Bharathidasan University

Tiruchirappalli- 620024,
Tamil Nadu, India

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Unit Iv - MOLECULAR FARMING IN PLANTS

DR. A. LAKSHMI PRABHA
Professor
Department of Botany

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- Brief History of Molecular Farming
- Why Molecular Farming ??
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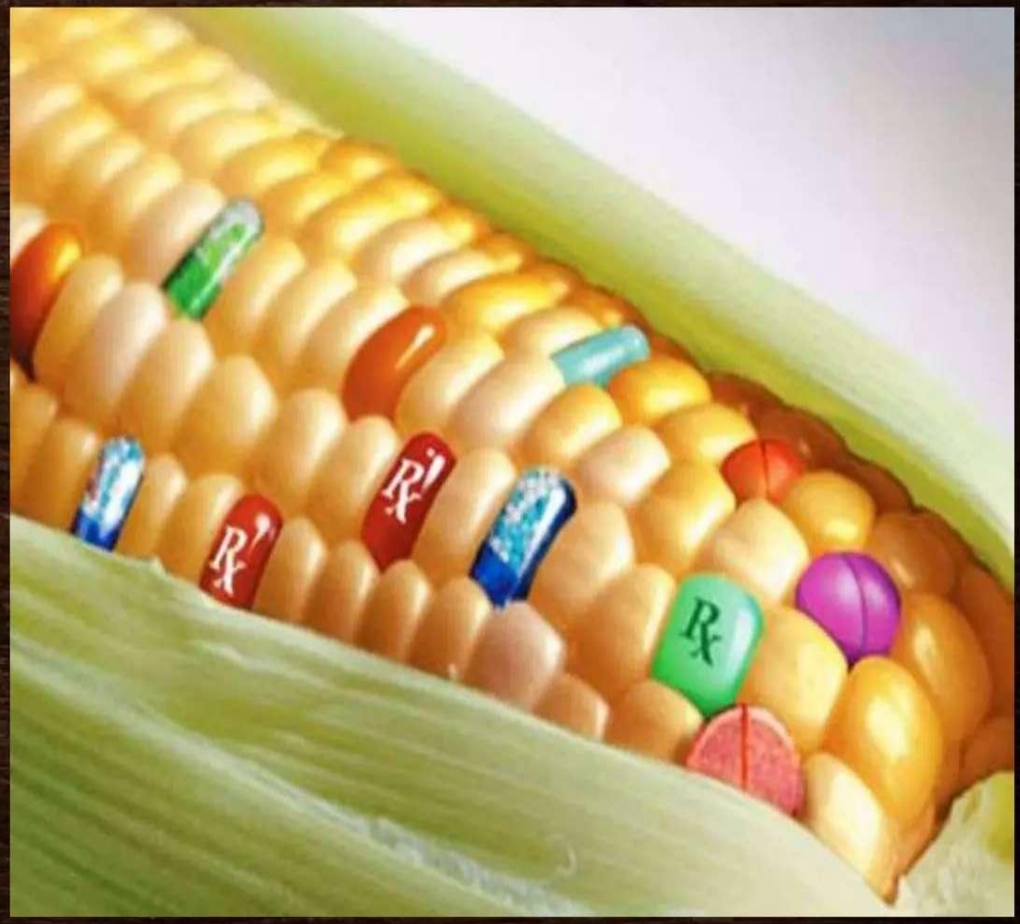
What is Molecular Farming ??

- The use of whole organisms, organs, tissues or cells or cell cultures, as bioreactors for production of commercially valuable products via recombinant DNA techniques.
- Molecular farming is an application of GM Technology.
- "Molecular pharming" is a technology that uses plants to produce large quantities of pharmaceutical substances such as vaccines and antibodies.



HISTORY OF MOLECULAR FARMING

1986 First plant - derived recombinant therapeutic protein- human GH in tobacco & sunflower. (A. Barta, D. Thompson et al.)

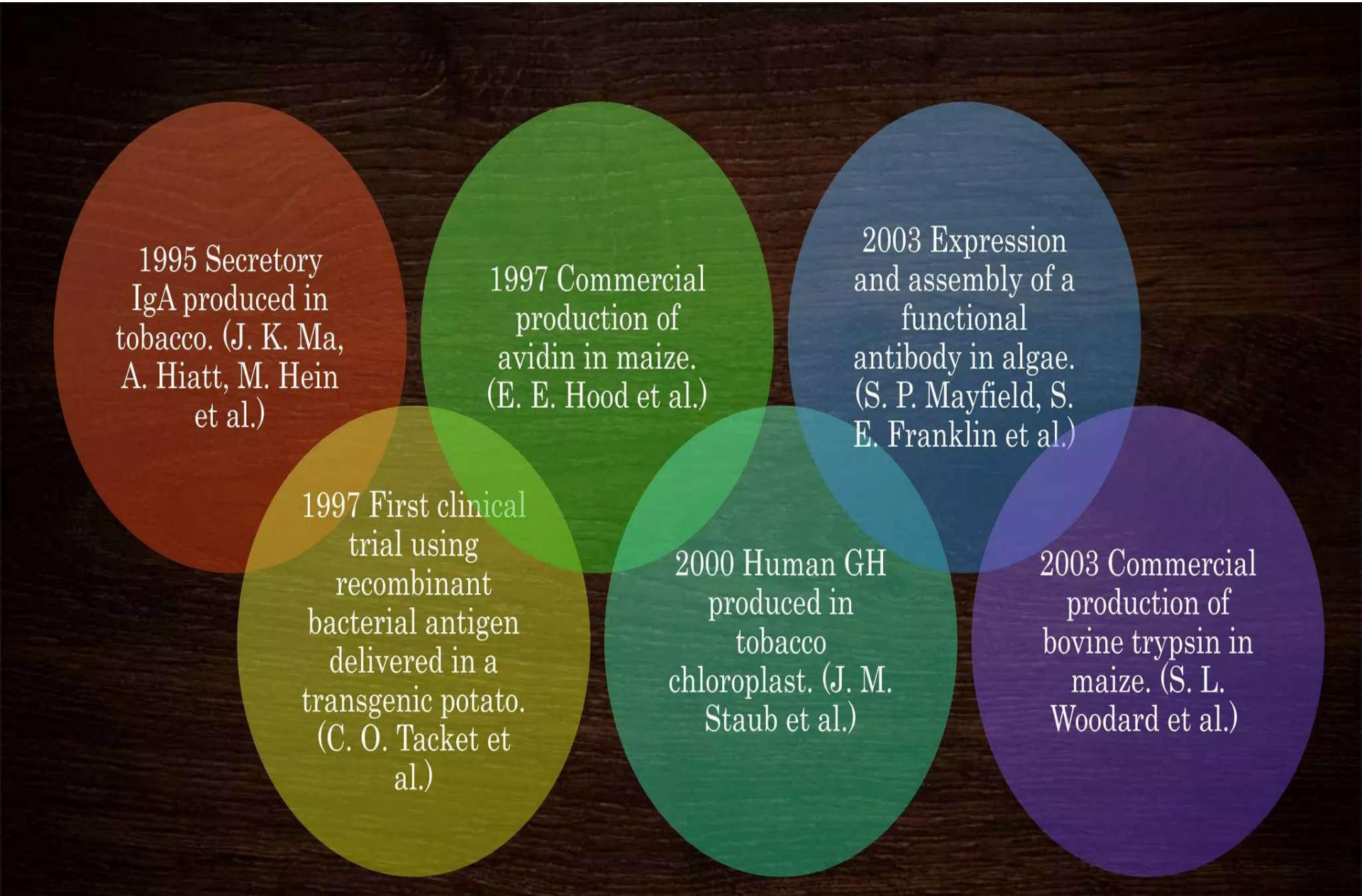
1990 First native human protein produced in plants – human serum albumin in tobacco & potato. (P. C. Sijmons et al.)

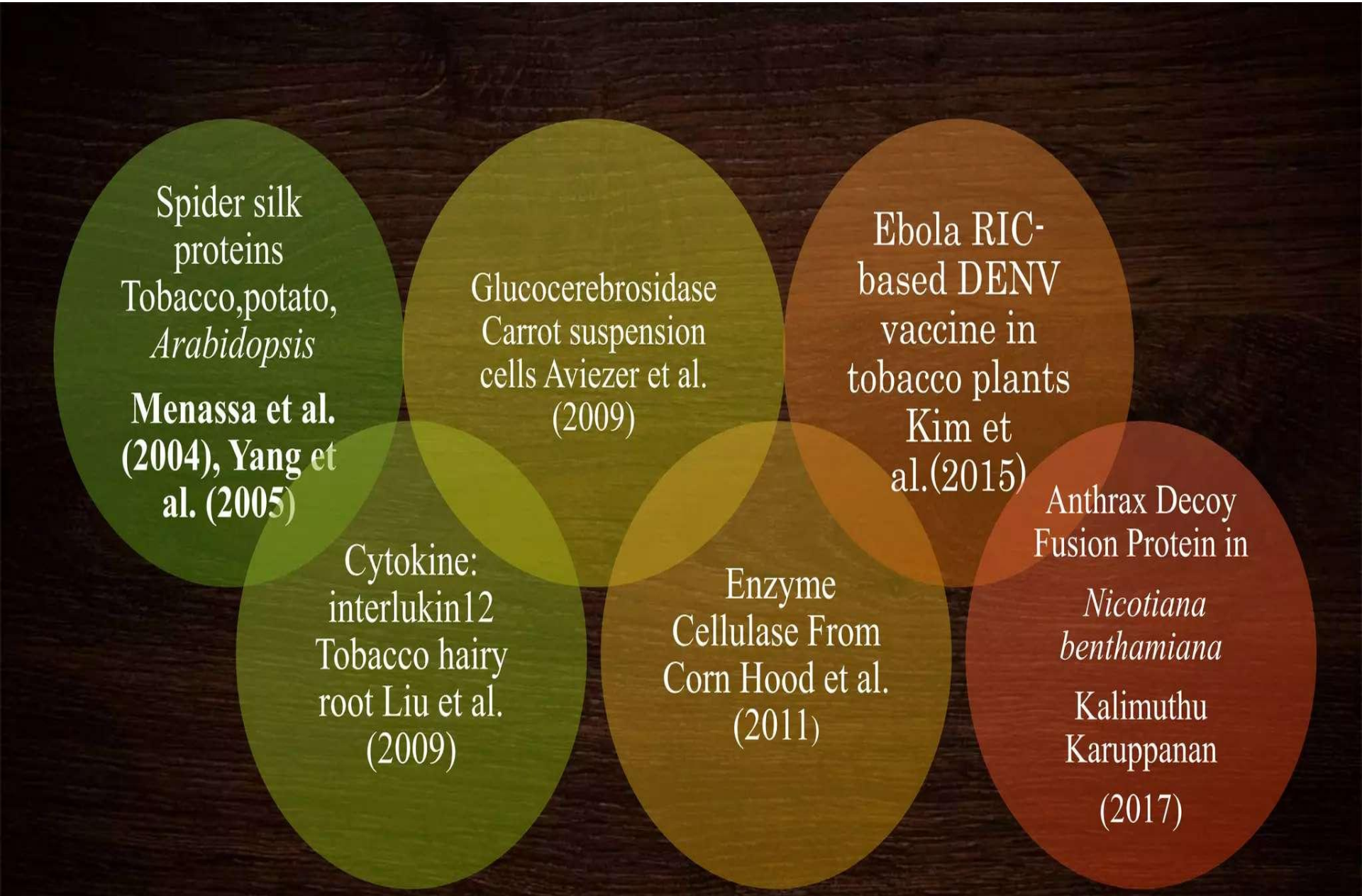
1992 First plant derived industrial enzyme – α- amylase in tobacco. (J.Pen, L. Molendijk et al.)

1989 First plant - derived recombinant antibody – full-sized IgG in tobacco. (A. Hiatt, K. Bowdish)

1992 First plant derived vaccine candidate – hepatitis B virus surface antigen in tobacco. (H. S. Meson, D. M. Lam)—

1996 First plant derived protein polymer – artificial elastin in tobacco. (X. Zhang, D. W. Urry, H. Daniel)


1995 Secretory IgA produced in tobacco. (J. K. Ma, A. Hiatt, M. Hein et al.)
1997 Commercial production of avidin in maize. (E. E. Hood et al.)
2003 Expression and assembly of a functional antibody in algae. (S. P. Mayfield, S. E. Franklin et al.)
1997 First clinical trial using recombinant bacterial antigen delivered in a transgenic potato. (C. O. Tacket et al.)
2000 Human GH produced in tobacco chloroplast. (J. M. Staub et al.)
2003 Commercial production of bovine trypsin in maize. (S. L. Woodard et al.)

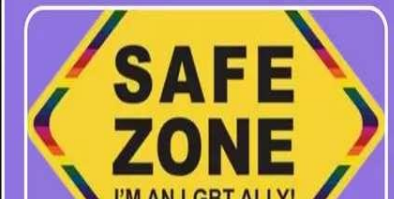

Spider silk proteins Tobacco,potato, *Arabidopsis*
Menassa et al. (2004), Yang et al. (2005)
Glucocerebrosidase Carrot suspension cells Aviezer et al. (2009)
Ebola RIC-based DENV vaccine in tobacco plants Kim et al.(2015)
Cytokine: interlukin12 Tobacco hairy root Liu et al. (2009)
Enzyme Cellulase From Corn Hood et al. (2011)
Anthrax Decoy Fusion Protein in *Nicotiana benthamiana*
Kalimuthu Karuppanan
(2017)

WHY MOLECULAR PHARMING??

A major advantage of transgenic plants for molecular farming is the comparatively low cost of large-scale production. It is estimated that recombinant proteins can be produced in plants at 2–10% of the cost of microbial fermentation systems and at 0.1% of the cost of mammalian cell cultures(Trends in biotechnology, Vol 21/Issue12, 2003).

Plant-based production can be modulated rapidly in response to market demand simply by using more or less land as required.

Recombinant protein expression in the seeds of transgenic cereal plants results in high levels of the product accumulation in a small volume, which minimizes the costs associated with processing. (An example is the oleosin-fusion platform developed by SemBioSys Genetics)



As a production system for pharmaceutical proteins, plants are considered to be much safer than both microbes and animals because they generally lack human pathogens, oncogenic DNA sequences, and

Another advantages of molecular pharming is the well-established technology for gene transfer and expression, high biomass yield, prolific seed production and the existence of a large-scale processing infrastructure.

Plant as a Bioreactor is easy to store the products like storage in seeds for longer durations. Another fundamental advantage of plants has always been the range and diversity of recombinant molecules that they can potentially produce

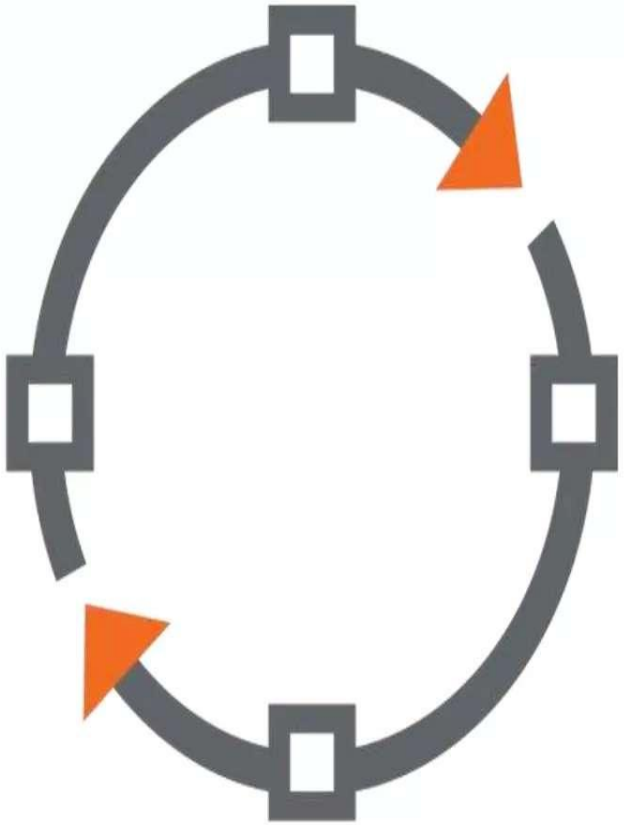
Easy to purify proteins and sometimes several types of recombinant protein can be used in unprocessed or partially processed material, therefore removing many of the downstream costs (can be consumed directly).

As a production system for pharmaceutical proteins, plants are considered to be much safer than both microbes and animals because they generally lack human pathogens, oncogenic DNA

Comparison of Expression Systems

Expressions System	Yeast	Bacteria	Plant viruses	Transgenic Plants	Animal Cell Cultures	Transgenic Animals
Cost of maintaining	inexpensive	inexpensive	inexpensive	inexpensive	expensive	expensive
Type of storage	-2.0°C	-2.0°C	-2.0°C	RT*	N_2**	N/A
Gene size (protein) restriction	Unknown	Unknown	Limited	Not limited	Limited	Limited
Production cost	Medium	Medium	Low	Low	High	High
Protein yield	High	Medium	Very high	High	Medium to high	High
Therapeutic risk	Unknown	yes	Unknown	Unknown	yes	yes

HOW TO DO MOLECULAR PHARMING??



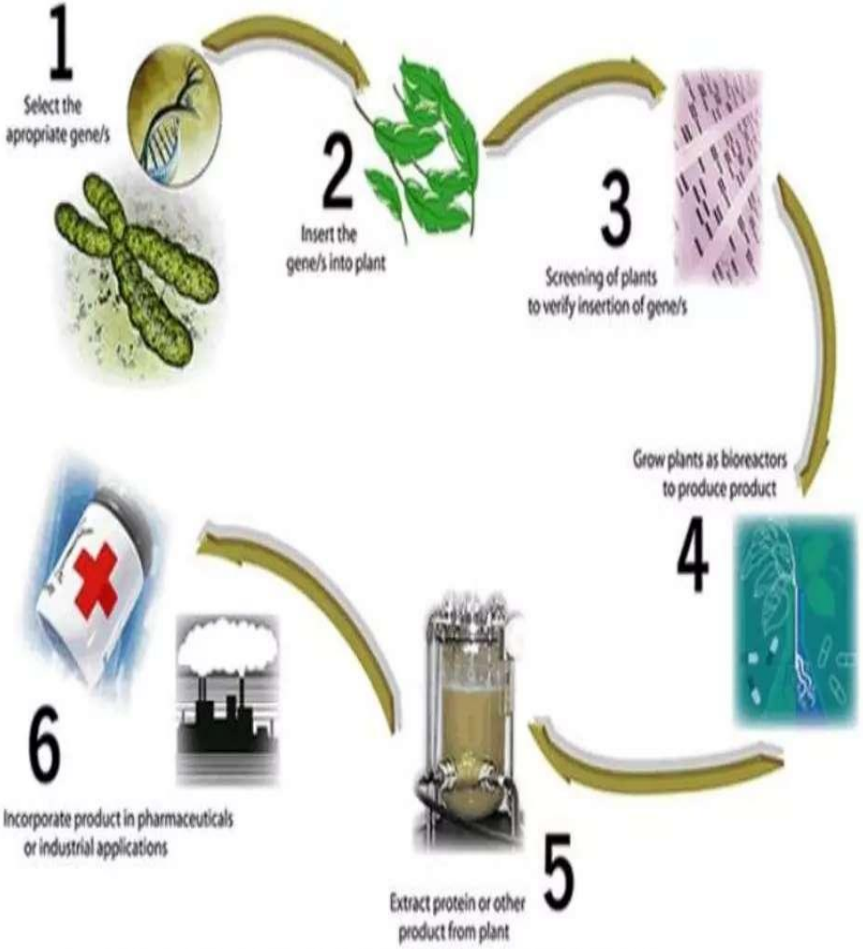
PROCESS

Steps In Molecular Pharming

Gene construct → Introduction Into Vector → Plant transformation → Plant regeneration

Selection of producers → Protein characterisation → Downstream processing → Pre-clinical and clinical trials

The diagram below is a simplified representation of the Molecular Farming aproach to the production of biomolecules



Presented in Credit Seminar (Division of Agricultural Physics, IARI, New Delhi) by Nirmal Kumar

PLANT Transformation strategies

Stable nuclear transformation

Stable nuclear transformation involves the incorporation of a foreign gene or genes (exogenous) of interest into the nuclear genome of the plant, thereby altering its genetic makeup, and leading to the expression of the transgene

Stable plastid transformation

Transformation of plastid genome. provides a valuable alternative to nuclear transformation because it eliminates the provision of a natural biocontainment of transgene flow by out-crossing (as plastids are inherited through maternal tissues in most species and the pollen does not contain chloroplasts, hence the transgene may not be transferable

Plant cell-suspension cultures.

Transient expression systems.

Fastest and the most convenient production platform for plant molecular farming

3 Types

Agroinfiltration method

(Agrobacterium-mediated)

Virus infection method

Tobacco mosaic virus (TMV) and potato virus X (PVX) are used as vectors to deliver foreign genes into plants, without integration

Magnifection Technology

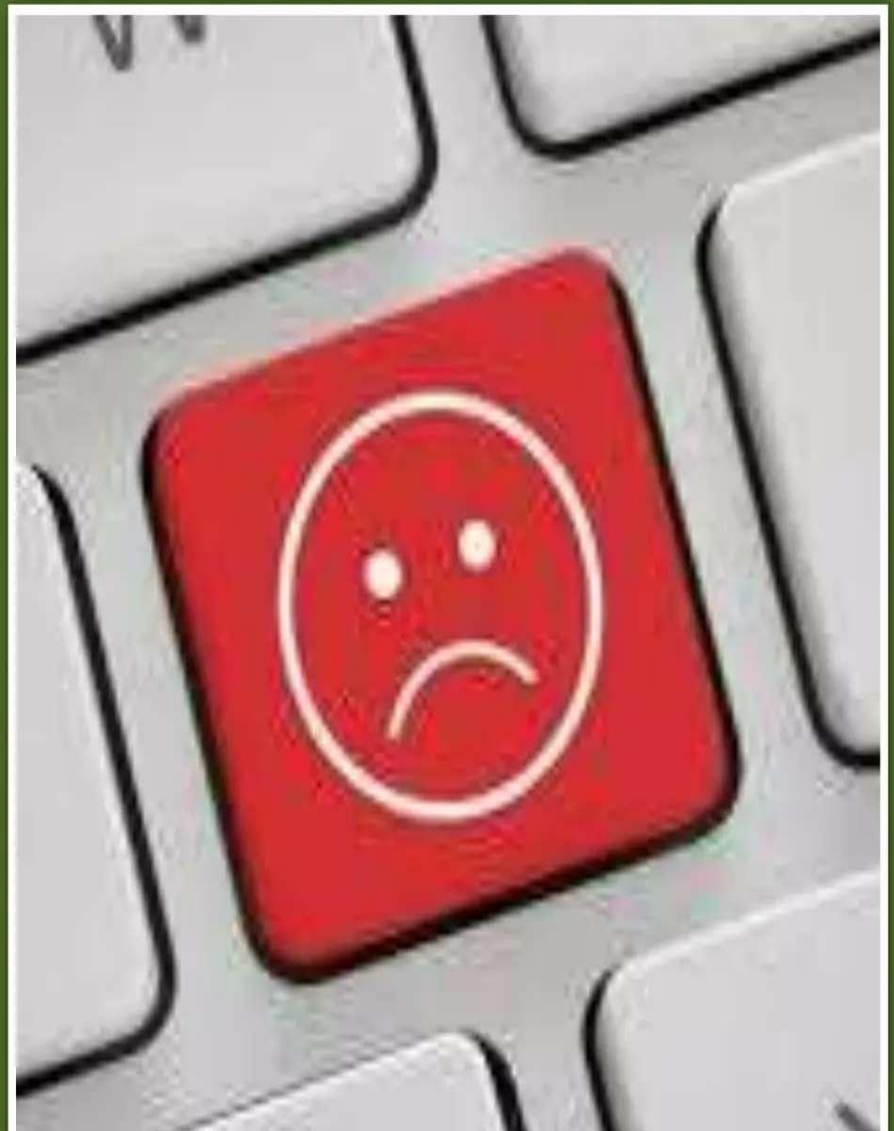
New robust transient expression system known as MagnICON® technology, (developed by Icon Genetics)

By removing the coat protein (responsible for systemic movement) of the noncompeting virus strains and the systemic delivery of the resulting viral vectors to the entire plant using Agrobacterium as the vehicle of delivery and primary infection

WHAT ARE ITS APPLICATIONS IN AGRICULTURE ??

- Production and use of bioplastics and Biopolymers in post harvest packaging of horticultural crops for long transport.
- Production of secondary plant metabolites (Jasmonic acid, salicylic acid etc) in the plants at triggered levels for disease resistance.
- Production of biofuels and over production of starch content in maize for production of sugar syrup.
- Production of edible vaccines or extraction of non-edible vaccines from plants.
- Production of enzymes and other proteins in the crop plants for there clinical use, which can be alternative use of agricultural crops other than food.
- over production of vitamins which are deficient in crop plants can be used to reduce the deficiency of vitamins in the target groups of

What are the Drawbacks of Molecular pharming??



- Biosafety concerns

1. horizontal transfer
2. Vertical gene transfer (Pollen mediated)
3. transformed plants can be eaten up by animals

- Ethical issues
- If consumed inadvertently, it could lead to desensitization of vaccines(direct consumption)
- Production problems (low productivity and complex production process)
- Time requirement (Require time for initial batch production when compared to microbial systems)

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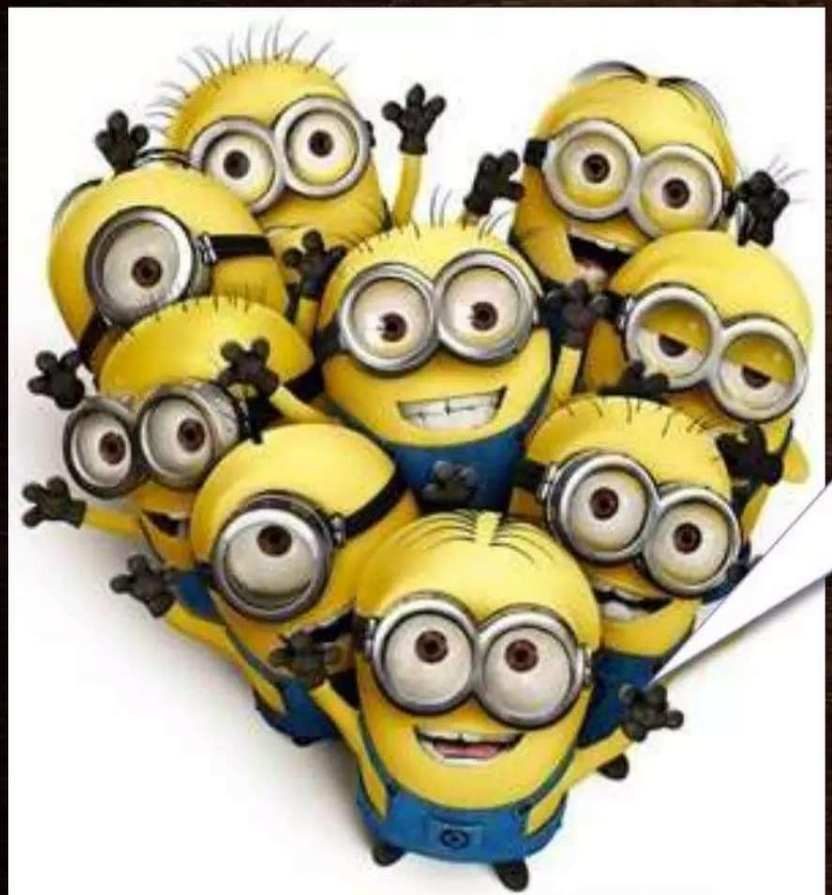

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