Contents

1. Propagation Of Planting Materials For Vegetatively Propagated Crops

Propagation Of Planting Materials For Vegetatively Propagated Crops

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Propagation techniques

Cuttings, in fruit trees and sugarcane, bamboo.

Runners in straw berry.

“Eye” of the potatoe tuber.

Division of the crown in some grasses and legumes.

Grafting in roses and fruit trees.

1. .

Splits in pyrethrum.

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Tissue culture

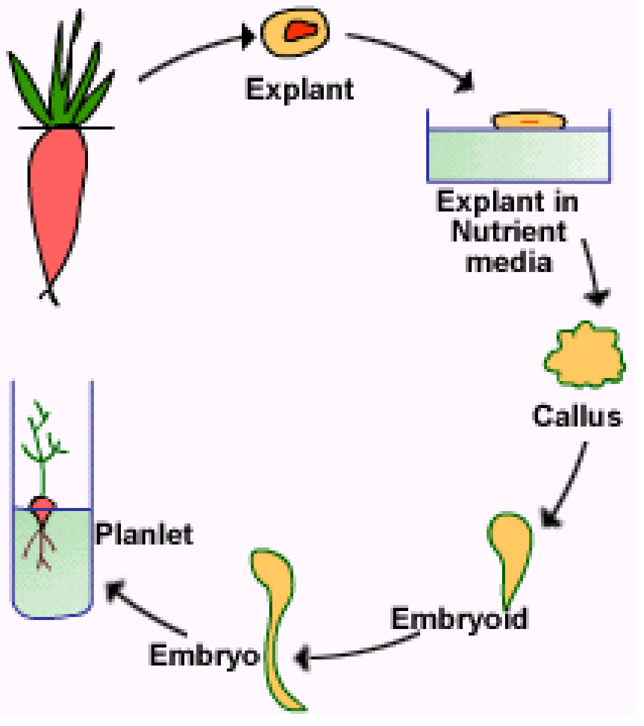
Tissue culture

- Plant cell and tissue culture involves the culture of isolated plant cells or attached fragments of plant tissue on a nutrient medium under aseptic conditions, to regenerate whole functional plants.

- Competency- endogenous potential of given cell/plant tissue to develop certain way(forming distinct plant organs eg. Stems leaf etc.

PlANT CELLS ARE TotipotenT

- Totipotency: ability of a cell or tissue or organ to grow and develop into a fully differentiated organism



CELLS ARE COMPETENT

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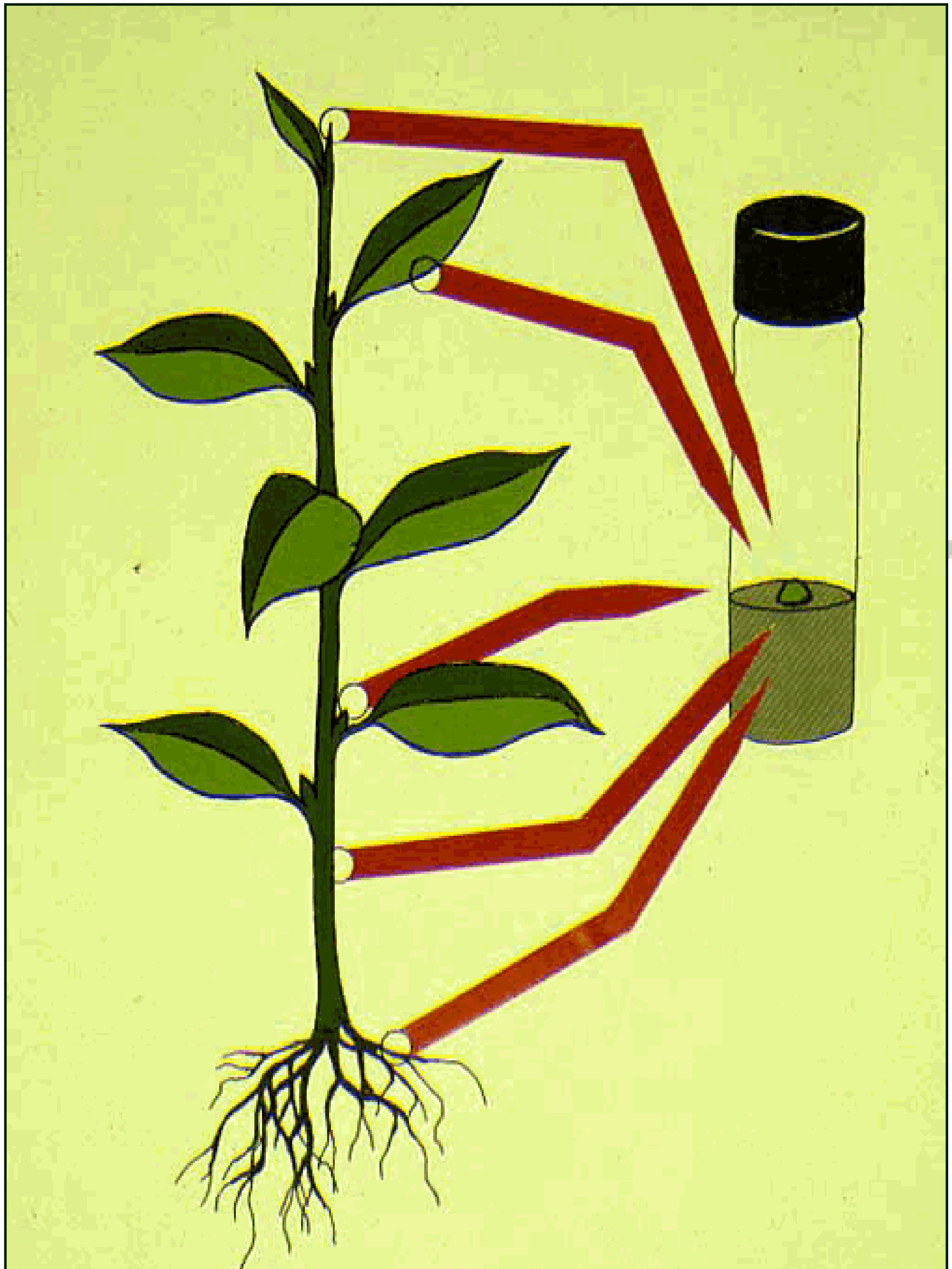


Explant

- Cell, tissue or organ of a plant that is used to start in vitro cultures
- Many different explants can be used for micropropagation, but axillary buds and meristems are most commonly used.

Explants cultured

- Explants- multicellular tissue fragments.
- Organ cultures
  - Meristem culture.
  - Shoot tip culture.
  - Root tip culture.
  - Seed culture.
  - Hypocotyl.
  - Auxillary bud.
  - Leaf culture.
- Callus culture.
- Haploid cultures.
  - Ovule culture.
  - Anther culture
- Protoplast culture.
- Embryo culture.
  - Zygotic ombryo culture.
  - Nucellar embryo culture.

Steps of Micropropagation

Stage 0 – Selection & preparation of the mother plant

sterilization of the plant tissue takes place

Stage I - Initiation of culture

explant placed into growth media

Stage II - Multiplication

explant transferred to shoot media; shoots can be constantly divided

Stage III - Rooting

explant transferred to root media

Stage IV - Transfer to soil

explant returned to soil; hardened off

CITRUS TISSUE CULTURE


Seeds extracted from young fruits


1 month old citrus seedlings


Excised seeds cultured on media


Mass propagation of citrus

CITRUS TISSUE CULTURE


2 months old citrus in GH


6 months old citrus budded


4 months old citrus in GH


18 month TC citrus in orchard

Tissue culture Media

- This is a solid or liquid nutrient base that provides the multiplying and differentiating cells and tissues with the necessary micro and macronutrients.

Tissue culture Media (components)

- Macronutrients.
- Micronutrients.
- Organic substances.
- Vitamins.
- Plant hormones (growth regulators).
- Carbon source (sucrose).
- Undefined natural substances.
- Gelling agents (Agar)

Plant growth regulators/ plant hormones

- Auxins.
- Cytokinins.
- Gibberellins.
- Abscisic acid.
- Polyamines.

Aseptic technique

All operations in tissue culture must be sterile. i.e. done in the absence of actively dividing micro-organisms.

Can be done at three levels, sterilization of the working area, sterilization of the media and the explant

Work area sterilization

- Laminar flow hood (filters)
- Open flame
- Dry heat (Oven) (100-150 ^{0}C)
- Chemical sterilization (Sodium hypochlorite, 95% ethanol, commercial disinfectants eg. Lysol and zephiram.
- U.V. light.

Aseptic technique cont

Media sterilization

Autoclave (Moist heat) 121^0C

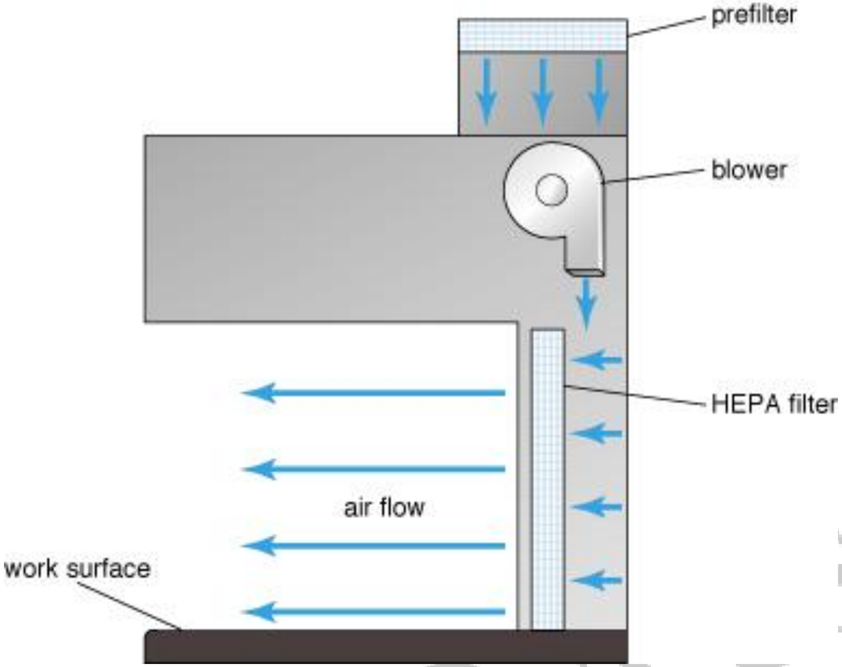
Filter Sterilization (using suction and microfilters)

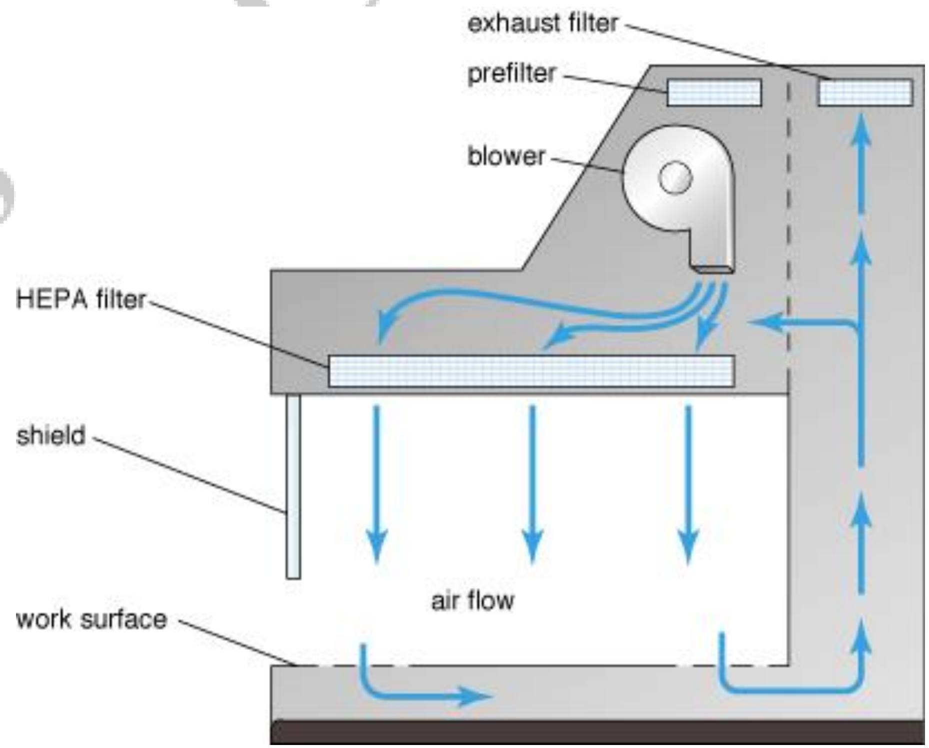
Explant sterilization

Chemical sterilization (Sodium hypochlorite, 95% ethanol)

Its vital to use sterile glassware and sterile equipment which can be sterilized using the above methods.

Laminar flow hood


prefilter
blower
HEPA filter
air flow
work surface


exhaust filter
prefilter
blower
HEPA filter
shield
air flow
work surface

Applications of T.C. (1)

Clonal propagation,

All explants are propagated from somatic tissue, thus they have similar genetic composition and diploid set of chromosomes.

Can produce thousands of similar seedlings from single plant (cloning)

Techniques used to regenerate PLANTS

Mass production of disease free rootstocks, scions and whole plants.

Invitro propagation through Callus;-mass actively dividing undiferentiated cells produced by explant

Invitro propagation via somatic embryogenesis; embyos induced on vegetative tissue

Reduces the time required to produce sufficient planting material.

Callus

Somatic embryos

Propagation

Disease elimination from rootstocks, scions and whole plants.

Isolation of pathogen-free plant materials.

Bacterial- Citrus greening disease

Viral- Passion fruit woodiness virus; Cassava Mosaic Virus; Sweet potatoe viruses.

Provide farmers with clean planting material.

Applications of T.C.

- **<u>Germplasm conservation</u>**
- *invitro* collection.
- Characterization.
- Indexing of germplasm (conventional and molecular techniques.
- Safe movement or shipment of germplasm.
- Inline with KEPHIS requirements for safe movement of plant materials across borders.

GMOS

DEVELOPMENT OF A GMO.

Scientists needs to isolate specific parts of DNA.

Cut DNA at precise locations using specific enzymes.

Introduce the DNA into plant cells.

Select the transformed tissues and plants.

INDIRECT GENE TRANSFER TECHNIQUES

Agrobacterium-Mediated Gene Transfer

Agrobacterium tumefaciens and A. rhizogenes, respectively.

Ti plasmids.

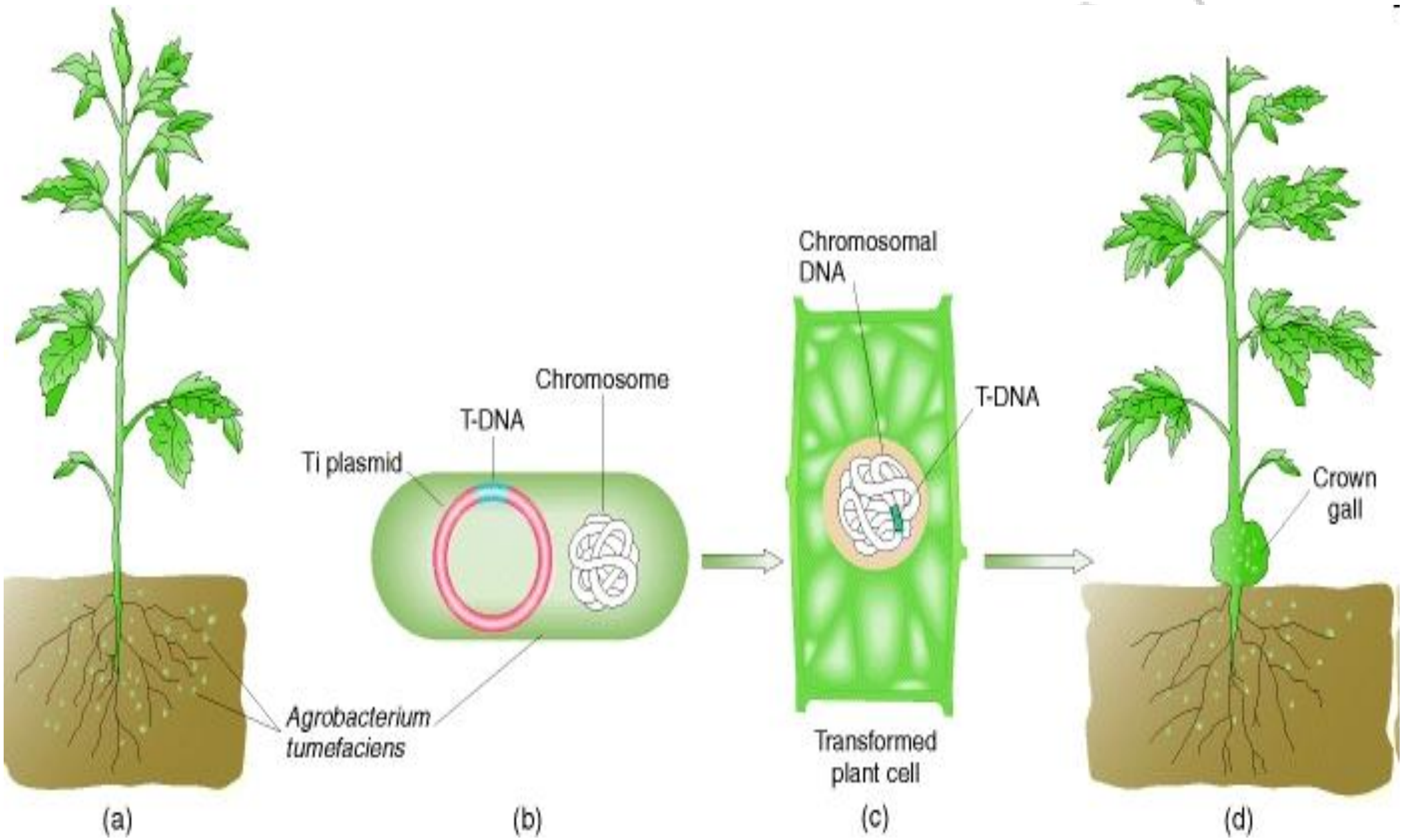
T-DNA

Agrobacterium mediated transformation.

Soil born gram negative bacteria

unique ability to
 introduce part of DNA
 into plant cells

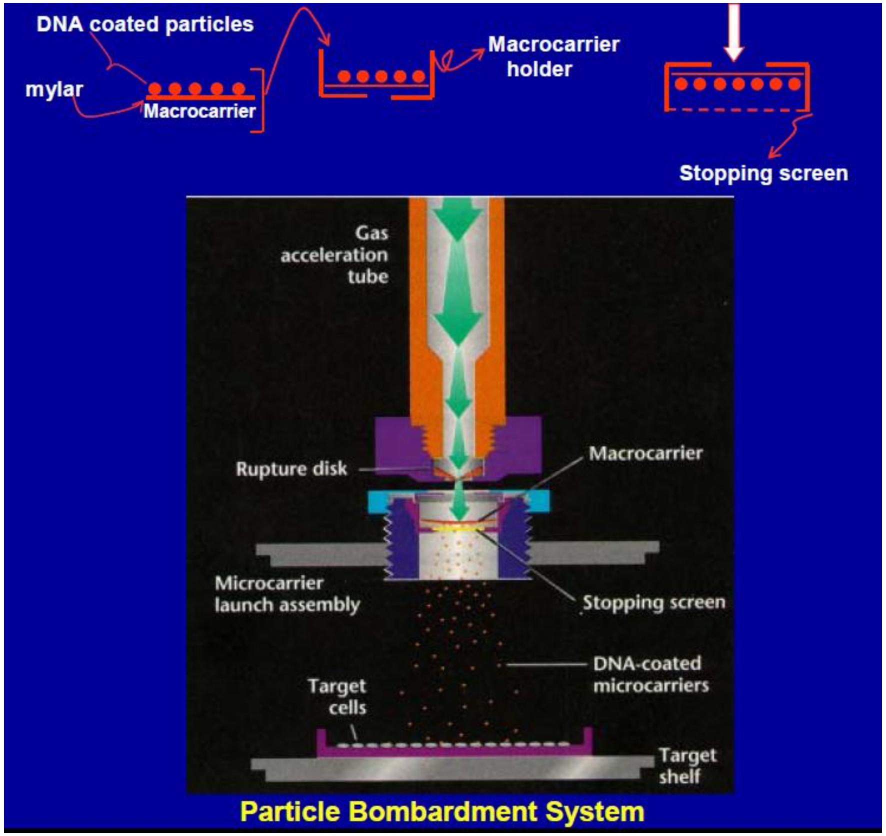
gene transfer



Direct gene transfer

- Polyethylene glycol (PEG).
- Electroporation.
- Microinjection
- Microprojectile bombardment (Biolistic).

The Particle Bombardment PROCESS.

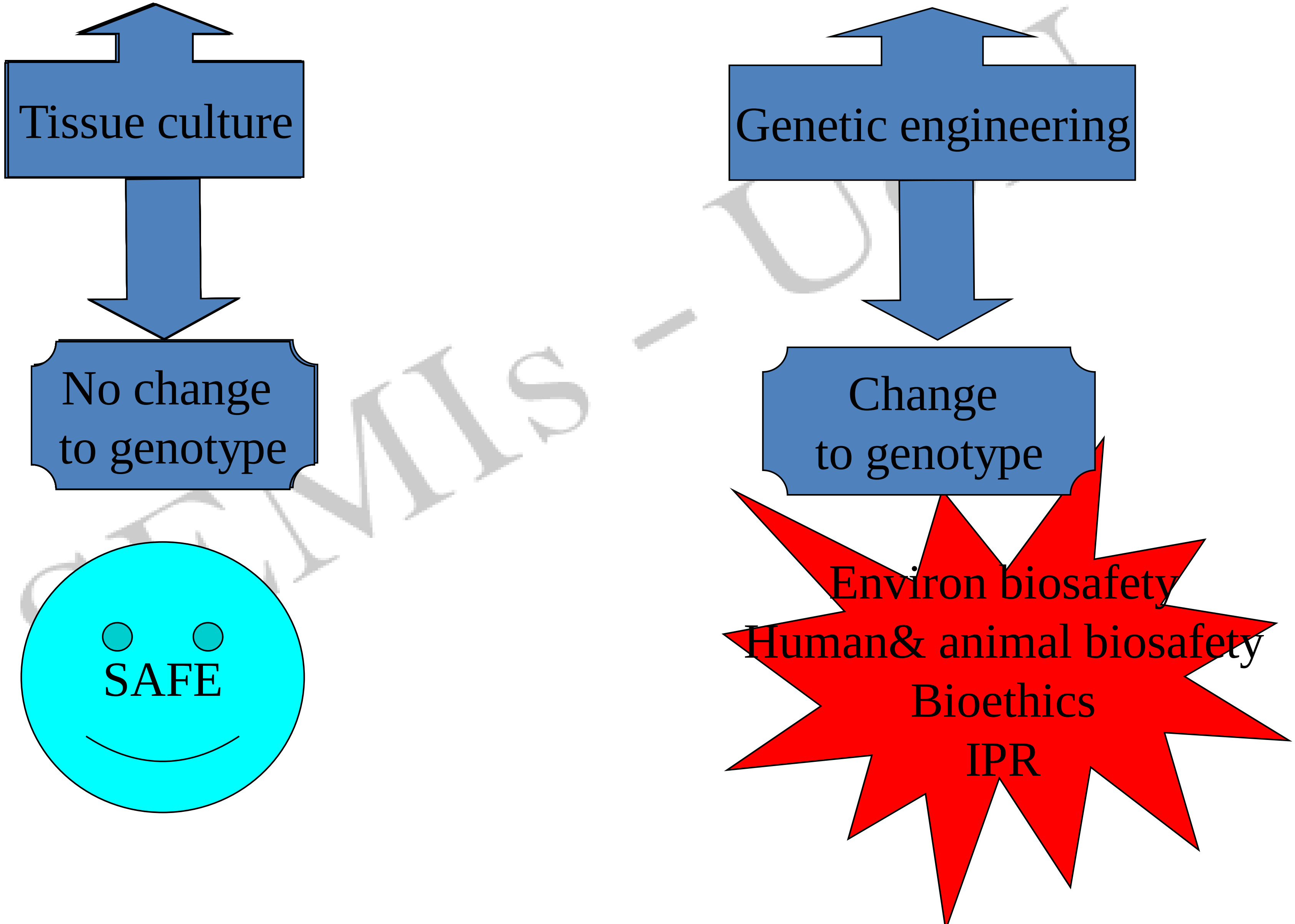


CONCERNS IN GENETIC ENGINEERING

- Negative impact to environment
  - weediness of weedy relatives
  - Bt crop toxins destroy non-target beneficial insects
  - Contamination of the “centers of origin” eg mexico

- Safety to humans &animals
  - Health and allergy related risks
- Bioethics
- Intellectual Property Rights

BIOTECHNOLOGY



Most controversial!

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Food and Chemical Toxicology

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Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

Gilles-Eric Séralini [a,*], Emilie Clair [a], Robin Mesnage [a], Steeve Gress [a], Nicolas Defarge [a], Manuela Malatesta [b], Didier Hennequin [c], Joël Spiroux de Vendômois [a]

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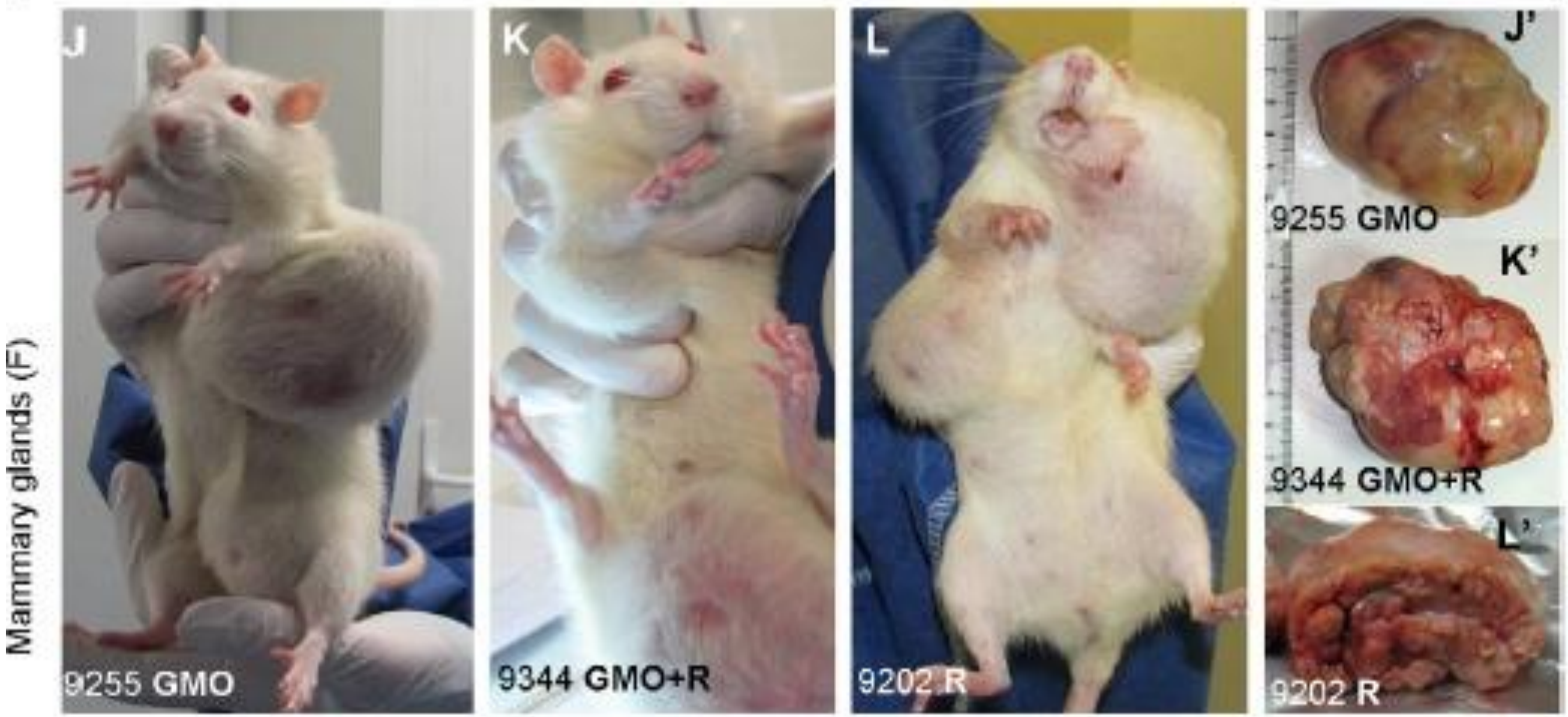
Endocrine disrupting effects

ABSTRACT

The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.

SPECULATIONS!



Critique !

It is also worth noting that tumors are frequent in Sprague–Dawley Rats: a spontaneous tumor incidence of 45% was previously recorded during a 1.5 year period (Prejean *et al*., 1973).

The images of GMM fed rats with large tumors presented by Seralini *et a*l. are misleading as they imply that such tumors do not normally occur or occur less frequently in untreated rats.

Such tumors may occur in rats that are not fed GMO and Seralini *et al*. provide no statistical evidence that the incidence of tumors in general or any specific kind of tumor is increased in GMO fed rats.

Alexander Y. Panchin; Sector of Molecular Evolution.
Food and Chemical Toxicology 53 (2013) 475

PAPER RETRACTED

The Journal Food and Chemical Toxicology retracted the article "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize," which was published in November 2012.

This call back came after a thorough and time-consuming analysis of the published article and the data it reports, along with an investigation into the peer-review behind the article.

The Editor in-Chief deferred making any public statements regarding this article until this investigation was complete, and the authors were notified of the findings.