

**COURSE SEMINAR(ENT-699)**  
**ON**  
**Role of Transgenic plants in Pest management**



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# Introduction

- Transgenic plants are the ones, whose DNA is modified using **genetic engineering techniques**.
- Transgenic plants are the results of **modern biotechnology**.
- An organism containing a **transgene** introduced by technological (not breeding ) methods is called transgenic.
- The process of producing transgenic organism is called **transgenesis**.
- The aim is to introduce a new trait to the plant which does **not occur** naturally in the species.

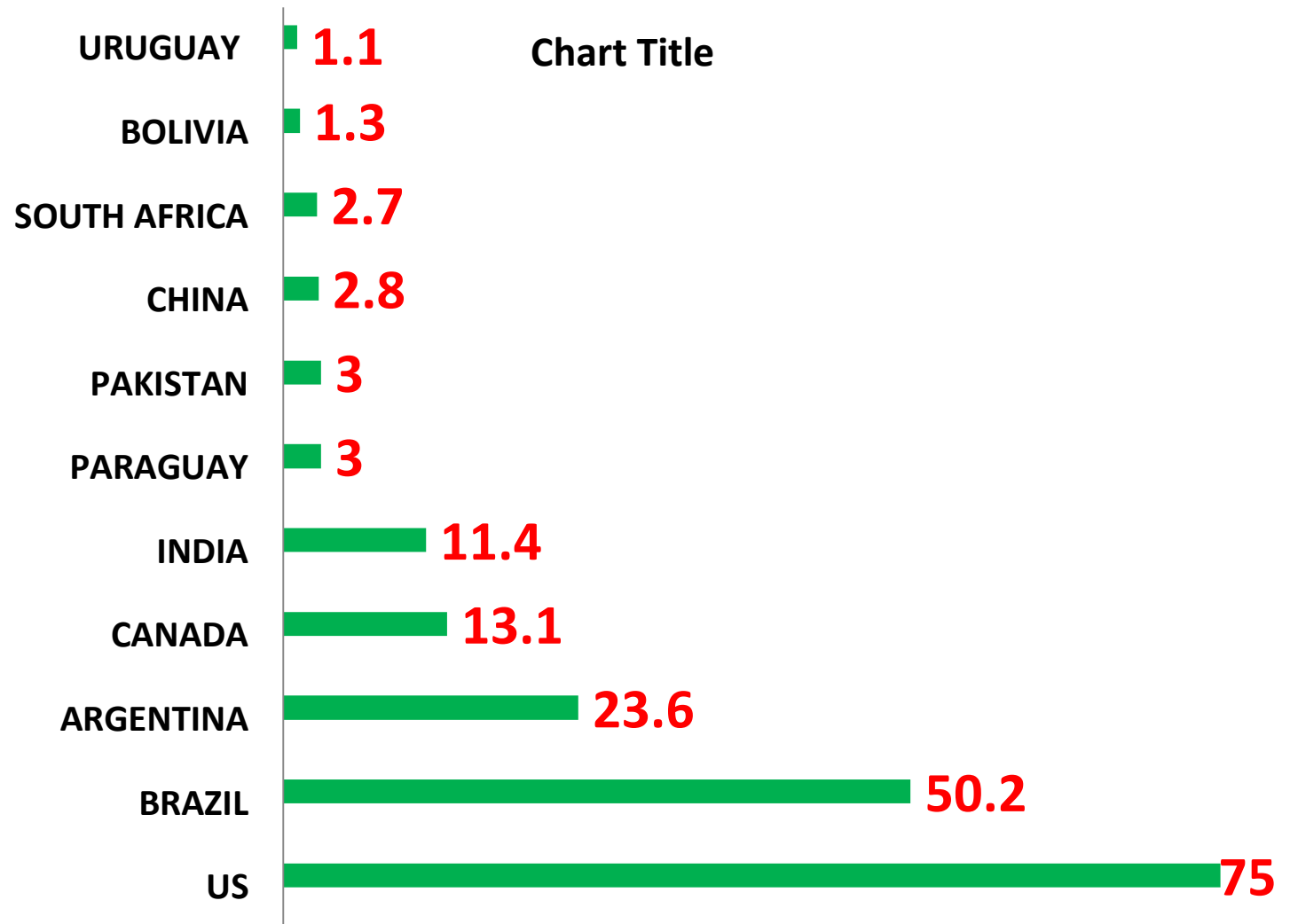
Cont.....

- The purpose of inserting a combination of genes in a plant, is to make it as useful and productive as possible.
- This process provides **advantages** like improving shelf life, higher yield, improved quality, pest resistance, tolerant to heat, cold and drought resistance against a variety of biotic and a biotic stresses.

Conti.....

- Acreage increased from 1.7 m. hectares in 1996 to 185.1 m. hectares in 2016, some 12% of global cropland.
- As of 2016, major crop ( soybean, maize, canola and cotton).
- Traits consist of herbicide tolerance (95.9 million hectares), insect resistance (25.2 million hectares) or both (58.5 million hectares).
- In 2015, 53.6 million ha of GM maize were under cultivation ( almost 1/3 of the maize crop).

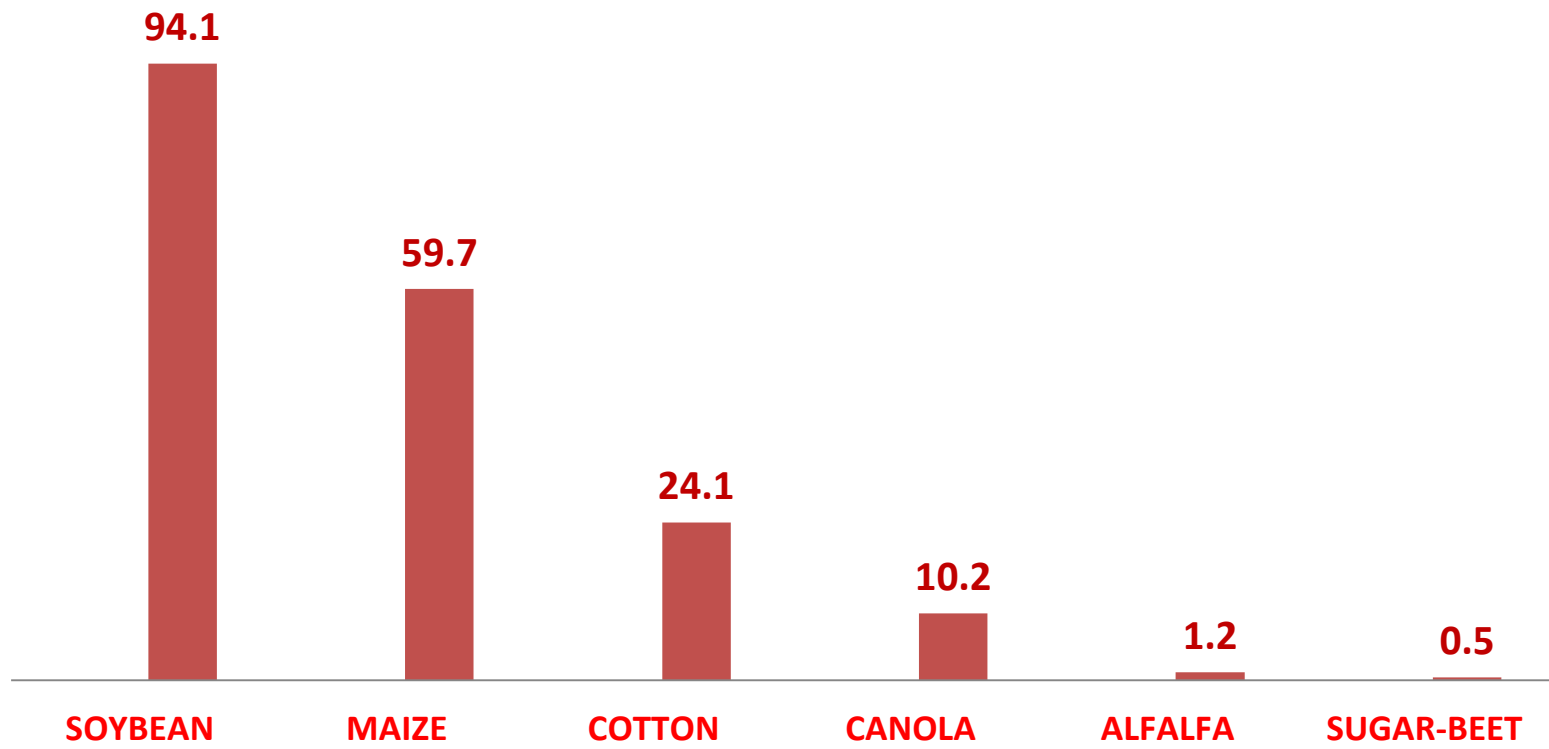
# TOP GM CROP GROWING COUNTRIES (M.Ha.)



# Reported by global crop biotech advocacy organization

Chart Title

■ mh



# GM crops and their related traits

<b>Traits</b>	<b>GM crops</b>
Insect resistance	Cotton, tomato, potato, maize
Herbicide resistance	Maize, rice, cotton, canola, chicory, soybean, flax, linseed, tobacco
Male sterility	Canola
Fertility restoration	Canola, chicory, maize
Delayed ripening	Melon, tomato
Viral resistance	Papaya, squash, potato
Oil modification	Canola, soybean



# History

- ❖ The first genetically engineered crop plant was tobacco, reported in 1983. It was developed creating a **chimeric gene**.
- ❖ The first field trials of genetically engineered plants occurred in **France and the US in 1986** tobacco plants were engineered to be **resistant to herbicides**.
- ❖ In 1987 Plant Genetic System, founded by **Marc Van Montagu** and **Jeff Schell**, was the first company to genetically engineer insect-resistant plants by incorporating genes that produced insecticidal proteins from *Bacillus thuringiensis* (Bt) into tobacco.

❖ The People's Republic of China was the first country to commercialize transgenic plants, introducing a **virus-resistant** tobacco in **1992**.

❖ By 2010, 29 countries had planted commercialized genetically modified crops and a further 31 countries had granted regulatory approval for transgenic crops to be imported.

- ❖ The first genetically modified animal to be commercialized was the **GloFish**, a **Zebra Fish** with **fluorescent gene**.
- ❖ In 1994 Calgene attained approval to commercially release the **Flavr Savr** tomato, a tomato engineered to have a longer shelf life.
- ❖ In 1995 **Bt Potato** was approved safe by the Environmental Protection Agency.

## History conti....

❖ **1995** Canola with modified oil composition (calgene), (Calgene ), Bt cotton (Monsanto), glyphosate-resistant soybeans (Monsanto), virus-resistant squash (Asgrow), and additional delayed ripening tomatoes (DNAP, Zeneca/Peto, and Monsanto) were approved.

❖ **2000** Golden rice with beta-carotene developed with increased nutrient value.

❖ Vitamin A-enriched golden rice, was the first food with increased nutrient value.

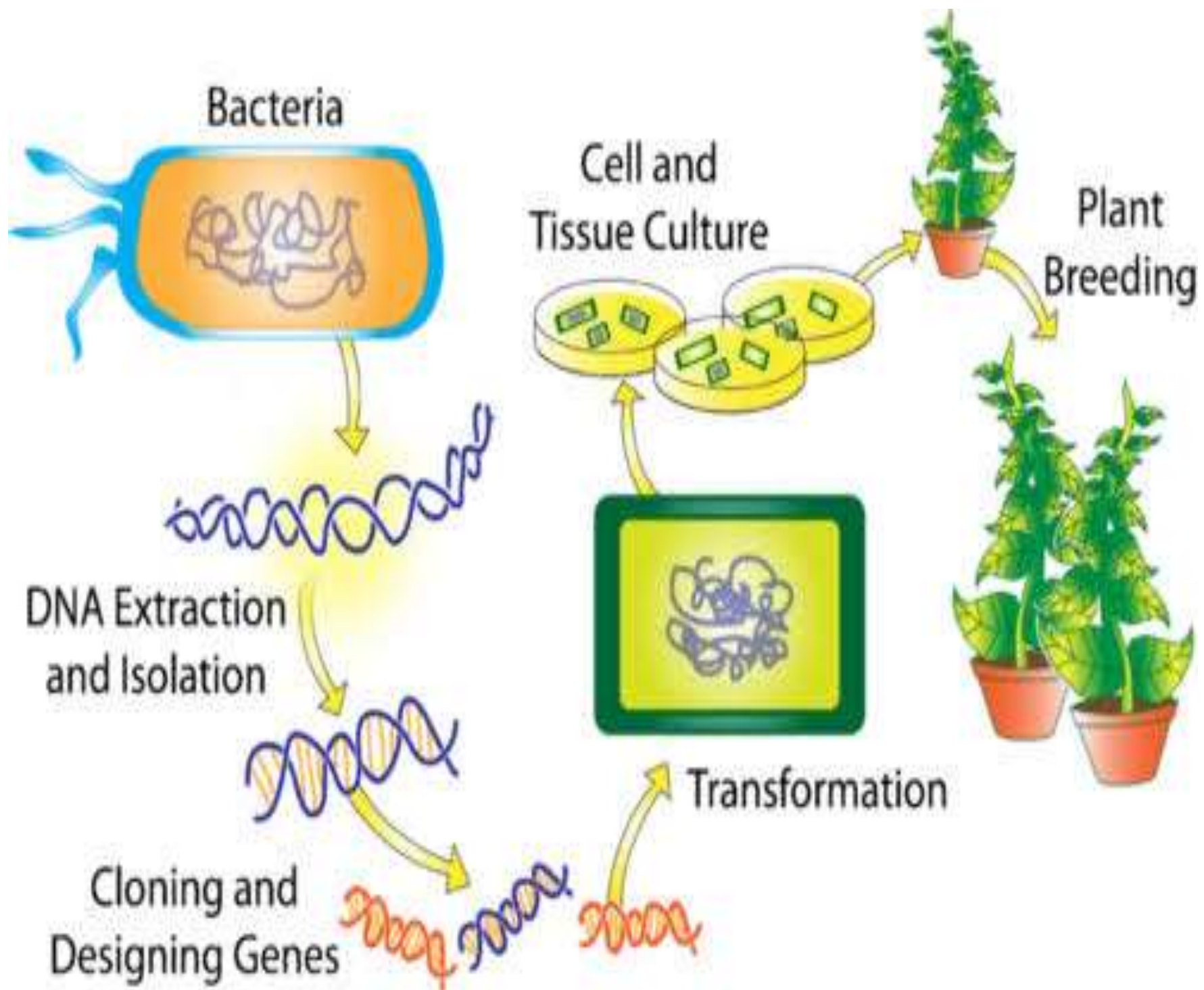
## METHODS

### □ **GENE GUN METHOD**

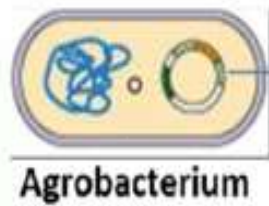
This method was first used by Klein *et al.* 1987 to transform cell of *Allium cepa*.

▪ Also known as the “**Micro-Pro-jectile Bombardment**” or “**Biolistic**” method is most commonly used in the species like corn and rice.

▪ ***ipt* gene Method**



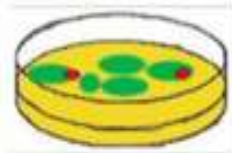
## Agrobacterium-mediated transformation



Ti plasmid carrying desired genes

Agrobacterium

Co-cultivation of Agrobacterium with plant tissues



Chromosomes with integrated DNA carrying desired genes

Transformed plant cell



Proliferation of transformed cells (callus formation)



Shoot regeneration



Regenerated plant expressing medical proteins

Direct consumption of edible plant tissues

## Particle bombardment method

Metal particles coated with DNA carrying desired genes



Gene gun

Bombardment of plant tissues with DNA-coated particles

# Insect Resistance

## 1. Resistance gene from micro-organism

- Bt gene from *Bacillus thuringiensis*.
- ipt gene from *Agrobacterium tumefaciens*.

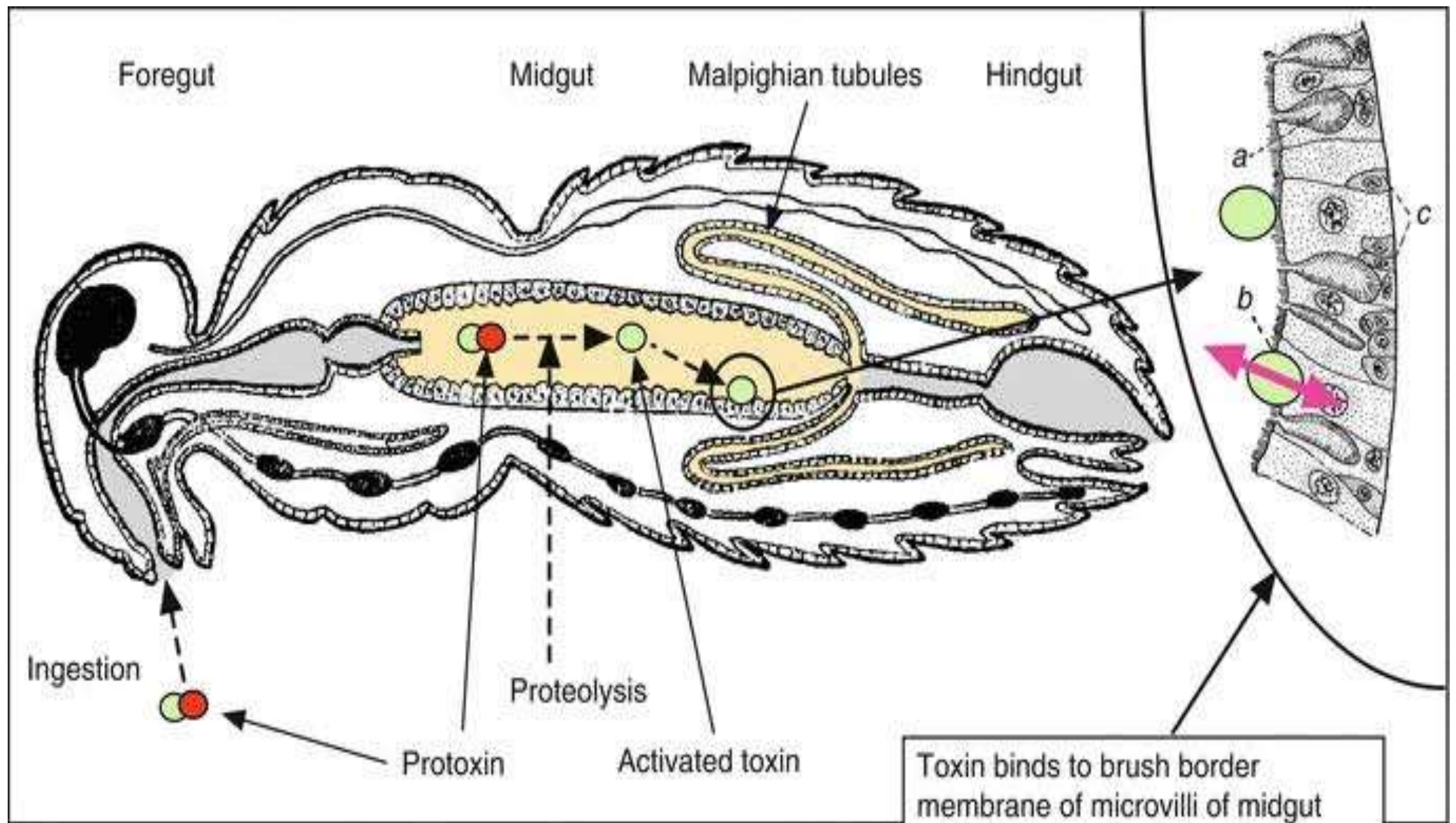
## 2. Resistance gene from highest plant.

- Proteinase inhibitor.
- Lectin.



## *Bacillus thuringiensis*

- It is gram negative soil bacteria produce **parasporal** crystalline protein.
- This protein are responsible for the insecticidal activity of the bacterial strain.
- Cry protein are solublized in the alkali ( pH 7.5-8.5) environment of insect midgut.
- They are converted to active form upon infection by susceptible tissue then killing the insect by disruption of ion transport across the membrane of susceptible insect.



Toxin binds to brush border membrane of microvilli of midgut epithelial cells; insertion of toxin into membrane forms an open pore leading to collapse of ionic gradients across cell membrane and leakage of components

# *Bacillus thuringiensis*

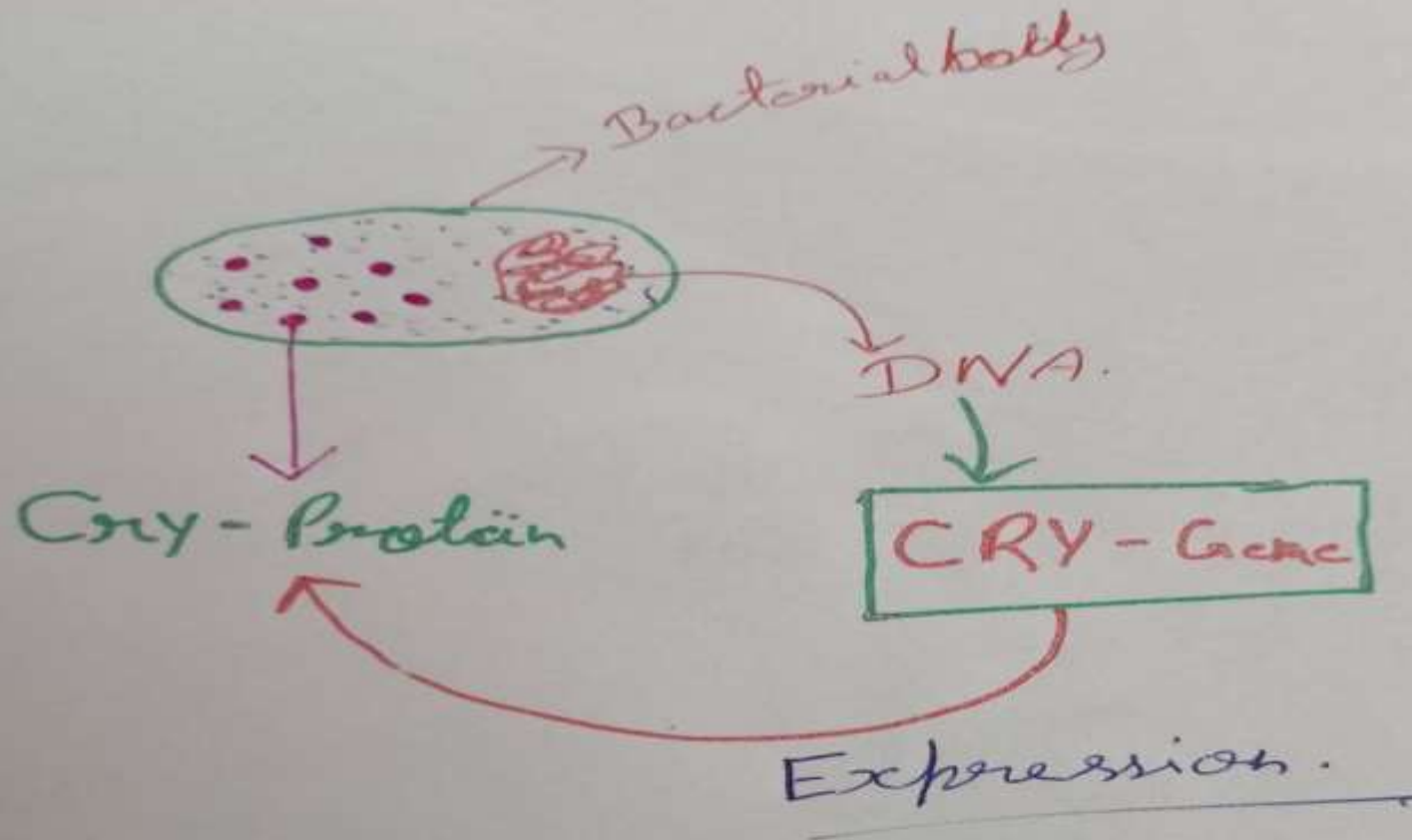
## ➤ **Bt Endotoxins and their Genes**

Initially, Bt toxins were classified into 14 distinct groups and 4 classes (Höfte and Whiteley classification [Höfte and Whiteley, 1989]) based on their host range.

➤ These are: ·

- **CryI** (active against Lepidoptera [“Cry” stands for “crystalline” reflecting the crystalline appearance of the d-endotoxin; “Cry” is used to denote the protein whereas “cry” denotes the respective gene]), ·
- **CryII** (Lepidoptera and Diptera),
- **CryIII** (Coleoptera) and
- **CryIV** (Diptera).

# Bacillus thuringiensis



Formation of Cry-Protein.

Cry protein	Origin (Bt subspecies)	Major Target Insects Order	Common names
CryIA(a)	<i>kurstaki</i>	L	Silk worm, Tobacco horn worm, European corn borer
CryIA(b)	<i>berlineri</i>	L & D	Tobacco horn worm, Cabbage worm, Mosquito
CryIA(c)	<i>kurstaki</i>	L	Tobacco budworm, Cabbage looper, Cotton bollworm
CryIA(d)	<i>aizawai</i>	L	Several Lepidoptera
CryIA(e)	<i>alesti</i>	L	Tobacco budworm
CryIB	<i>thuringiensis</i>	L	Cabbage worm
CryIB(c)	<i>morrisoni</i>	L	Several Lepidoptera
CryIC	<i>entomocidus</i>	L & D	Cotton leaf worm, Mosquito
CryIC(b)	<i>galleriae</i>	L	Beet army worm
CryID	<i>aizawai</i>	L	Beet army worm, Tobacco horn worm
CryIE	<i>kenyae</i>	L	Cotton leaf worm
CryIE(b)	<i>aizawai</i>	L	Several Lepidoptera
CryIF	<i>aizawai</i>	L	European corn borer, Beet army worm
CryIG	<i>galleriae</i>	L	Greater wax moth

Cry protein	Origin (Bt subspecies)	Major Target Insects Order	Common names
CryIIA	<i>kurstaki</i>	L & D	Gypsy moth, Mosquito
CryIIB	<i>kurstaki</i>	L	Gypsy moth, Cabbage looper, Tobacco horn worm
CryIIC	<i>shanghai</i>	L	Tobacco horn worm, Gypsy moth
CryIIIA	<i>san diego</i>	C	Colorado potato beetle
CryIIIA(a)	<i>tenebrionis</i>	C	Colorado potato beetle
CryIIIB	<i>tolworthi</i>	C	Colorado potato beetle
CryIIIC	N/a	C	Spotted cucumber beetle
CryIIID	<i>kurstaki</i>	C	N/a
CryIVA	<i>israelensis</i>	D	Mosquito (Aedes and Culex)
CryIVB	<i>israelensis</i>	D	Mosquito (Aedes)
CryIVC	N/a	D	Mosquito (Culex)
CryIVD	N/a	D	Mosquito (Aedes and Culex)
CryV	N/a	L & C	European corn borer, Spotted cucumber beetle

Cry protein	Origin (Bt subspecies)	Major Target Insects Order	Common names
CryIX	<i>galleriae</i>	L	Greater wax moth

**Rajamohan and Dean (1995) and Crickmore *et al.* (1996)**

- **Bt Endotoxins (Cry) and their Activity against Specific Insect Species**

## Proteinase Inhibitor

- Plants contain peptides acting as protease inhibitors.
- Protease inhibitors (PIs) are generally small proteins which are mainly abundant in storage tissues such as tubers and seeds, but are also found in the aerial parts.
- Protease inhibitors are widely distributed throughout the plant kingdom and they play important roles in the defense against herbivores and pathogens.
- The protease inhibitors are divided into four classes, i.e. serine, cysteine, aspartic and metallo-protease inhibitors.
- Of these, the most abundant are serine PIs and are present in seeds, leaves and tubers of several members of the Fabaceae, Poaceae and Solanaceae.



## Lectins

- Lectins are glycoprotein of nonimmune origin that recognize and bind carbohydrates.
- These proteins are found in a wide variety of species (viruses, bacteria, fungi, seaweed, animals, and plants). Plant Lectins have been widely studied, and in this group, the legume Lectins have been related to insecticidal activities.

# Definition and general features of Lectins

➤ The term Lectins is derived from the Latin word *legere* meaning “to choose” or “select”

➤ The Lectins are commonly called hemagglutinins.

➤ Lectins may be obtained from plant and may be soluble or membrane bound. In nature, Lectins play a role in biological recognition phenomena involving cells and proteins and thereby protect plants against external pathogens such as fungi and other organisms.

# Biological activities of plant Lectins

- Lectins are mainly present in seeds of plants but they are also identified in vegetative tissues such as bulbs, tubers, rhizomes, roots, bark, stems, fruits, and leaves.
- Plant Lectins can be divided into four classes basis on their number domain and characteristics.

**I. Merolectins**

**II. Hololectins**

**III. Chimerolectins.**

**IV. Superlectins**

## Insecticide activity of plant lectins

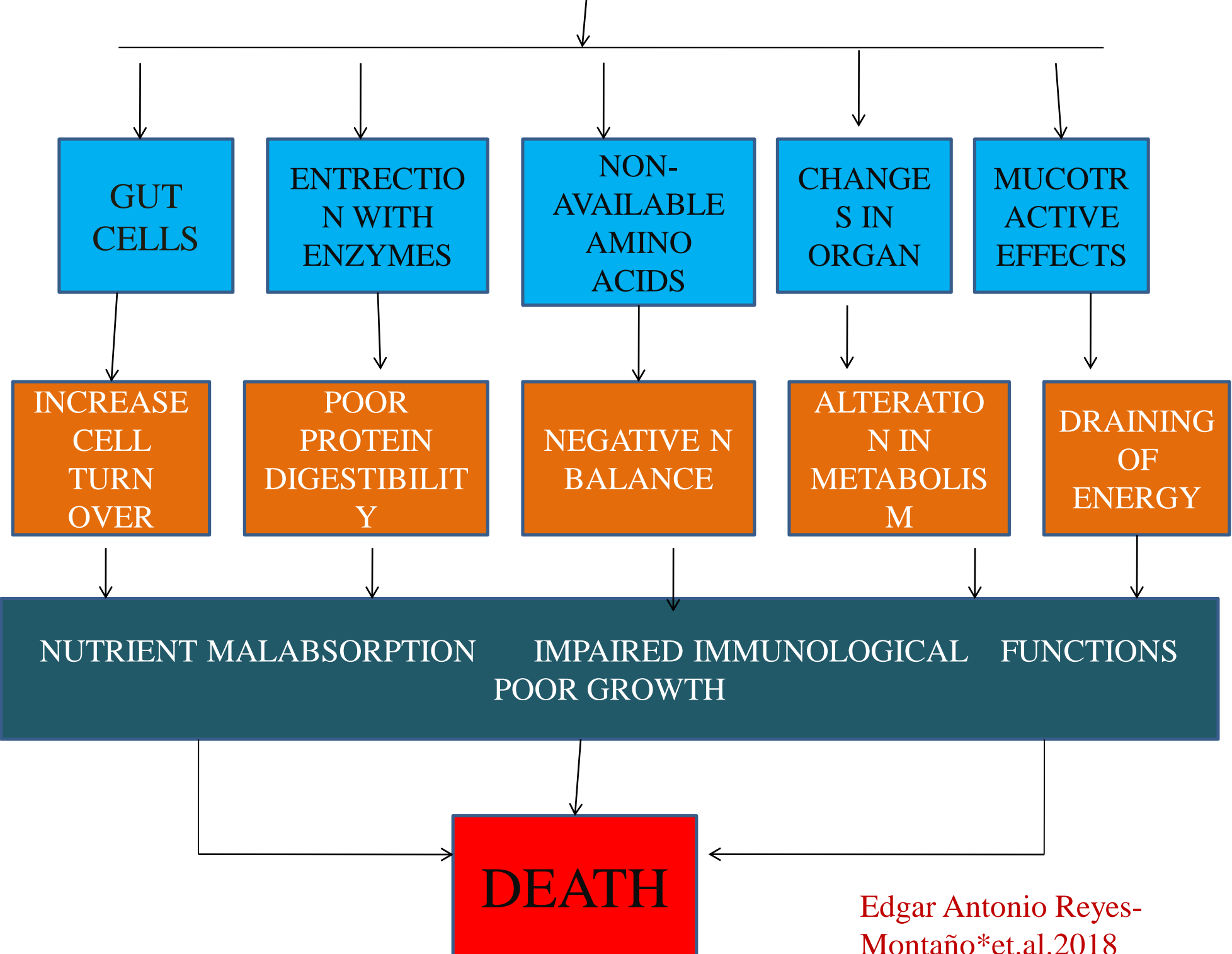
- To date a great number of studies have shown lectin toxicity in insects belonging to different orders, including **Lepidoptera, Coleoptera, and Hemiptera**.
- Lectins are currently receiving a significant interest as insecticidal agents against sap-sucking insects including **aphids and leaf and plant hoppers**, with no effect on human metabolism.
- Lectins act on insects by binding to glycoprotein's present in insect gut epithelium, eventually causing death of insect by inhibiting absorption of nutrients.

## Conti....

➤ Lectins from legume family have shown insectistatic and insecticidal activity . The lectins from seeds of *Canavalia brasiliensis*, *Dioclea grandiflora*, *Dioclea rostrata*, and *Phaseolus vulgaris* have shown to protect seeds against the beetle *Callosobruchus maculatus*.

➤ Preliminary evidence of Gleheda's (*Glechoma hederacea*) insecticidal activity against Colorado potato beetle larvae (*Leptinotarsa decemlineata*) has been obtained using a single dose of lectin .

# LECTINS



Edgar Antonio Reyes-Montaña\*et.al.2018

## Advantages of transgenic plants

- Improvement in nutritional value of food.
- Increase in farmer's income.
- Increase in food supply.
- Resistance to insect.
- Tolerance to specific herbicides.
- Imply lower pollution.

## Disadvantages of transgenic plants

- Damage to human health.
- Disruption of current practices of farming and food production in developed countries
- Disruption of traditional practices and economies in less development countries.
- Lack of research on consequences of transgenic crops.



## Conclusion

- Transgenic plants have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by yield and reducing reliance upon chemical pesticides and herbicides.
- Transgenic technology can be easily integrated with other control methods like biological, cultural, mechanical, pheromones and even chemical pesticides.
- Effective dissemination of correct information and proper guidance is a prerequisite to remove any misconception or apprehension about this remarkable new technology.

- A meta-analysis concluded that GM technology adoption had reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%.
- This reduction in pesticide use has been ecologically beneficial, but benefits may be reduced by overuse.

# Reference

- Abdallah, N.A., Prakash, C.S., McHughen, A.G., 2015. Genome editing for crop improvement: challenges and opportunities. *GM Crop. Food* 6 (4), 183\_205.
- Azizoglu, U., Yilmaz, S., Ayvaz, A., & Karabörklü, S. (2015). Effects of *Bacillus thuringiensis* subsp. *kurstaki* HD1 spore-crystal mixture on the adults of egg parasitoid *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae). *Biotechnology & Biotechnological Equipment*, 29(4), 653-658.
- Bakhsh, A., Dinc, T., Hussain, T., Demirel, U., Aasim, M., & Çalışkan, M. E. (2018). Development of transgenic tobacco lines with pyramided insect resistant genes. *Turkish Journal of Biology*, 42(2), 174-186.
- Bakhsh, A., Siddique, S., Husnain, T., 2012. A molecular approach to combat spatiotemporal variation in insecticidal gene (*Cry1Ac*) expression in cotton. *Euphytica* 183 (1), 65\_74.
- Crickmore, N., Zeigler, D. R., Feitelson, J., Schnepf, E. S., Van Rie, J., Lereclus, D., & Dean, D. (1996). Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and molecular biology reviews*, 62(3), 807-813.

- Down, R.E., Ford, L., Woodhouse, S.D., Raemaekers, R.J.M., Leitch, B., Gatehouse, J.A. & Gatehouse, A.M.R. (2000). Snowdrop lectin (GNA) has no acute toxic effects on a beneficial insect predator, the 2-spot ladybird (*Adalia bipunctata* L.). *Journal Insect Physiology*. 46: 379-391.
- EPA (2001). International Center for Technology Assessment, FIFRA Scientific Advisory Panel, Subpanel on Bt Plant-Pesticides Risk and Benefit Assessments (SAP Report No. 2000-07). <http://www.epa.gov/scipoly/sap/2000/october/octoberfinal.pdf>
- Hågvar, E.B. (2002). Some studies on the effects of gene modified (gm) plants on insects. Proceedings from Vietnamese-Norwegian workshop on Biological control of crop pests, *Plant. Ås. Grønn Forsk.* 13: 93-96.
- Hilder, V.A., Gatehouse, A.M., Boulter, D., 1993. Transgenic plants conferring insect tolerance: protease inhibitor approach. In: *Transgenic Plants*. Academic Press, New York. pp. 317\_338.
- Höfte, H., & Whiteley, H. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological reviews*, 53(2), 242-255.
- Kannan, M., Uthamasamy, S. & Mohan, S. (2004). Impact of insecticides in sucking pests and natural enemy complex of transgenic cotton. *Current Science*. 86: 726-727.

- Klein, T. M., Arentzen, R., Lewis, P. A., & Fitzpatrick-McElligott, S. (1992). Transformation of microbes, plants and animals by particle bombardment. *Bio/technology*, 10(3), 286-291.
- McBride, K.E., Svab, Z., Schaaf, D.J., Hogan, P.S., Stalker, D.M., Maliga, P., 1995. Amplification of a chimeric Bacillus gene in chloroplasts leads to an extraordinary level of an insecticidal protein in tobacco. *Biotechnology* 13 (4), 362.
- Montagu, V.M., & Schell, J. (1987). Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. *Nature*, 303(5914), 209-213.
- Naranjo, S.E., Head, G. & Dively, G.P. (2005). Field studies assessing arthropod nontarget effects in *Bt* Transgenic crops: introduction. *Environment Entomology*. 34: 1178-1180.
- Nikolay, K., Omar A., 2019. New CRISPR-based technology developed to control pests with precision-guided genetics. ,<https://phys.org/news/2019-01-crispr-based-technology-pests-precision-guided-genetics.html>..
- Poppy, G.M. & Sutherland, J.P. (2004). Can biological control benefit from genetically-modified crops? Tritrophic interactions on insect-resistant transgenic plants. *Physiology Entomology*. 29: 257- 268.

- Qaim, M., Zilberman, D., 2003. Yield effects of genetically modified crops in developing countries. *Science* 299 (5608), 900\_902.
- Rajamohan, F.& Dean, D. H. (1995). Single amino acid changes in domain II of *Bacillus thuringiensis* CryIAb delta-endotoxin affect irreversible binding to *Manduca sexta* midgut membrane vesicles. *Journal of bacteriology*, 177(9), 2276-2282.
- Reyes-Montaño, E. A., & Vega-Castro, N. A. (2018). Plant lectins with insecticidal and insectistatic activities. *Insecticides-Agriculture and Toxicology*; IntechOpen: London, UK, 17-42.
- Romeis, J., Babendreier, D., Wäckers, F.L. (2003). Consumption of snowdrop lectin (*Galanthus nivalis* agglutinin) causes direct effects on adult parasitic wasps. *Oecologia* 134: 528-536.
- Romeis, J., Meissle, M. & Bigler, F. (2006). Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology*. 24: 63-71.
- Sayyed, A. H., Cerda, H., & Wright, D. J. (2003). Could Bt transgenic crops have nutritionally favourable effects on resistant insects?. *Ecology letters*, 6(3), 167-169.

- Watkinson, A.R., Freckleton, R.P., Robinson, R.A. & Sutherland, W.J. (2000). Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science* 289: 1554- 1557.
- Wu, Y., 2014. Detection and mechanisms of resistance evolved in insects to Cry toxins from *Bacillus thuringiensis*. *Advanced Insect Physiology*. 47, 297\_342.
- Xu, C., Wang, B.C., Yu, Z., Sun, M., 2014a. Structural insights into *Bacillus thuringiensis* Cry, Cyt and parasporin toxins. *Toxins* 6 (9), 2732\_2770.
- Zwahlen, C., Hilbeck, A., Gugerli, P., & Nentwig, W. (2003). Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Molecular Ecology*, 12(3), 765-775.

**THANK  
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