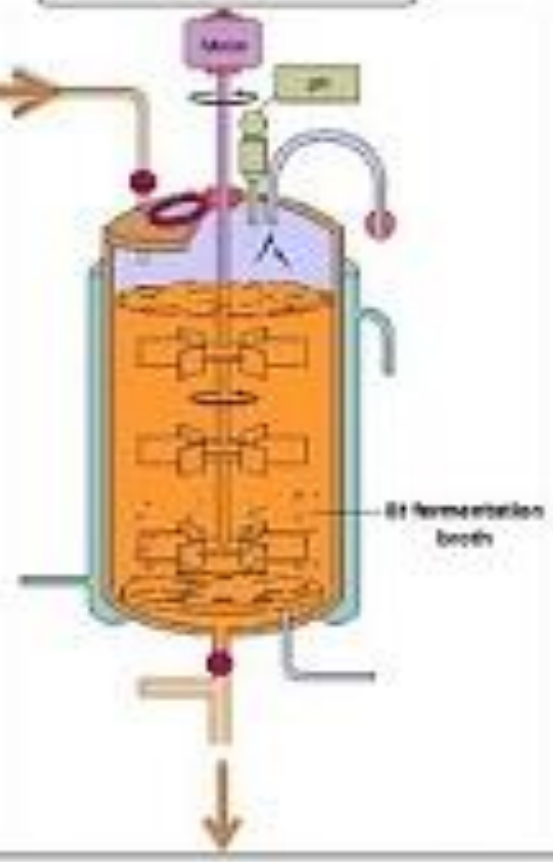
was a bioinsecticide formulation dispensed as a dry powder or tablet. Formulations contained *B. thuringiensis* var. *israelensis* and chemical dryers, dispersing agents, binding agents and moisturizing agents, protectors against sunlight; and optionally, diluents, lubricant and neutralizing agents.


DIPEL 2X
XenTari GD
BACTOSPEINE
NUEVO
DELFIN
SIN PLAZOS DE SEGURIDAD
THURICIDE HP
INSECTICIDA BIOLÓGICO
Recomendado en tratamientos de lucha integrada y biológica.
Polvo mojable
SANDOZ AGRO, S.A.E.
1 kg
SANDOZ

Integration of production process of Bt-based biopesticide using membrane separation technology

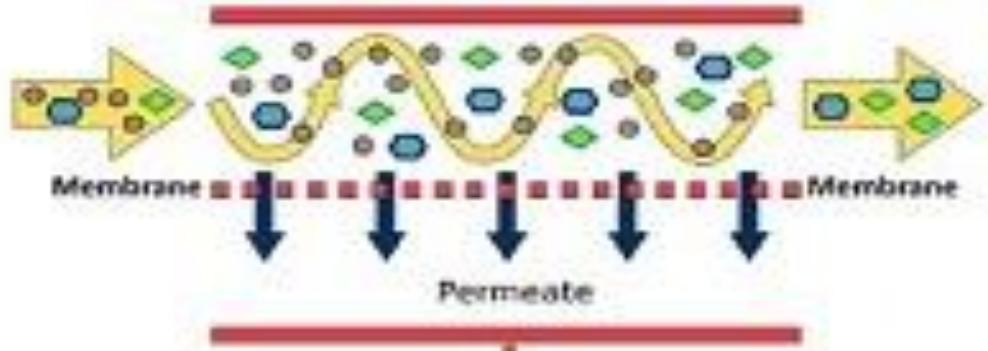
- Higher production rate of purified spore-crystal compounds
- More compact equipment for purification step
- Easier scale-up considerations for downstream processing step
- Safer, cleaner and more trustable purification
- Lower overall costs

Bt fermentation process



Mixture of spores, toxic parasporal crystals, cell debris, inclusion bodies, enzymes and other residual solids

Harvesting spore-crystal complex (scc) of Bt by membrane technology



Bt formulation and final pesticide product

Re-used broth for farther fermentation step