BT403

AGRICULTURAL BIOTECHNOLOGY

HANDOUTS

VIRTUAL UNIVERSITY OF PAKISTAN

1. Agriculture Biotechnology Introduction

Biotechnology: it is use of biological processes, organisms, or systems. It is defined as a set of tools that uses living organisms (or parts of organisms) to make or modify a product, improve plants, trees or animals, or develop microorganisms for specific uses.

Benefits of Biotechnology

It is used for all these three purposes

HEAL THE WORLD

Biotechnology heals the world by creating more precise tools for disease detection and by reducing rates of infectious disease.

FUEL THE WORLD

It fuels the world by using biofuels, improving manufacturing process efficiency and by reducing the use of and reliance on petrochemicals

FEED THE WORLD

It feeds the world by generating higher crop yields, using biotech crops and by improving food and crop oil content.

Agriculture Biotechnology is collection of scientific techniques to improve plants, animals and microorganisms, to manipulate the genetic makeup, for the production or processing of

agricultural products, to Increase agricultural productivity, to enhance breeders' ability to make improvements in crops and livestock and to enable improvements that are not possible with traditional crossing of related species alone.

Agricultural biotechnology is the term used in crop and livestock improvement through biotechnology tools. This monograph will focus only on agricultural crop biotechnology. Biotechnology encompasses a number of tools and elements of conventional breeding techniques, bioinformatics, microbiology, molecular genetics, biochemistry, plant physiology, and molecular biology.

2. History of agriculture biotechnology

A brief history

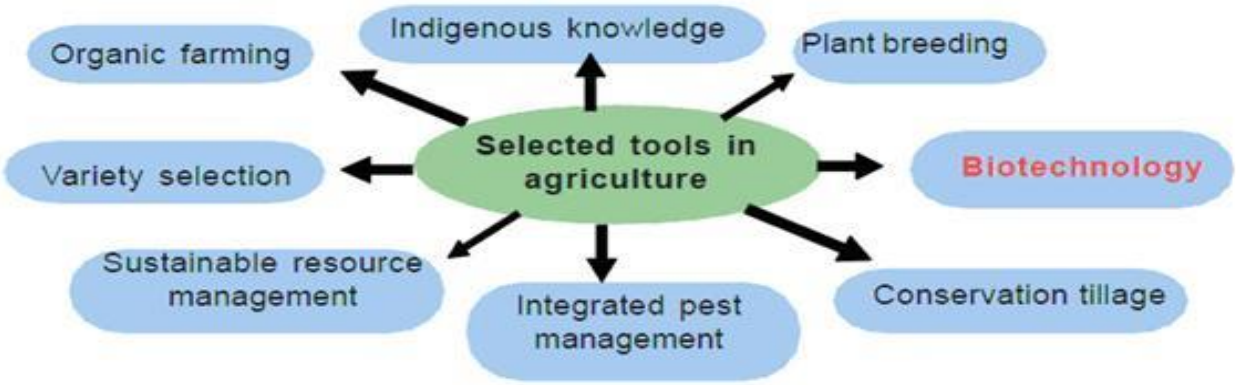
- It is practiced for so long about 8-10,000 years ago to improve organisms by: Selection and breeding
- **Selection:** it is selection and saving of the best looking plants and seeds, the selection features are: Faster growth, higher yields, pest and disease resistance, larger seeds, or sweeter fruits
- **Breeding**: it is done by artificially mating, cross-pollination, and desirable characteristics from different parent plants could be combined in the offspring. Superior plants are selected and breed them to create new and improved varieties of different crops.
- In 1990 -The first food product of biotechnology (an enzyme used in cheese production and a yeast used for baking) appeared on the market.
- In1995, farmers have been growing genetically engineered (GE) crops.
- In 2003, 7 million farmers in 18 countries more than 85 percent of them resource-poor farmers in the developing world were planting biotech crops.

Why is agricultural biotechnology important?

In a world where 7 Billion people, living mostly in rural areas, go hungry every day, Food demand is set to double in the next thirty years and arable land is limited, Advances in agriculture are critical if we are to reduce hunger and promote growth and development in a socially acceptable and environmentally sustainable way.

Benefits of agriculture biotechnology

Agricultural biotechnology has been used to protect crops from disastrous diseases. Biotech crops can make farming more profitable by increasing crop quality and may in some cases increase yields. Biotech crops may provide enhanced quality traits such as increased levels of beta-carotene in rice to aid in reducing vitamin A deficiencies. Agriculture biotechnology produces herbicide-tolerant crops.The tools of agricultural biotechnology have been invaluable for researchers in helping to understand the basic biology of living organisms. Biotechnology has helped to make both insect pest control and weed management safer and easier while safeguarding crops against diseases.

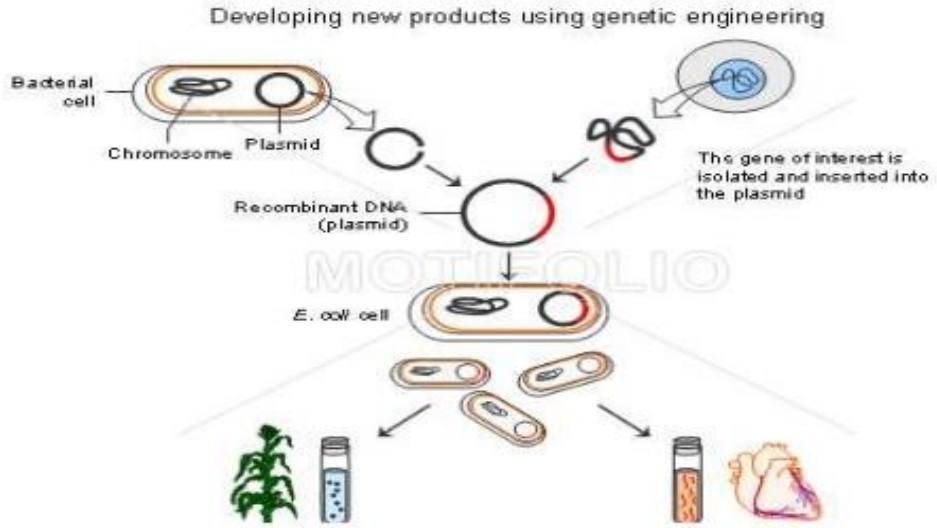


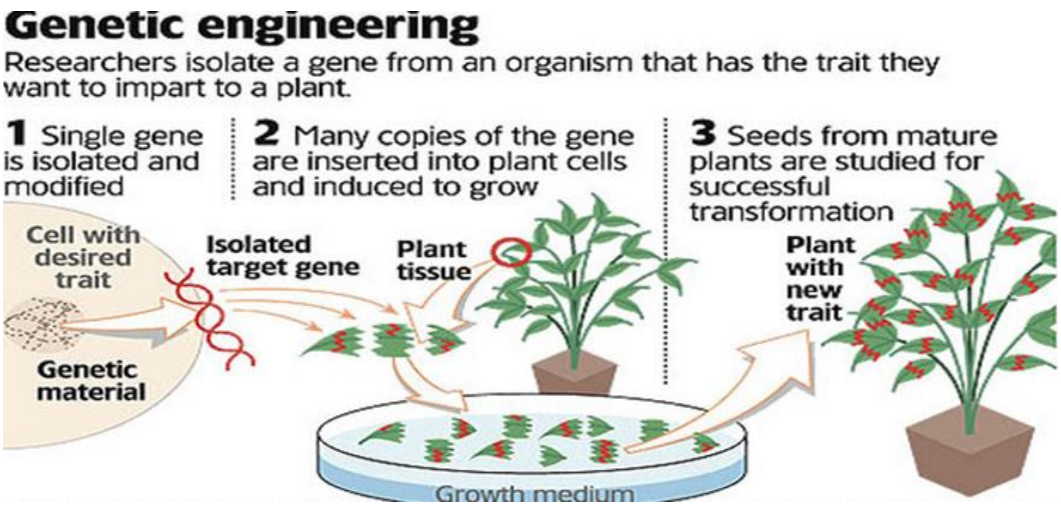
3. Applications of agriculture biotechnology I

Genetic engineering:

Genetic engineering is also called genetic modification, is the direct manipulation of an organism's genes using biotechnology. All crops improved with transferred DNA to date have been developed to aid farmers to increase productivity by reducing crop damage from weeds, diseases or insects. It inserts fragments of DNA into chromosomes of cells, uses tissue culture to regenerate the cells into a whole organism with Different genetic

composition from the original cells. This is also known as rDNA technology that produces transgenic organisms.

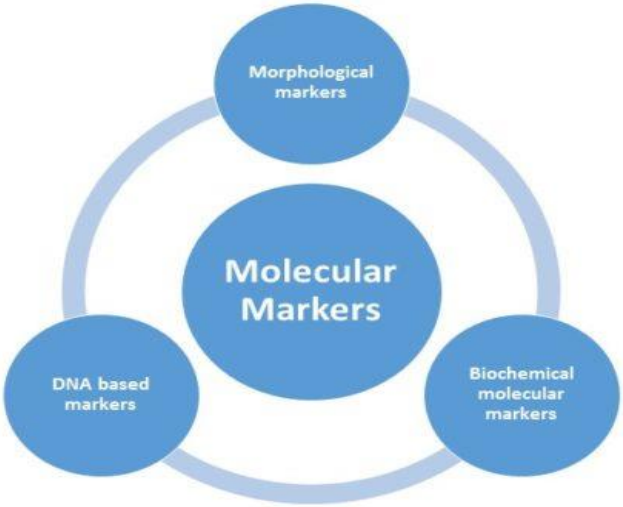




Molecular markers: Scientists can use molecular markers to select plants or animals that possess a desirable gene, even in the absence of a visible trait. Thus, breeding is more precise and efficient.

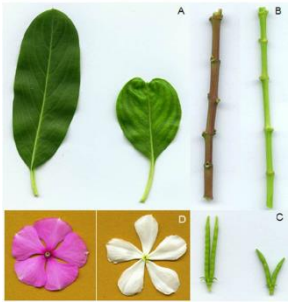
Types of Molecular markers:

There are three types of molecular markers:



Morphological markers:

Morphological markers are those traits that are scored visually, or morphological markers are those genetic markers whose inheritance can be followed with the naked eye.



Biochemical molecular markers: A molecular marker is a molecule contained within a sample taken from an organism (Biochemical molecules). The first biochemical molecular markers used were the protein based markers. One of the earliest protein based markers to be used was Isozyme. These are different forms of an enzyme exhibiting the same catalytic activity but differing in charge and electrophoretic mobility.

DNA based markers:

The sequence of nucleotides in DNA of an individual is unique and thus determines its identity. The ultimate difference between individuals lies in the nucleotide sequence of their DNA. These can be used to diagnose the presence of the gene without having to wait for gene effect to be seen.

4. Applications of agriculture biotechnology II

Molecular diagnostics: Molecular diagnostics are methods to detect genes or gene products that are very precise and specific. Molecular diagnostics are used in agriculture to more accurately diagnose crop/livestock diseases.

Vaccines;

Biotechnology-derived vaccines are used in livestock and humans. They may be cheaper, better and/or safer than traditional vaccines. They are also stable at room temperature, and do not need refrigerated storage. Biotechnology-derived vaccines are used in livestock and humans. They are cheaper, better, safer than traditional vaccines, stable at room temperature and do not need refrigerated storage

Tissue culture

Tissue culture is the regeneration of plants in the laboratory from disease-free plant parts. This technique allows for the reproduction of disease-free planting material for crops. Examples of crops produced using tissue culture include citrus, pineapples, avocados, mangoes, bananas, coffee and papaya.

Flowers

Gene identification and transfer techniques used to improve the color, smell, size and other features of flowers. It is used to make improvements to other common ornamental plants, in particular, shrubs and trees. Some of these changes are similar to those made to crops, such

as enhancing the cold resistance of a breed of tropical plant, so it can be grown in northern gardens.

Nutrient Supplementation

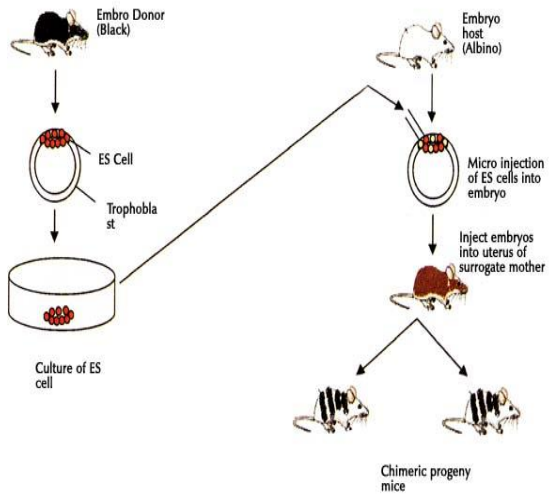
In an effort to improve human health, nutrient supplementation is needed, particularly in underdeveloped countries, scientists are creating genetically altered foods that contain nutrients known to help fight disease or malnourishment. An example of this is *Golden Rice*, which contains beta-carotene, the precursor for Vitamin A production in our bodies.

Pest resistant crops

Pest resistant GM crops (primarily cotton and maize) are genetically modified so they are toxic to certain insects. They are often called Bt crops. The introduced genes were originally identified in a bacterial species called Bacillus thuringiensis.

5. Tissue culture

Introduction: Tissue culture is the term used for "the process of growing cells artificially in the laboratory" Tissue culture involves both plant and animal cells. Tissue culture produces clones, in which all product cells have the same genotype (unless affected by mutation during culture).



Overview of the Tissue Culture Process

Brief history: Tissue culture had its origins at the beginning of the 20th century with the work of 1- Gottleib Haberlandt (plants) and 2-Alexis Carrel (animals).

The first commercial use of plant clonal propagation on artificial media was in the germination and growth of orchid plants, in the 1920's

In the 1950's and 60's there was a great deal of research, but it was only after the development of a reliable artificial medium (Murashige & Skoog, 1962) that plant tissue culture really 'took off' commercially

Critical requirements:

Tissue culture of both plant and animal has several critical requirements.

1. Appropriate tissue (some tissues culture better than others)
2. A suitable growth medium containing energy sources and inorganic salts to supply cell growth needs.
3. This can be liquid or semisolid
4. Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can over run a culture
5. Growth regulators - in plants, both auxins & cytokinins. In animals, this is not as well defined and the growth substances are provided in serum from the cell types of interest

6. Frequent subculturing to ensure adequate nutrition and to avoid the build up of waste metabolites

Steps of tissue culturing

1. selection of explant:

Explants are small pieces of plant parts or tissues that are aseptically cut and used to initiate a culture in a nutrient medium. Explants can be taken from different parts of a plant such as shoots, leaves, stems, flowers, roots, and from many types of mature cells provided they are able to de-differentiate into totipotent cells.

2. Establishment of the explant

Establishment of the explant in a culture medium The medium sustains the plant cells and encourages cell division. It can be solid or liquid

3. Multiplication

The explant gives rise to a callus (a mass of loosely arranged cells) which is manipulated by varying sugar concentrations and the auxin (low): cytokinin (high) ratios to form multiple shoots.The callus may be subdivided a number of times Dividing shoots.Warmth and good light are essential

4. Root formation

The shoots are transferred to a growth medium with relatively higher auxin: cytokinin ratios. Promote root formation.Ready to transfer to soil

6. Growth Regulators

Growth

Growth is an irreversible change in Mass, i.e. increase in size, volume and weight of any part of plant's body. It means quantitative increase in plant body e.g. Cell division Cell enlargement.

Development

Development is an irreversible change in state. It means the qualitative change in plant body e.g. Seed Seedling Vegetative maturation Flowering. Growth is a continuous process Development is phase to phase process.

Plant growth regulating compounds

- Plant growth regulating compounds are:
- Natural and Synthetic
- Natural- found naturally in plants
- Synthetic- human made
- Both groups regulate or influence:
- Cell division
- Cell differentiation
- Root and shoot growth
- Senescence (plant aging)

Plant growth regulators

Plant Growth regulators (PGR) refers to natural or synthetic substances influence the growth and development.

Classification of PGR On the Basis of Origin

Natural hormone: natural hormones are produced by some tissues in the plant they are also called endogenous hormones. e.g. IAA (Indol acetic acid)

Synthetic hormone: synthetic hormones are produced artificially and similar to natural hormone in physiological activity. They are also clso called Exogenous hormones. e.g. 2,4- D, NAA (Naphthalene acetic acid).

Growth promoting hormones/Growth promoter: they increase the growth of plant. e.g. Auxins. Gibberellins, Cytokinins etc.

Growth inhibiting hormones/Growth retardant: they inhibit the growth of plant. e.g. ABA, Ethylene.

Auxins

It is derived from the Greek word "auxein" means- "to grow/increase". Auxins may be defined as growth promoting substances which promote growth along the vertical axis when applied in low concentration to the shoot of the plant. Auxins are synthesized in the stem and root

apices and transported through the plant axis. They occur universally in all plants as Active growth = Auxin production.

Role of Auxins: Auxins stimulate cell elongation and influence a host of other developmental responses, they are involved in root initiation, vascular differentiation, tropic responses, apical dominance and in development of auxiliary buds, flowers and fruits

Auxins in plant tissue culture are used to induce callus from explants , and cause root and shoot morphogenesis and parthenocarpy

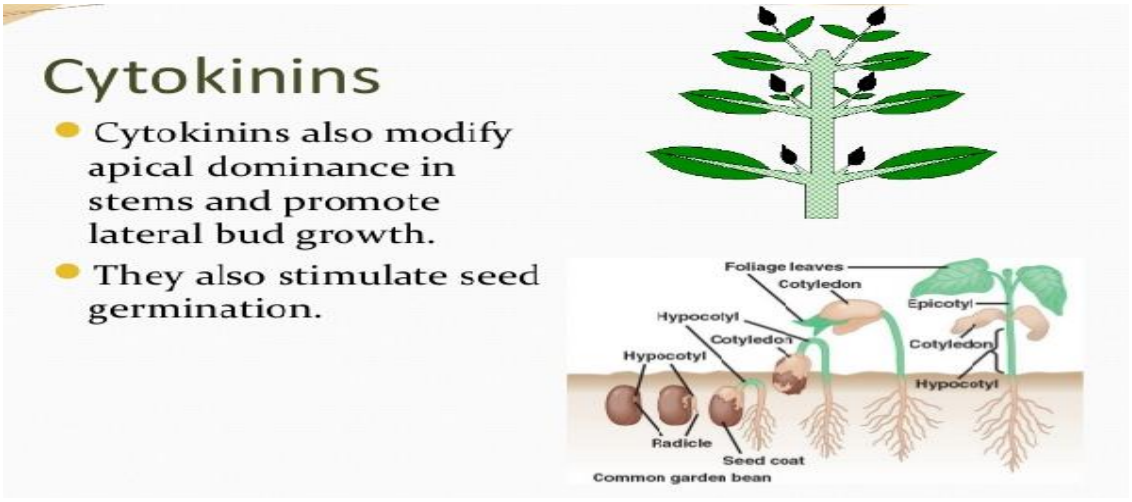
Gibberellin: They have a regulatory function and are produced in the shoot apex primarily in the leaf primordial (leaf bud) and root system.

Role of gibberellins: They are used to stimulates stem growth dramatically**,** they also stimulate cell division, Cell elongation (or both) and controls enzyme secretions, they are involved in overcoming dormancy in seeds and buds also used commercially in increasing fruit size of seedless grapes and Stimulating seed germination & seedling growth

Cytokinins :

Roles of cytokinins are : Promotion of cell division also found in all tissues with considerable cell division. Ex: embryos (seeds) and germinating seeds, young developing fruits roots supply cytokinins upward to the shoots. They also interact with auxins to influence differentiation of tissues (may be used to stimulate bud formation).

Effect of cytokinins



Ethylene

It is a growth retardant. Ethylene promotes ripening , it is gaseous hormone, it is produced in the actively growing meristems of the plant, in senescing ripening or ageing fruits, in senescing (ageing or dying) flowers, in germinating seeds and in certain plant tissues as a response to bending, wounding or bruising. Ethylene as a gas, diffuses readily throughout the plant. It may promote leaf senescing and abscission (leaf fall). Increases female flowers in cucumbers (economically - will increase fruit production). It is also responsible for de greening of oranges, lemons and grapefruit – ethylene gas breaks down chlorophyll and lets colors show through.

Abscissic acid

It is also a growth retardant which induces stomata closing, also involved in nhibition of bud growth and shoot formation. It is widespread in plant body – moves readily through plant. ABA appears to be synthesized by the leaves, it Interacts with other hormones in the plant, counteracting the growth - promoting the effects of auxins & gibberellins. It is also involved with leaf and fruit abscission (fall), onset of dormancy in seeds and onset of dormancy (rest period) in perennial flowers and shrubs. ABA is effective in inducing closure of stomata in leaves, indicating a role in the stress physiology in plants. (ex: increases in ABA following water, heat and high salinity stress to the plant)

7. Sterile Techniques

Sterile techniques are used to **clean equipment** and for **surface sterilization of explants.**

Clean Equipment

It is used for successful tissue culture requires the maintenance of a sterile environment. All tissue culture work is done in a laminar flow hood. The laminar flow hood filters air with a dust filter and a high-efficiency particulate air (HEPA) filter. It is important to keep the hood clean, which can be done by wiping it with 70% alcohol. The instruments used should also be dipped in 70% ethanol and sterilized using flame or glass beads. Hands should be disinfected with ethanol before handling cultures in order to avoid contamination.

It is imperative to maintain axenic conditions throughout the life of cultures: from explant to the production of whole plants. Entire experiments have been lost because of an episode of fungal or bacterial contamination at any stage of culture. Especially problematic are fungal contaminants that are propagated by spores that might blow into a hood from an environmental source. Therefore, it is important to work away from the unsterile edge of a laminar flow hood.

Surface Sterilization of Explants

Plant tissues inherently have various bacteria and fungi on their surfaces. It is important that the explant be devoid of any surface contaminants prior to tissue culture since contaminants can grow in the culture medium, rendering the culture non sterile. In addition, they compete with the plant tissue for nutrition, thus depriving the plant tissue of nutrients. Bacteria and especially fungi can rapidly overtake plant tissues and kill them. The surface sterilants are chosen for an experiment typically depend on the type of explant and also plant species. Explants are commonly surface-sterilized using sodium hypochlorite (household bleach), ethanol, and fungicides when using field-grown tissues. The time of sterilization is dependent on the type of tissue; for example, leaf tissue will require a shorter sterilization time than will seeds with a tough seed coat. Wetting agents such as Tween added to the sterilant can improve surface contact with the tissue.

Although surface contamination can be eliminated by sterilization, it is very difficult to remove contaminants that are present inside the explant that may show up at a later stage in culture. This internal contamination can be controlled to a certain extent by frequent transfer to fresh medium or by the use of a low concentration of antibiotics in the medium. Overexposing tissues to decontaminating chemicals can also kill tissues, so there is a balancing act between sterilizing explants and killing the explants themselves.

Culture Conditions and Vessels

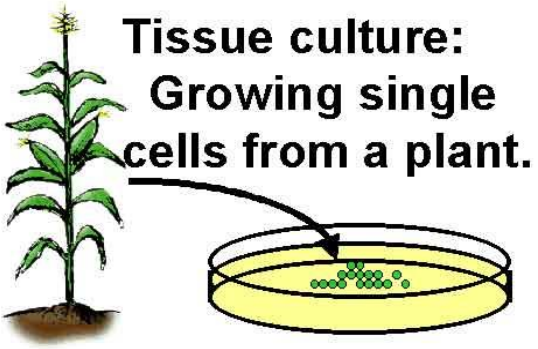
Cultures are grown in walk-in growth rooms or growth chambers. Humidity, light, and temperature have to be controlled for proper growth of cultures. A 16-h light photoperiod is optimal for tissue cultures, and a temperature of 22–258C is used in most laboratories. A light intensity of 25–50$\mu mol.m^{-2}s^{-1}$ PAR (photosynthetically active radiation) is typical for

tissue cultures and is supplied by cool white fluorescent lamps. A relative humidity of 50–60% is maintained in the growth chambers. Some cultures are also incubated in the dark. Cultures can be grown in various kinds of vessels such as petri plates, test tubes, “Magenta boxes,” bottles, and flasks

8. Basic Steps of plant tissue culture

Plant tissue culture

- Plant tissue culture is a technique of growing plant cells, tissues, organs, seeds or other plant parts in a sterile environment on a nutrient medium



Basic techniques of plant tissue culture are

- Culture vessels
- Culture medium
- Sterilization
- Inoculation
- Incubation
- Induction of callus
- Morphogenesis = Organogenesis Embryogenesis
- Hardening

Culture vessels: Cultures can be grown in various kinds of vessels such as petri plates, test tubes, “Magenta boxes,” bottles, and flasks.

Culture medium: The important media used for all purpose experiment are Murashige and Skoog medium (MS medium). The culture medium is closed with cotton plug/ or aluminium foil sheet. The pH of the medium is adjusted to 5.8 (acidic range).

Composition of culture medium:

- Nutrient Medium
- Medium depends upon the type of plant tissue or cell used for culture
- Generally nutrient consist of
- inorganic salts (both micro & macro elements) a carbon source (usually sucrose)
- Vitamins (eg. nicotinic acid, thiamine, pyridoxine
- Amino acids (eg. arginine)
- Growth regulators (eg. auxins)
- An optimum pH (5.7) is also vary important

Sterilization

Sterilization Methods are used in Tissue Culture Laboratory. All the materials, e.g., vessels, instruments, medium, plant material, etc., used in culture work must be freed from microbes therefore, they are sterilized.

Sterilization techniques

- sterilization is achieved by one of the following approaches:
- dry heat treatment
- flame sterilization
- autoclaving
- filter sterilization
- wiping with 70% ethanol
- surface sterilization.

Inoculation

Transfer of explant (root, stem, leaf, etc.) on to a culture medium is called inoculation. The inoculation is carried out under aseptic condition for which an apparatus called laminar air flow chamber is used. Flamed and cooled forceps are used for transfer of plant materials to different culture media kept in glasswares.

Incubation

The culture medium with the inoculum is incubated at 26 - 28oC with the light intensity at 2000 to 4000 lux (unit of intensity of light) and allowing photoperiod of 16 hour of light and 8 hours of darkness.

Induction of callus: Due to activity of auxins and cytokinins, the explant is induced to form callus. The callus is an unorganized mass of undifferentiated tissue. The mechanism of callus formation is that auxin induce cell elongation and cytokinin induces cell division as a result of which masses of cells are formed.

- **Morphogenesis**

Formation of new organs from the callus under the influence of auxin and cytokinin is called morphogenesis.

Roots and shoots are differentiated from the callus.

Such embryos are called somatic embryos result in the formation of young plantlet.

- **Types of morphogenesis:**

Organogenesis

Embryogenesis

- **Organogenesis:** it is formation of new organs such as shoot and root is known as organogenesis. The development of shoot from the callus is called caulogenesis and formation of root is called rhizogenesis respectively.
- **Embryogenesis**

Formation of embryos (ie. bipolar structure having shoot and root) from the callus is called embryogenesis.

These embryos arise from somatic callus tissue and are called somatic embryos or embryoids or somaclonal embryos.

- **Hardening**

Exposing the plantlets to the natural environment in a stepwise manner is known as hardening.

Finally the plantlets are gradually transferred to the soil.

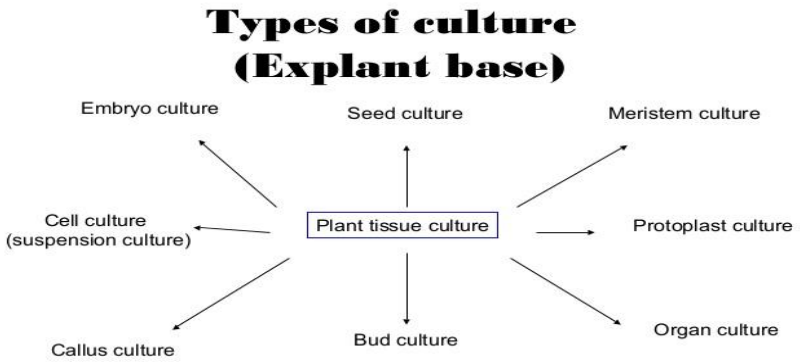
9. Types of tissue culture, callus culture

Types of tissue culture

- These are the types of in vitro cultures
- Callus Cultures
- Cell Suspension Culture

- Anther/Microspore Culture
- Protoplast Culture
- Embryo Culture
- Meristem Culture

Types of in vitro cultures



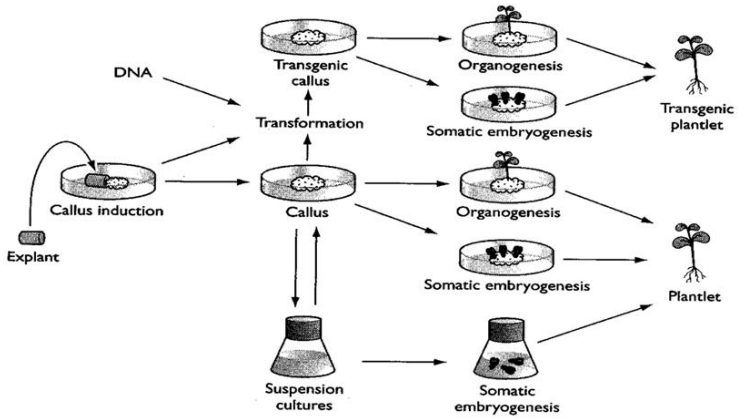
Callus Culture

It is an unorganized mass of thin-walled parenchyma cells which is involved in formation of wound callus is observed in all groups of living organisms. In Plant tissue culture, callus is produced by cultivation of plant tissue on nutrient media under in vitro conditions. Presence of growth hormones in the culture media promotes callus formation and proliferation

Role of Callus

In vitro callus provides totipotent cells for plant regeneration via organogenesis or somatic embryogenesis. Callus is used as a target tissue for genetic transformation. Callus formation is initiated for plant regeneration of other transformed tissues

Dispersal of friable callus into single cells is used for the initiating cell suspension cultures



Alternative applications of callus in plant tissue culture and genetic transformation.

Initiation and maintenance of callus cultures

(1) Selection of suitable parent material

(2) Choice of explant and method of isolation

(3) Culture medium and conditions required

(4) Optimization of culture conditions

Selection of suitable parent material

Parent plant must be healthy and free from decay or disease

Mother plant be actively growing and not be about to enter a period of dormancy

Choice of explant and method of isolation

- Any part of mother plant; Plant organs or specific plant tissue or plant cells
- Explant must contain living cells
- Younger tissue is more callus responsive due to the presence of large no. of actively dividing cells
- Explants isolated in sterile conditions
- Ensure proper sterilization methods for a particular explant

Culture medium and conditions required

- Culture the sterilized explants on suitable autoclaved culture medium
- Incubate cultures at 22-24 ± 2°C in light or dark
- Most callus will be initiated from the cut surfaces within 3-8 wks.

Optimization of culture conditions

- Consult literature to know previous callus initiation attempts for species under consideration

- For pioneering callus culture attempt, modify medium previously used for a related species
- Start with one of the defined media and manipulate hormone concentrations
- Set up a growth Latin Square of 25 culture plates with 5 each of auxin and cytokinin conc.
- Callus for this growth trial should be uniform and of large (20 mg) size.

Callus growth measurement

- Subculture vigorous growing callus (2-5 mm dia.)
- Slow growing callus plus explant transferred to fresh medium
- Callus growth assessment via fresh and dry wets.
- Plot a growth curve.

10. Cell Suspension Cultures

Cell suspension cultures are rapidly dividing suspensions of cells grown in liquid medium; they grow more rapidly than callus cultures and more ready to culture manipulations. They are comprised of cell aggregates and dispersed single cells

Techniques of cell culture

Initiation of cell suspension culture

Fragments of undifferentiated callus 2 - 3 g / 100 mL

↓

Liquid medium

↓

↓ **aeration** --------

↓

↓

Subculture

↓

↓ **agitation** --------

↓

Suspension cell cultures

Initiation of Cell Suspensions:

Initiation and establishment time depends on plant species and growth medium. Dicots are easier to establish in suspensions than monocot. It is initiated by agitating a fragment of callus in a vol. of liquid medium on a shaker, there are three procedures used for cell dispersion:

1. Initiate from friable callus
2. non-friable callus
3. Callus treated with cell wall degrading enzymes

Initiation from Friable Callus

It is most commonly used starting material, easily fragmented during agitation in liquid medium, achieved by:

- Callus passaged on a 7d cycle for 2-3 wks.
- Ratio of auxin/cytokinins altered, increase auxin concentration. Use 2-3 g of friable callus per 100 ml of liquid medium.
- Low levels of callus tissue fail to replicate.
- Subculture the cells to fresh medium at a ratio of 1:1. Filter the actively dividing cell suspensions to remove large callus aggregates to get fine suspensions

Initiation from non-friable callus

First repeatedly subculture or transfer callus fragments to semi-solid medium until friable, initiate and establish cell suspensions

- **Initiation from callus treated with enzymes**

- Pectinase
- Cellulase

(breaks down middle lamella of cell wall and separates plant cells)

Maintenance of Cell Suspensions

- Varieties of culture vessels are available, suitable ones allow large surface area to maximize gas exchange. E.g. 20 ml culture medium + 100 ml flask, 50-100 ml in a 250 ml flask, flasks enclosed with sterilized/dried aluminium foil caps. Shaker speed should be 100-120 rpm with optimum incubation conditions

Small batch culture

- Culture volume : in a small fixed volume : <100 ML

Growth Characteristics of Cell Suspensions

- Plant cell suspensions consist of cells with diverse morphology and state of aggregation. Two morphological types of cells can be distinguished:
- Cell aggregates made up of small cells
- Large and elongated single cells
- Proportion of the cell type depends on the passage of culture and nature of auxin

Monitoring the Growth of Culture

To monitor the growth following should be checked:

- Cell number
- PCV
- Fresh wt. and Dry wt.
- Cell Viability
- Medium conductivity & pH
- Use two methods simultaneously till stationary phase

Growth Curve of Suspension Cultures

- Plotted for fresh, dry wts. and PCV.
- Gives the length of culture cycle
- Used to decide the subculture interval of suspensions
- Lag Phase
- Exponential Phase
- Linear Phase
- Decelerating Phase
- Stationary Phase

Uses of Cell Suspensions

You can study various factors and compounds affecting growth and differentiation, cell division, rapid preparation of protoplasts, large scale production of commercial plants via somatic embryogenesis and commercial production of secondary metabolites.

11. Anther/Microspore Culture

Introduction

Anthers or pollens can be cultured on a suitable medium containing sucrose (usually 2%), iron, vitamins, hormones etc. The hormonal component of the medium is important for initiation of growth. Usually to the culture medium auxin, cytokinin etc. are added either singly or in various combinations. Low concentration of auxin stimulates callus formation. In a medium supplemented with auxin embryoid formation usually occurs at a faster rate as observed in

anther culture of Datura. Anthers cultured on a medium containing coconut milk or kinetin develop embryoids which later form haploid plantlets. Callus is formed from pollen grains on a medium supplemented with yeast extract or casein hydrolysate.

Factors Affecting Anther and Pollen Culture:

1. Activated charcoal:

It has a stimulatory effect on embryogenesis and this has been observed in anther cultures of potato, rye, tobacco, etc. This may be due to removal of inhibitory substances from agar by activated charcoal. Charcoal may absorb the degradation product (5-2-furfural) of sucrose. Anther cultures of Petunia and Nicotiana indicate that activated charcoal removes both exogenous and endogenous growth hormones from culture medium.

Temperature:

Temperature has significant effect on pollen embryoid development. In Datura embryoids are not formed if cultures are maintained at 20°C or below. In Nicotiana tabacum optimal temperature for embryoid growth is 25°C.

Pre-treatment of anthers at 3—10°C for 2—30 days stimulates embryogenesis. Wenzel (’77) observed that buds of Secale cerale pretreated at 6°C for 6—10 days develop embryoids. In N. tabacum if the buds are pre- treated at 5°C for 72 hours than 58% anthers produce embryoids. Sometimes pre-treatment at high temperature helps embryoid formation. In Brassica campestris pre-treatment of anthers at 35°C for 24 hours helps embryoid formation.

Centrifugation of the anthers at 3—5°C for approximately 30 minutes helps embryoid formation

Stage of the anther:

- Particular stage of the anther at the time of culture is important. Usually anthers just before or immediately after pollen mitosis are most suitable for culture.
- Suitable stages of anthers for culture are pre-mitotic, mitotic and post-mitotic.

a. **Pre-mitotic stage:**

Anthers at this stage have microspores which have just completed the first meiotic division and the pollens are immature, uninucleate and starch-free.

Anthers of Hordeum vulgare and Hyocyamus at this stage are suitable for culture. According to Nitsch (’72) and Sunderland (’71) anthers with uninucleate pollens are suitable for culture.

b. Mitotic stage:

In some plants, anthers at first pollen division stage are most suitable for culture, as observed in Nicotiana tabacum and Datura innoxia.

c. Post-mitotic stage:

Early bi-cellular stage of pollen development is most suitable for culture in Atropa belladonna and Nicotiana sp. etc. Mature anthers are usually unsuitable for culture, but in Brassica oleracea mature anthers are the proper stage for anther culture. Anthers of proper stage are chosen by selecting flower buds of definite length under fixed environmental conditions.

Photoperiod and light intensity:

Higher number of embryoids are formed when anthers are taken from plant grown under short days and high light intensities.

Flowering time:

Anthers taken from flowers at the beginning of the flowering period of the plant are most suitable for culture.

Endogenous auxin:

Embryogenic pollens are found near the tapetum within the anthers. The tapetum may release some substance which initiates embryogenic development in pollens. This is observed in Hyoscyamus niger by Raghavan (’78).

Age of the plant:

Usually anthers from younger plants are more suitable for culture.

Methods of Anther culture

1. Selected plants are cultivated until they reach flower bud stage.

2. In some cases flower buds are chilled few days prior to culture.

3. Flower buds of proper size and developmental stage are taken and surfaces sterilized with alcohol or hypochlorite solution for 10—20 minutes. Buds are rinsed several times in sterile double distilled water.

4. The anthers are carefully excised from flower buds using force and dissecting needle. Filaments must be removed prior to culture; otherwise callus may be formed at the cut ends.

5.Anthers may be cultured either on agar-solidified culture medium or placed on a filter paper bridge over a liquid medium.

6. Anthers are cultured at 25°G in presence or absence of light. Light is essential after plantlets are formed. Continuous illumination from cool white fluorescent lamp of 300 lux is satisfactory.

7. After a period of 4—5 weeks in culture plantlets are formed. From a single anther many plantlets are formed

8. These plantlets are carefully separated quite early and cultured on a fresh root-inducing medium containing 0.5% agar and all other components in half-strength to that of the anther culture medium.

9. After formation of proper root system they are transplanted to pots. These pots are preferably kept in humid condition for few days

12. Protoplast Culture

Protoplasts

Protoplasts are naked plant cells without the cell wall, but they possess plasma membrane and all other cellular components. They represent the functional plant cells but for the lack of the barrier, cell wall. Protoplasts of different species can be fused to generate a hybrid and this process is referred to as somatic hybridization (or protoplast fusion). Cybridization is the phenomenon of fusion of a normal protoplast with an enucleated (without nucleus) protoplast that results in the formation of a cybrid or cytoplast (cytoplasmic hybrids).

Protoplast isolation: It refers to the separation of protoplast from plant tissue, it is important to isolate viable and uninjured protoplast as gently and as quickly as possible, it involves two methods:

a. Mechanical

b. Enzymatic

Mechanical method:

Tissue is immersed in 1.0 M sucrose until protoplasm shrunk away from their enclosing cell wall (Plasmolysis). Plasmolysed tissue is cut with a sharp knife at such a thickness that only cell walls are cut Plasmolysed cell. Undamaged protoplast in strips are released by osmotic swelling when placed in a low concentration of sucrose solution. Problem encountered: some cells release uncut complete protoplast while the rest produces broken dead protoplasts

Enzymatic method:

it refers to the use of enzymes to dissolve the cell wall for releasing protoplasts. It involves two methods:

I. Direct method (One step only)

II. Sequential method (Two step method)

- **Direct method :**
- Incubation of leaf segments overnight in enzyme solution
- Mixture is filtered and centrifuged
- Protoplast forms pellet
- Then washed with sorbitol and re-centrifuged
- Clean protoplasts float
- They are pipetted out

Sequential method

Two enzyme mixtures(mixture A and mixture B) are used one after the other. Leaf segments with mixture A (Macerozyme in manifold at pH 5.8) are vacuumed infiltrated for 5 mins, transferred to a water bath at 25°C and subjected to slow shaking. The enzyme mixture is then replaced by fresh 'enzyme mixture A' and leaf segments are incubated for another hour. he mixture is filtered using nylon mesh and centrifuged for 1 min, washed 3 times with 13% mannitol . Cells are then incubated with 'enzyme mixture B' (Cellulase in mannitol solution

at pH 5.4) for above 90 mins at 30°C. The mixture is centrifuged for 1 min so that protoplast form a pellet and clean 3 times with sorbitol

Purification of protoplast

Protoplasts are purified by removing: Undigested material (debris),Bursts protoplasts , and enzymes. Debris are removed by filtering the preparation through a nylon mesh . Enzymes are removed by centrifugation whereby the protoplasts settle to the bottom of the tube and the supernatant removed with the help of a pipette. Intact protoplasts are separated from broken protoplasts through centrifugation and removed by a pipette as they are collected at the top of tube

Protoplast Culture

Isolated protoplast can be cultured in an appropriate medium to reform cell wall and generate callus. Optimal culture conditions are:

- Optimal density to the culture.
- Optimal auxin to cytokinin ratio, glucose and sucrose.
- Maintain osmoprotectant in the medium
- Temperature: 20-28°C pH: 5.5-5.9 0.25% Casein hydrolysate BAP and NAA

Culture of protoplasts

Protoplasts cultured in suitable nutrient media first generate a new cell wall. The formation of a complete cell with a wall is followed by an increase in size, number of cell organelles, and induction of cell division. The first cell division may occur within 2 to 7 days of culture. It results in small clumps of cell, also known as micro colony, within 1 to 3 weeks. From such clumps, there are two routes to generate a complete plant (depending on the species). Plants are regenerated through organogenesis from callus masses. The micro calli can be made to develop into somatic embryos, which are then converted into whole plant through germination

Importance of Protoplast Culture

The protoplast in culture can be regenerated into a whole plant. Hybrids can be developed from protoplast fusion. It is easy to perform single cell cloning with protoplasts. Genetic transformations can be achieved through genetic engineering of protoplast DNA. Protoplasts are excellent materials for ultra-structural studies.

Isolation of cell organelles and chromosomes is easy from protoplasts. Protoplasts are useful for membrane studies (transport and uptake processes).Isolation of mutants from protoplast cultures is easy.

13. Embryo Culture

What is embryo?

A seed plant embryo is part of a seed, consisting of precursor tissues for the leaves, stem and root as well as one or more cotyledons. The young sporophyte of a seed plant usually comprising a rudimentary plant with plumule, radicle, and cotyledons

What is Embryo Culture?

The embryo of different developmental stages, formed within the female gametophyte through sexual process, can be isolated aseptically from the bulk of maternal tissues of ovule, seed or capsule and cultured in vitro under aseptic and controlled physical conditions in glass vials containing nutrient solid or liquid medium to grow directly into plantlet

Culturing method

The general method of embryo culture follows the following steps.

1. Pluck healthy and mature fruits from the field and wash thoroughly in running water for about an hour.
2. Surface sterilize with 0.01% Tween-20 for 15 min, rinse seeds several times with distilled water and finally treat with 0.01% $HgCl_2$ solution for 10-15 min.
3. Finally rinse it for six times with sterile distilled water.

4. Incubate the cultures at 22-25°C under a 16 h photoperiod of 2000 lux luminous intensity.
5. After two weeks of inoculation the embryo begins to swell on callus proliferation medium. Distinct callus growth is observed after 4 weeks.
6. After 8 weeks of inoculation transfer the callus on shoot regeneration medium. Within 4 weeks of transfer into second medium the callus turns green and produces soft spongy tissue. Some of these tissues are differentiated into embryoids.
7. The embryoids produce cluster of budlets when subcultured onto shoot regeneration medium.
8. The budlets grow into shoots and produce 2-3 leaf appendages within 12 weeks.
9. Thereafter, they are separated into individual shoots and then subcultured into a fresh medium of the same composition until shoots develop.

Types of Embryo Culture

There are two types of embryo culture:

1. **Mature Embryo Culture**

Mature embryos are isolated from ripe seeds and cultured in vitro. Mature embryo cultures are carried out in the following conditions

Conditions

1. When the embryos remain dormant for long periods.
2. Low survival of embryos in vivo.
3. To avoid inhibition in the seed for germination.
4. For converting sterile seeds to viable seedlings.

2. **Embryo Rescue**

Embryo rescue involves the culture of immature embryos to rescue them from unripe or hybrid seeds which fail to germinate. This approach is very useful to avoid embryo abortion and produce a viable plant. Wild hybridization involving crossing of two different species of plants from the same genus or different genera often results in failure. This is mainly because the normal development of zygote and seed is hindered due to genetic barriers.

Applications of Embryo Culture:

Embryo culture can be used in:

- Prevention of Embryo Abortion
- Overcoming Seed Dormancy
- Shortening of Breeding Cycle
- Production of Haploids
- Overcoming Seed Sterility
- Clonal Propagation

14. Meristem/shoot tip culture

Meristem

A meristem is the tissue in most plants containing undifferentiated cells (meristematic cells), found in zones of the plant where growth can take place

What is Meristem Culture?

Meristem culture is the in vitro culture of a generally shiny special dome-like structure measuring less than 0.1 mm in length and only one or two pairs of the youngest leaf primordia, most often excised from the shoot apex.

Principle

The excised shoot tips and meristem can be cultured aseptically on agar solidified simple nutrient medium or on paper bridges dipping into liquid medium and under the appropriate

condition will grow out directly into a small leafy shoot or multiple shoots. Alternatively the meristem may form a small callus at its cut case on which a large number of shoot primordia will develop. These shoot primordia grow out into multiple shoots. Once the shoots have been grown directly from the excised shoot tip or meristem, they can be propagated further by nodal cuttings. This process involves separating the shoot into small segments each containing one node. The axillary bud on each segment will grow out in culture to form yet another shoot.

Protocol

- Remove the young twigs from a healthy plant. Cut the tip (1 cm) portion of the twig.
- Surface sterilize the shoot apices by incubation in a sodium hypochlorite solution (1% available chlorine) for 10 minutes. The ex- plants are thoroughly rinsed 4 times in sterile distilled water.
- Transfer each explants to a sterilized petri dish.
- Remove the outer leaves from each shoot apices with a pair of jeweler's forceps. This lessens the possibility of cutting into the softer underlying tissues.
- After the removal of all outer leaves, the apex is exposed. Cut off the ultimate apex with the help of scalpel and transfer only those less than 1 mm in length to the surface of the agar medium or to the surface of filter-paper Bridge. Flame the neck of the culture tube before and after the transfer of the excised tips. Binocular dissecting microscope can be used for cutting the true meristem or shoot tip perfectly.
- Incubate the culture under 16hrs light at 25°C
- As soon as the growing single leafy shoot or multiple shoots obtained from single shoot tip or meristem, develop root, transfer them to hormone free medium
- The plantlets formed by this way are later transferred to pots containing compost and kept under greenhouse conditions

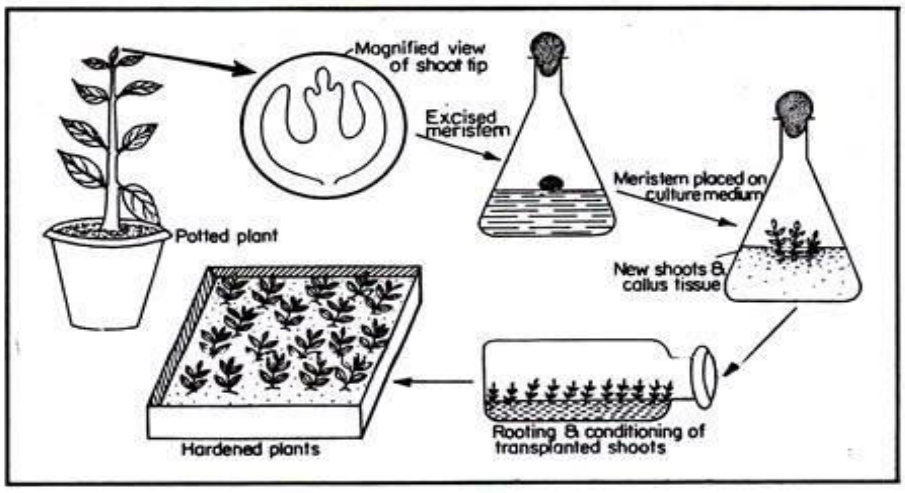


Fig 2.3

Flow diagram illustrating the technique of shoot tip or meristem culture

Importance of Shoot Tip/Meristem Culture

The uses of shoot tips and meristem in tissue culture are very varied and include mainly:

1. Virus eradication,
2. Micro-propagation and
3. Storage of genetic resources.

Virus Eradication

This technique is also valuable for the maintenance of carefully defined stocks of specific varieties and cultivars in disease Free State. The size of the meristem explant is critical for virus eradication.

Micro Propagation

A sexual or vegetative propagation of whole plants using tissue culture techniques is referred to as micro-propagation. Shoot tip or meristem culture of many plant species can successfully be used for micro-propagation.

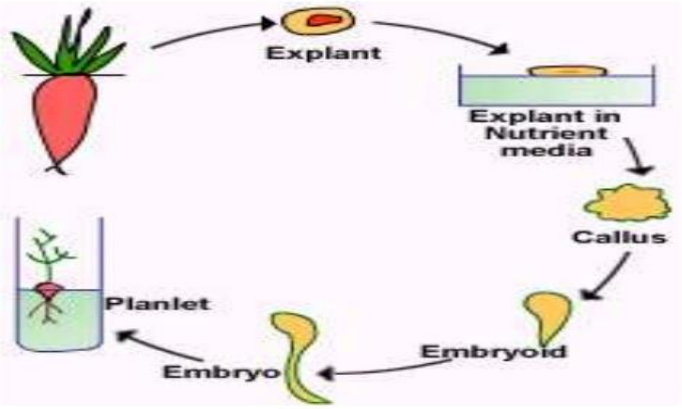
Storage of Genetic Resources

Many plants produce seeds that are highly heterozygous in nature or that is recalcitrant. Such seeds are not accepted for storing genetic resources. So, the meristem from such plants can be stored in vitro.

15. Regeneration Methods of Plants in Culture

Plant regeneration

The process of growing an entire plant from a single cell or group of cells. Regeneration is possible because plant cells can be made totipotent using hormones. Differentiated tissue: stems, leaves, roots, etc. Undifferentiated (embryonic) cells are totipotent: can become a whole new plant by differentiating into a whole new plant.



Plant regeneration

The main objective in plant cultures is to regenerate a plant or plant organ from the callus culture. The regeneration of plant or plant organs only taken place by the expression of cellular totipotancy of the callus tissues. In agriculture biotechnology, tissue culture is most important for the regeneration of transgenic plants from single transformed cells

Organogenesis

Organogenesis is the formation of organs: either shoot or root. In vitro organogenesis depends on the balance of auxin and cytokinin and the ability of the tissue to respond to phytohormones during culture. It takes place in three phases.

In the first phase the cells become competent;

Next, they dedifferentiate.

In the third phase, morphogenesis proceeds independently of the exogenous phytohormone.

Indirect Organogenesis

Formation of organs indirectly via a callus phase is termed indirect organogenesis. Induction of plants using this technique does not ensure clonal fidelity, but it could be an ideal system for selecting somaclonal variants of desired characters and also for mass multiplication. Induction of plants via a callus phase has been used for the production of transgenic plants in which

the callus is transformed and plants regenerated or

the initial explant is transformed and callus and then shoots are developed from the explant.

Direct Organogenesis

The production of direct buds or shoots from a tissue with no intervening callus stage is termed direct organogenesis. Plants have been propagated by direct organogenesis for improved multiplication rates, production of transgenic plants, and—most importantly—for clonal propagation. Typically, indirect organogenesis is more important for transgenic plant production.

16. Somatic Embryogenesis

Introduction

Somatic embryogenesis is an artificial process in which a plant or embryo is derived from a single somatic cell or group of somatic cells. Somatic embryos are formed from plant cells that are not normally involved in the development of embryos, i.e. ordinary plant tissue.

Somatic embryoid formation

It may be formed from:

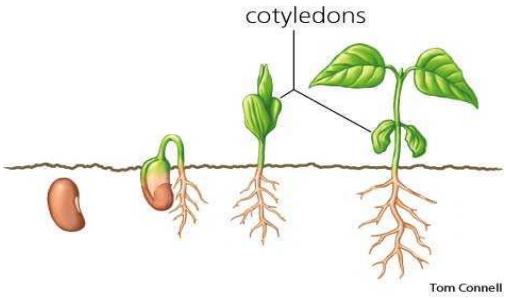
- Vegetative cells of a mature plant
- Reproductive cells other than zygote or
- Cotyledons, hypocotyl or young plantlets

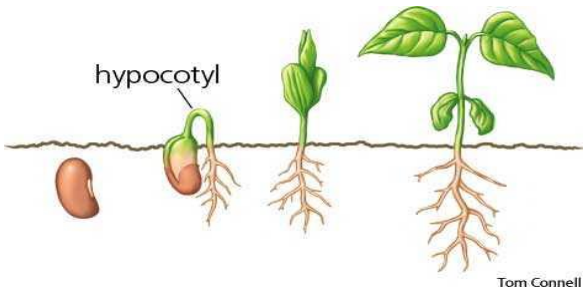
Hypocotyl, Cotyledon

The hypocotyl (short for "hypocotyledonous stem"meaning "below seed leaf") is the stem of a germinating seedling, found below the cotyledons (seed leaves) and above the radicle (root).It is the part of the stem of an embryo plant, beneath the stalks of the seed leaves or cotyledons

and directly above the root. An embryonic leaf in seed-bearing plants, one or more of which are the first leaves to appear from a germinating seed.

A cotyledon is a significant part of the embryo within the seed of a plant, and is defined as "the embryonic leaf in seed-bearing plants, one or more of which are the first to appear from a germinating seed."





Steps of somatic embryogenesis

The steps which are involved in somatic embryogenesis are:

- Initiation of embryogenic culture
- Prolifertion of embryogenic culture
- Pre-maturation of somatic embryos
- Maturation of somatic embryos
- Plant development

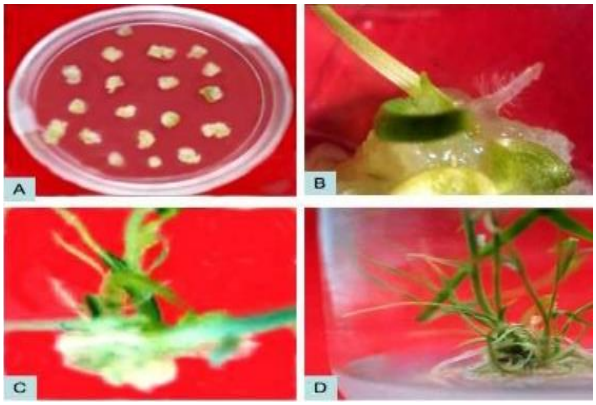
Types of Somatic Embryogenesis

There are two types of somatic embryogenesis

- Direct somatic embryogenesis
- Indirect somatic embryogenesis

Direct somatic embryogenesis

In this type of embryogenesis, the embryos initiate directly from explants in the absence of callus formation.



Indirect Embryogenesis

In this type of embrogenesis, the embryos are developed through cell proliferation i.e., callus formation. The cells from which embryos arise are called as 'Induced embryogenic determined cells' (IEDC). Here growth regulators with specific cultural conditions are required for initiation of callus and then redetermination of those cells into the embryo development.

Advantages:

Advantages of somatic embryogenesis are:

- Higher propagation rate
- Suitable for suspension culture

- Artificial seed production
- Somaclonal variation
- Germplasm conservation
- Labour savings

Disadvantages

- Disadvantages of somatic embryogenesis are:
- Low frequency embryo production
- Incomplete embryo production
- May create unwanted genetic variation
- Inability to generate large number of normal , free living plantlet
- Plantlets are weaker
- Respone tissue specific

Factors influencing somatic embryogenesis

The factors that influence somatic hybridization are:

1. Auxin
2. Cytokinin
3. Nitrogen
4. Activated charcoal
5. Age of culture

1. **Auxin**

In medium having relatively high concentration of auxin embryonal budding or embryonal clumps have been observed. For cell differentiation the medium should contain auxin and

reduced nitrogen.Subsequent development takes place in medium with no auxin or low concentration of auxin and reduced nitrogen. In some plants first and second stages occur in the first medium and plantlet development takes place in the second medium.

2. **Cytokinin**

- The role of cytokinin in embryogenesis is not clear. Embyogenesis in carrot cell suspension is stimulated by addition of zeatin in medium lacking auxin but inhibited by the addition of kinetin. Inhibitory effect of exogenous cytokinin may be due to an increase in endogenous cytokinin in growing embryoids. The role of cytokinin in embryogenesis is not clear. Embyogenesis in carrot cell suspension is stimulated by addition of zeatin in medium lacking auxin but inhibited by the addition of kinetin. Inhibitory effect of exogenous cytokinin may be due to an increase in endogenous cytokinin in growing embryoids

3. **Nitrogen**

The ratio of nitrogen to auxin is an important factor controlling embryogenesis. Embryo development can be initiated on White's medium with low nitrogen content only in absence of auxin. At low nitrogen concentration organic nitrogen is more suitable than inorganic nitrogen.Substances used as a source of nitrogen are potassium nitrate, ammonium chloride, glutamine, glutamic acid, alanine, urea etc.

4. **Activated charcoal**

Presence of activated charcoal in the medium helps embryogenesis in several cases. Activated charcoal may adsorb the inhibitory substances present in the medium.

5. **Age of the culture**

Embryogenesis usually occurs in short-term cultures. With older cultures this ability decreases and ultimately it is completely lost. This may be due to either the inability to synthesise some embryogenetic substances or changes in the ploidy level which may lead to loss of morphogenetic potential.

17.Application of plant cell Culture in crop improvement

Introduction

Plant tissue culture comprises a set of in vitro techniques, methods and strategies that are part of the group of technologies called plant biotechnology. Tissue culture has been exploited to create genetic variability from which crop plants can be improved, to improve the state of health of the planted material and to increase the number of desirable germplasms available to the plant breeder.

Germplasm are living genetic resources such as seeds or tissues that are maintained for the purpose of plant breeding, preservation, and other research uses. Tissue culture protocols are available for most crop species, although continued optimization is still required for many crops, especially cereals and woody plants. Tissue culture techniques, in combination with molecular techniques, have been successfully used to incorporate specific traits through gene transfer. In vitro techniques for the culture of protoplasts, anthers, microspores, ovules and embryos have been used to create new genetic variation in the breeding lines, often via haploid production.

Haploid is the term used when a cell has half the usual number of chromosomes. Cell culture has also produced somaclonal and gametoclonal variants with crop improvement potential.

Somaclonal variation is the variation seen in plants that have been produced by plant tissue culture. Chromosomal rearrangements are an important source of this variation

Gametoclonal variation has been defined as the variation among plants regenerated from gametic cells in culture. The culture of single cells and meristems can be effectively used to eradicate pathogens from planting material and thereby dramatically improve the yield of established cultivars. Large scale micro propagation laboratories are providing millions of plants for the commercial ornamental market and the agricultural, clonally propagated crop

market. With selected laboratory material typically taking one or two decades to reach the commercial market through plant breeding, this technology can be expected to have an ever increasing impact on crop improvement as we approach the new decade.

Applications

- The applications of various tissue culture approaches to crop improvement are following:
- Breeding & biotechnology
- Wide hybridization
- Haploidy
- Somaclonal variation
- Micropropagation
- Synthetic seed
- Pathogen eradiction
- Germplasm preservation

18.Plant Breeding and Biotechnology

Plant breeding can be conveniently separated into two activities: manipulating genetic variability and plant evaluation. Historically, selection of plants was made by simply harvesting the seeds from those plants that performed best in the field. In spite of the general lack of integration of most plant biotechnology and plant breeding programs, field trials of transgenic plants have recently become much more common. More than 50 different plant species have already been genetically modified, either by vector dependent (e.g. Agrobacterium) or vector independent (e.g. biolistic, micro-injection and liposome) methods. In almost all cases, some type of tissue culture technology has been used to recover the modified cells or tissues. In fact, tissue culture techniques have played a major role in the development of plant genetic engineering. Tissue culture will continue to play a key role in the genetic engineering process for the predictable future, especially in efficient gene transfer and transgenic plant recovery.

19. Wide Hybridization

Definition

Hybridization between individuals from different species, belonging to same genus or different genera, is termed as distant or wide hybridization. A critical requirement for crop improvement is the introduction of new genetic material into the cultivated lines of interest, whether via single genes, through genetic engineering, or multiple genes, through conventional hybridization or tissue culture techniques. During fertilization in angiosperms, pollen grains must reach the stigma of the host plant, germinate and produce a pollen tube. The pollen tube must penetrate the stigma and style and reach the ovule. The discharge of sperm within the female gametophyte triggers syngamy and the two sperm nuclei must then fuse with their respective partners. The egg nucleus and fusion nucleus then form a developing embryo and the nutritional endosperm, respectively. This process can be blocked at any number of stages, resulting in a functional barrier to hybridization and the blockage of gene transfer between the two plants.

Barrier for crossing:

There are two barriers for crossing

Pre-Zygotic

Post-Zygotic

Pre –zygotic Barriers

Post- zygote barriers are those which occur prior to fertilization, they are due to the genic differences in different species. There is failure of pollen germination, slow growth of pollen tube, inability of the pollen to reach the ovary and arrest of pollen tube in style ovary and ovule.

Post- Zygotic barriers

Post- zygote barriers are those which occur after fertilization, hybrid in viability and weakness leading to chromosome elimination, lethality and embryo abortion, hybrid sterility and hybrid breakdown with weak or sterile individuals in F2 owing to recombination of genes complements of the parental species.

Techniques to overcome isolation barriers

Pre-zygotic barriers can be overcome by: In-vitro fertilization, Protoplast fusion, Embryo culture, Use of growth hormones (IAA, NAA) and adopting bridging species technique.

In vitro Fertilization

IVF has been used to facilitate both interspecific and intergeneric crosses, to overcome physiological based self-incompatibility and to produce hybrids. A wide range of plant species has been recovered through IVF via pollination of pistils and self and cross-pollination of ovules. This range includes agricultural crops, such as tobacco, clover, com, rice, cole, canola, poppy and cotton. The use of delayed pollination, distant hydridization, pollination with abortive or irradiated pollen, and physical and chemical treatment of the host ovary have been used to induce haploidy.

Protoplast Fusion

Protoplast fusion has often been suggested as a means of developing unique hybrid plants which cannot be produced by conventional sexual hybridization. Protoplasts can be produced from many plants, including most crop species. However, while any two plant protoplasts can be fused by chemical or physical means, production of unique somatic hybrid plants is limited by the ability to regenerate the fused product and sterility in the interspecific hybrids rather than the production of protoplasts. Perhaps the best example of the use of protoplasts to improve crop production is that of Nicotiana, where the somatic hybrid products of a chemical fusion of protoplasts have been used to modify the alkaloid and disease-resistant traits of commercial tobacco cultivars.

Protoplast fusion should focus on four areas:

Agriculturally important traits

Achieving combinations that can only be accomplished by protoplast fusion

A somatic hybrids integrated into a conventional breeding programme and

The extension of protoplast regeneration to a wider range of crop species

Embryo Culture

The most common reason for post-zygotic failure of wide hybridization is embryo abortion due to poor endosperm development. Embryo culture has been successful in overcoming this major barrier as well as solving the problems of low seed set, seed dormancy, slow seed germination, inducing embryo

growth in the absence of a symbiotic partner, and the production of monoploids of barley. Interspecific and intergeneric hybrids of a number of agriculturally important crops have been successfully produced, including cotton, barley, tomato, rice, jute, Hordeum X Secale,Triticum x Secale,Tripsacumx lea and some Brassicas.

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Bridging species technique

When direct cross between tow species with the same or different ploidy levels are difficult to accomplish a third specie (Bridge specie) is used to make such crosses possible

Pre-zygotic barriers

Pre- zygote barriers can be overcome by: Tissue culture techniques, Back crossing, Doubling of chromosomes and Embryo rescue.

Backcrossing

Backcrossing is a **crossing** of a hybrid with one of its parents or an individual genetically similar to its parent, in order to achieve offspring with a genetic identity which is closer to that of the parent.It is used in horticulture, animal breeding and in production of gene knockout organisms.

Doubling of chromosomes

Artificial production of **doubled** haploids is important in plant breeding. Haploid cells are produced from pollen or egg cells or from other cells of the gametophyte, then by induced or spontaneous **chromosome doubling**, a **doubled** haploid cell is produced, which can be grown into a **doubled** haploid plant.

Embryo rescue

The term "**embryo rescue**" refers to a number of in vitro techniques whose purpose is to promote the development of an immature or weak **embryo** into a viable plant. **Embryo rescue** has been widely used for producing plants from hybridizations in which failure of endosperm to properly develop causes **embryo** abortion. **Embryo rescue** is one of the earliest and successful forms of in-vitro culture techniques that is used to assist in the development of plant **embryos** that might not survive to become viable plants

20. Haploids

Introduction

A haploid is a cell or organism that has a single set of chromosomes that are not paired. The haploid gamete is normally produced during plant cell division. During fertilization, these cells normally merge with other similar haploid cells. A haploid cell only has half the number of chromosomes as are present in diploids.

Applications of Haploid Plants are:

- In Vitro production of haploids can solve some problems in genetic studies
- The following points highlight the top applications of haploid plants

Development of Pure Homozygous Line

In plant breeding, it is very much essential to get the pure homozygous line which is generally obtained through selfing for 6-7 generations. But by the use of anther/pollen culture it can be reduced to few months or a year. These genetically pure homozygous lines are used for breeding as well as genetic research purpose. This technique is also helpful for breeding of these plants which have more elongated juvenile phase.

Selection of Mutants Resistance to Diseases

Selection of mutants with resistance to disease is of prime importance in crop improvement. Haploids provide a relatively easier system for the induction of mutations. Some examples of using anther culture technique in mutant successfully are tobacco mutants resistant to black shank disease and wheat lines resistant to scab.

Transfer of Desired Alien Gene

Chromosomal instability in haploids makes them potential tools for introduction of alien chromosomes on genes during wider crossing programs.

Induction of Mutagenesis

Haploid cell cultures are useful material for induction of mutations and to study the effect of mutation. This method can overcome the masking effect of presence of dominant gene. The screening method for detection of mutational effect Is also easier in this technique.

Induction of Genetic Variability

The pollen/microspore is easy explant for production of genetically variable types by introducing the different foreign genes through different transformation procedure. These transformed or transgenic haploids can be used further in breeding program.

Development of Aneuploids

Haploids have been used in the production of aneu plaids like monosomies in wheat, trisomies in potato. In tobacco nullisomics were derived from haploids obtained from monosomies which could

not produce nullisomics on selfing. **Nullisomic** is a genetic condition involving the lack of both the normal chromosomal pairs for a species (2n-2).

21.Somaclonal Variation

Somaclonal variations

The genetic variations found in the in vitro cultured cells are collectively referred to as somaclonal variations. These are genetic variations in plants that have been produced by plant tissue culture and can be detected as genetic or phenotypic traits.

Basic features of somaclonal variations

Variations in number and structure of chromosomes are commonly observed. Regenerated plants with altered chromosomal changes often show changes in leaf shape and colour, growth rate and habit, and sexual fertility. It is generally heritable mutations and persists in plant population even after plantation into the field.

Mechanism of Somaclonal Variations

- **Genetic (Heritable Variations)**
- Pre-existing variations in the somatic cells of explant
- Caused by mutations and other DNA changes
- Occur at high frequency
- **Epigenetic (Non-heritable Variations)**
- Variations generated during tissue culture
- Caused by temporary phenotypic changes
- Occur at low frequency

Causes of Somaclonal Variations are:

1. Physiological Cause

2. Genetic Cause

3. Biochemical Cause

1. Physiological Cause

Physiological causes are: Exposure of culture to plant growth regulators and Culture conditions.

2. Genetic Cause

- Genetic causes are: **Change in chromosome number:** Aneuploidy, gain or loss of 1 or more chromosomes, Polyploidy, gain or loss of an entire genome, Translocation, arms of chromosomes switched, inversion, piece of chromosome inverted.
- **Change in chromosome structure**: Deletion, Inversion, Duplication, Translocation,
- **Gene Mutation**: Transition, Traversions, Insertion and Deletion
- **Plasmagene Mutation :** It is a self-replicating extra nuclear determiner of hereditary characteristics.
- **Transposable element activation :** Transposable elements (TEs), also known as "jumping genes" or transposons, are sequences of DNA that move (or jump) from one location in the genome to **another.**
- **DNA sequence :** Change in DNA **and** Detection of altered fragment size by using Restriction enzyme
- **Change in Protein :** Loss or gain in protein band and Alteration in level of specific protein
- **Methylation of DNA:** Methylation inactivates transcription process.

Biochemical Cause

It is the Lack of photosynthetic ability due to alteration in carbon metabolism e.g. biosynthesis of starch via carotenoid pathway and Nitrogen metabolism processes.

- **Advantages of Somaclonal Variations are:** help in crop improvement, creation of additional genetic variations, increased and improved production of secondary metabolites, selection of plants resistant to various toxins, herbicides, high salt concentration and mineral toxicity, suitable for breeding of tree species.

Applications of Somaclonal Variations

Production of agronomically useful plants: As a result of somaclonal variations, several novel variants of existing crops have been developed, e.g., pure thorn-less blackberries, somaclonal variations are useful and improved morphological characters in different crops.

Resistance to diseases: Somaclonal variations have largely contributed towards the development of disease resistance in many crops e.g. rice, wheat, maize, sugarcane, tobacco, apple, tomato.

- **Resistance to abiotic stresses:** It has been possible to develop biochemical mutants with abiotic stress resistance.
- i. Freezing tolerance e.g. wheat.
- ii. Salt tolerance e.g., rice, maize, tobacco.
- iii. Aluminium tolerance e.g., carrot, sorghum, tomato.

Resistance to herbicides

Certain somaclonal variants with herbicide resistance have been developed. E.g.

i. Tobacco resistant to glyphosate, sulfonylurea and picloram.

- ii. Carrot resistant to glyphosate.
- iii. Lotus resistant to 2, 4-dichlorophenoxy acetic acid

Improved seed quality

A new variety of Lathyrus sativa (grass Pea) seeds with a low content of neurotoxin has been developed through somaclonal variations. **Neurotoxins** are an extensive class of exogenous

chemical neurological insults that can adversely affect function in both developing and mature nervous tissue.

22.Micropropagation

Micropropagation is the practice of rapidly multiplying reserve plant material to produce a large number of progeny plants, using modern methods of plant tissue culture. Micropropagation is used to multiply noble plants, such as those that have been genetically modified or raised through conventional plant breeding methods. It is also used to provide a sufficient number of seedlings to plant from a common plant that does not produce seeds, or does not respond well to vegetative reproduction.

Micropropagation Technique

Micropropagation is a complicated process and comprises mainly 4 stages (I, II, III and IV). But initial step 0 is also necessary.

Step 0: This is the initial step in micro-propagation, and involves the selection and growth of common plants for about 3 months under controlled conditions.

Stage I – Establishment: In this stage, the initiation and establishment of the culture is achieved in a suitable medium. The selection of appropriate explants is important. The most

commonly used explants are organs, shoot tips and axillary shoots. The explant selected is surface sterilized and washed prior to use.

Stage II – Multiplication: At this stage, the main activity of Micro propagation occurs in a defined culture medium. Phase II mainly involves the multiplication of shoots or the rapid formation of explant embryos.

Stage III – Rooting: This stage involves the transfer of shoots to a medium for rapid development in shoots. Sometimes sprouts are planted directly on the ground to develop roots. In vitro rooting of shoots is preferred while simultaneously handling a large number of species.

Stage IV – Acclimatization: This stage involves the establishment of seedlings in the soil. This is done by transferring seedlings from stage III of the laboratory to the environment of the greenhouse. For some plant species, stage III is omitted, and un rooted shoots of stage II are planted in pots or in a suitable compost mix.

Factors Affecting Micro-propagation: there is need of optimization of several factors is necessary for success in clonal propagation in vitro (micro-propagation).

Genotype of the plant: Selection of the correct genotype of plant species (by screening) is necessary to improve micropropagation. In general, plants with a vigorous germination and branching ability are more suitable for micro-propagation.

Physiological status of explants: The explants (plant materials) of the most recently produced parts of plants are more effective than those of the older regions. A good knowledge of the process of natural propagation of donor plants, with special reference to the stage of growth and seasonal influence, will be useful in the selection of explants.

Culture media: Conventional plant tissue culture media are suitable for micropropagation during stage I and stage II. However, for stage III, certain modifications are essential. The addition of growth regulators (auxins and cytokinins) and alterations in mineral composition is essential. This depends largely on the type of crop.

Light: The photosynthetic pigment in cultured tissues absorbs light and, therefore, influences micropropagation. Variations in daytime lighting also effect micropropagation. In general, a lighting of 16 hours of day and 8 hours of night is suitable for the proliferation of shoots.

Temperature: The majority of micropropagation culture necessitates optimum temperature around 25 °C. However, there are some exceptions.

Micropropagation applications are:

- Suitable alternative to traditional methods
- High prevalence of plants
- The production of disease-free plants
- Seed production in some crops
- Cost effective process
- Automated micro propagation
- Very small size explants can be used
- Only practical method of multiplying genetically modified cells or cells after protoplast fusion.
- Ease in keeping, packing and transport of material multiplied by micropropagation.

23.Synthetic Seed

Synthetic seed: Many fruit crops are difficult to multiply by conventional propagation methods and improve through traditional breeding programs. Among the innovative techniques of micro propagation, the concept of somatic embryogenesis with synthetic seed production or artificial seed technology is very promising. Synthetic seed is referred to as encapsulated somatic embryos, which functionally mimic seeds and can develop into seedling under suitable conditions. Synthetic seeds are defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be used for sowing as a seed and that possess the ability to convert into a plant under in vitro or ex vitro conditions and that retain this potential also after storage. In simple words synthetic seed contains an embryo produced by somatic embryogenesis enclosed within an artificial medium that supplies nutrients and is encased in an artificial seed covering.

Why Synthetic Seeds:

In some of the horticultural crops seeds propagation is not successful due to; heterozygosity of seeds particularly in cross pollinated crops, minute seed size e.g.; orchids, presence of reduced endosperm, some seeds require mycorrhizal fungi association for germination e.g.: orchids, no seeds are formed.

Characteristics of Synthetic Seeds:

i. High volume. Large scale propagation method

ii. Maintains genetic uniformity of plants

iii. Direct delivery of propagules to the field, thus eliminating transplants

iv. Lower cost per plantlet

v. Rapid multiplication of plants

Advantages of Synthetic Seeds are:

i. Ease of handling while in storage

ii. Easy to transport

iii. Has potential for long term storage without losing viability

iv. Maintains the clonal nature of the resulting plants

v. Serves as a channel for new plant lines produced through biotechnological advances to be delivered directly to the green house or field

vi. Allows economical mass propagation of elite plant varieties

24.Germplasm conservation

Germplasm: A germplasm is a collection of genetic resources for an organism. Germplasm is a living tissues from which new plants can be grown. It can be a seed or another plant part-a leaf,a piece of stem,pollen or even just a few cells that can be turned into the whole plant. For plants, the germplasm may be stored as a seed, stem, Callus, Whole plant in nurseries.

Germplasm conservation: Plant germplasm is genetic source material in the form of Seeds, Cultured cells Callus, Pollens. The in-situ /ex-situ preservation of this material is known as "Germplasm conservation". Germplasm provide the raw material (genes) which the breeder uses to develop commercial crop varieties.

What is the need of Preservation: Preservation/Conservation of plant biodiversity is an important issue. Storage of Economically important, endangered, rare species and make them available when needed. The conventional methods of storage failed to prevent losses caused due to various reasons.

Methods of Germplasm conservation are:

1. In-situ Preservation
2. Ex-situ Preservation

In-situ Preservation: it is preservation of the germplasm in their natural habitat, conservation of domesticated and cultivated species in the farm or in the surroundings. However, there is a heavy loss or decline of species, populations and ecosystem composition, which can lead to a loss of biodiversity, due to habitat destruction and the transformations of these natural environments; therefore, in situ methods alone are insufficient for saving endangered species.

Ex-situ preservation: 1. It is used to maintain the biological material outside their natural habitats, for storage in seed banks, field gene collections, in vitro collections and botanical gardens. Ex situ conservation is a viable way for saving plants from extinction, and in some cases, it is the only possible strategy to conserve certain species. In vitro conservation is especially important for vegetatively propagated and for non-orthodox seed plant species. Non-Orthodox seeds are seeds which do not survive drying and/or freezing during ex-situ conservation.

Approaches for the in vitro conservation of germplasm:

- Cryopreservation (freeze-preservation)
- Cold storage
- Low-pressure and low-oxygen storage
- **Cryopreservation:** Cryopreservation (Greek, krayos-frost) literally means preservation in the frozen state. The principle involved in cryopreservation is to bring the plant cell and tissue cultures to a zero metabolism or non-dividing state by reducing the temperature in the presence of cryoprotectants In this case the cells are preserved in the frozen state. The germplasm is stored at a very low temperature using
- Solid carbon dioxide (at -790C)
- Using low temperature deep freezers (at -800C)
- Using vapour nitrogen (at- 1500C)
- Liquid nitrogen (at-1960C).

Cold Storage: Cold storage is a slow growth germplasm conservation method. It conserves the germplasm at a low and non-freezing temperature (1- 9°C). The growth of the plant material is slowed down in cold storage in contrast to complete stoppage in cryopreservation. Thus it prevents cryogenic injuries. Long term cold storage is simple, cost effective. It yields germplasm with good survival rate. Virus free strawberry plants could be preserved at 10°C for about 6 years. Several grape plants have been stored for over 15 years by using a cold storage at temperature around 9°C and transferring them in the fresh medium every year.

Low pressure and low oxygen storage: In low- pressure storage, the atmospheric pressure surrounding the plant material is reduced. In the low oxygen storage, the oxygen concentration is reduced. The lowered partial pressure reduces the in vitro growth of plants.In the low-oxygen storage, the oxygen concentration is reduced and the partial pressure of oxygen below 50 mmHg reduces plant tissue growth. Due to the reduced availability of 0_2, and reduced production of CO_2, the photosynthetic activity is reduced. It inhibits the plant tissue growth and dimension. This method has also helped in increasing the shelf life of many fruits, vegetables and flowers. The germplasm conservation through the conventional methods has several limitations such as short-

lived seeds, seed dormancy, seed-borne diseases, and high inputs of cost and labour. The techniques of cryo-preservation (freezing cells and tissues at -1960c) and using cold storages help us to overcome these problems.

Applications or significance of germplasm conservation are: The conservation of germplasm involves the preservation of the genetic diversity of a particular plant or genetic stock. It can be used at any time in future.It is important to conserve the endangered plants or else some of the valuable genetic traits present in the existing and primitive plants will be lost. Main crops produce recalcitrant or short lived seeds. Similarly, in case of clonal crops seeds are not the best material to conserve due to their genetic heterogeneity and unknown worth. Their genes need to be conserved. The roots and tubers loose viability rapidly. Their storage requires large space, low temperature and is expensive. In addition, materials modified by genetic engineering may some, times be unstable. Such materials are needed to be conserved intact for future use.

25.Genetic Markers in Plant Breeding

Genetic marker: A genetic marker is a gene or DNA sequence with a known location on a chromosome. It can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed. Any phenotypic difference controlled by genes, that can be used for studying recombination processes or selection of a more or less closely associated target gene Anything in the genome that is variable and can be used to compare individuals. Detectable allelic variation on a chromosome it can be a phenotype, can also be a unique detectable sequence of DNA. They are points of variation that can be used to identify individuals or species, or may be used to associate an inherited disease with a gene through genetic linkage with nearby but possibly unidentified or uncharacterised genes. Examples include single nucleotide polymorphisms (SNPs) and minisatellites.

Polymorphism: In biology and zoology is the occurrence of two or more clearly different morphs or forms, also referred to as alternative phenotypes, in the population of a species.

Single nucleotide polymorphisms (SNPs): A single-nucleotide polymorphism, often abbreviated to SNP. A variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population. For example, at a specific base position in the human genome, the C nucleotide may appear in most individuals, but in a minority of individuals, the position is occupied by an A. This means that there is an SNP at this specific position, and the two possible nucleotide variations – C or A – are said to be alleles for this position. As SNPs are thought to play a major role in the induction of phenotypic variation in plants, so it is very important to identify the functional SNPs regarding crop improvements. SNPs also can identify the genomic diversity of species to demonstrate the speciation and evolution, and associate genomic variations with phenotypic traits

Types of genetic markers are:

Molecular markers

Morphological markers

26.Molecular Markers

Molecular markers

It is a sequence of DNA or protein that can be screened to reveal key attributes of its state or composition and thus used to reveal genetic variation, also known as "Genetic Marker". Genetic markers are the sequences of DNA which have been traced to specific location on the chromosomes and associated with particular traits.

Classification of molecular markers:

Molecular Markers are classified as:

1. Protein Based Markers/ Biochemical Markers

2. DNA Based Markers

Protein Based Markers/ Biochemical Markers: Plant Breeding Markers related to the variations in protein and amino acid banding pattern.

Isozyme markers: Multiple forms of the same enzyme coded by the different genes

Allozyme : one enzyme, one locus; two or more alleles in a population

Advantages: advantages of molecular markers are:

They are simple, inexpensive, electrophoretically resolvable, and detectable, does not require DNA extraction or the availability of sequence information, primers or probes, quick and easy to use, codominant markers that have high reproducibility.

Disadvantages of molecular markers are: they are relatively low abundance and low level of polymorphism, can be affected by environmental conditions, they may change depending on the type of tissue used for the analysis**.**

Applications of molecular markers are:

They are used for detection of the gene introgression (gene movement) and recombination, for comparative mapping, for determination of the genetic diversity and phylogenetic relationships.

27. DNA based markers

DNA Markers

A gene or other fragment of DNA whose location in the genome is known is called DNA marker. It refers to any unique DNA sequence which can be used in DNA hybridization, PCR or restriction mapping experiments to identify that sequence. It can be identified by a range of molecular techniques such as RFLPs, RAPDs, AFLP, SNPs, SCARs, microsatellites etc.

Advantages of DNA markers:

Advantages of DNA markers are presented below.

- They are highly polymorphic.
- They have simple inheritance (often co-dominant).
- They abundantly occur throughout the genome.
- They are easy and fast to detect.
- They exhibit minimum pleiotropic effect.
- Their detection is not dependent on the developmental stage of the organism.

Properties of DNA Marker: An ideal DNA marker should have some properties or characteristics

Polymorphism:

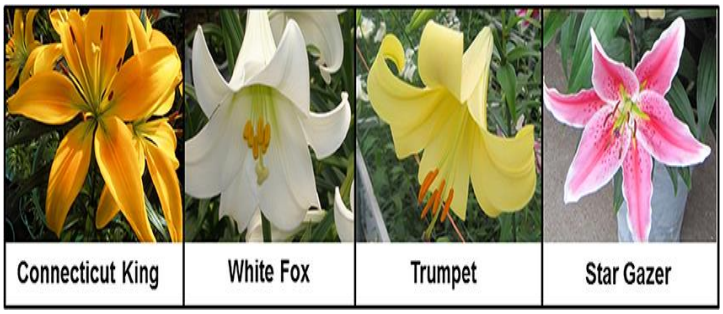
- Markers should exhibit high level of polymorphism.
- In other words, there should be variability in the markers.
- It should demonstrate measurable differences in expression between trait types and/or gene of interest.

Co-Dominant:

- Marker should be co-dominant.
- It means, there should be absence of intra-locus interaction.
- It helps in identification of heterozygotes from homozygotes.

Multi-Allelic:

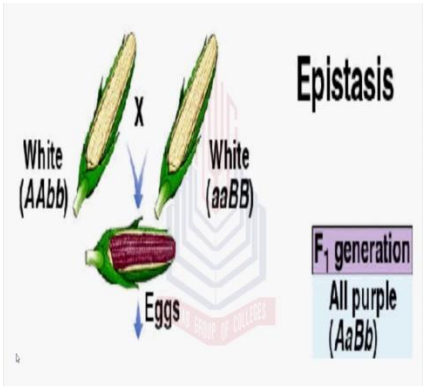
- The marker should be multi-allelic.
- It useful in getting more variability/ polymorphism for a character.
- A multiallelic site is a specific locus in a genome that contains three or more observed alleles, again counting the reference as one, and therefore allowing for two or more variant alleles.



Different flower colors due to allelic variation in multiple genes

No Epistasis:

- There should be absence of epistasis.
- It makes Identification of all phenotypes (homo- and heterozygotes) easy.
- Epistasis: the interaction of genes that are not alleles, in particular the suppression of the effect of one such gene by another.



Neutral:

- The marker should be neutral.
- The substitution of alleles at the marker locus should not alter the phenotype of an individual.
- This property is found in almost all the DNA markers

No Effect of Environment: Markers should be insensitive to environment. This property is also found in almost all the DNA markers.

Applications of DNA Marker in Crop Improvement are:

- i. DNA markers are useful in the assessment of genetic diversity in germplasm, cultivars and advanced breeding material.
- ii. DNA markers can be used for constructing genetic linkage maps.
- iii. DNA markers are useful in identification of new useful alleles in the germplasm and wild species of crop plants.
- iv. DNA markers are used in the marker assisted or marker aided selection. MAS has several advantages over straight selection.
- v. DNA markers are useful in the study of crop evolution.

28. Morphological markers

Morphology:

Morphology is a branch of biology dealing with the study of the form and structure of organisms and their specific structural features.

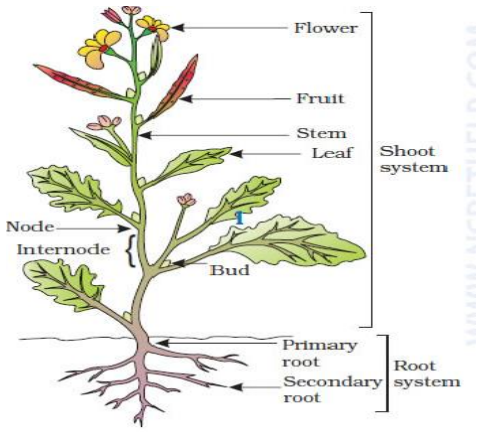
Plant Morphology:

Plant morphology or phyto morphology is the study of the physical form and external structure of plants. This is usually considered distinct from plant anatomy, which is the study of the internal

structure of plants, especially at the microscopic level. Plant morphology is useful in the visual identification of plants.

Morphological features of plants:

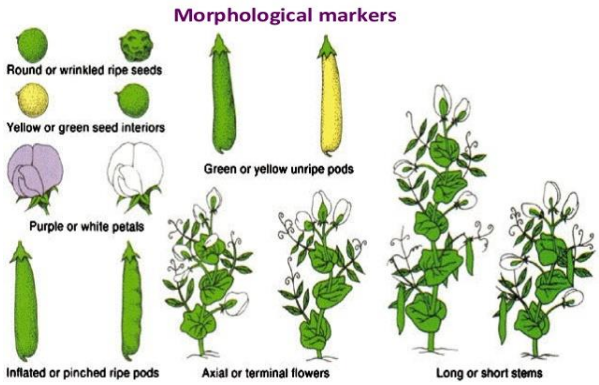
- Plants are characterized by following features:
- Roots
- Shoots
- Stem
- Leaves
- Flowers
- Fruits



Morphological Markers

They are visually characterized phenotypic characters. e.g.

- Flower colour ,seed shape , growth habit, pigmentation
- It involves Germplasm characterization and indirect selection



Advantages

Some advantages of morphological markers are:

- They are inexpensive to score and ready to experiments in natural populations

Disadvantages

Some disadvantages of morphological markers are:

- visible polymorphisms relatively rare

- Most genetic variation not so easily observed (Variants are ambiguous)
- Genetic basis of variation can be complex, and is not necessarily easy to determine.

Limitations: some limitations of morphological marks are enlisted here:

- They do not represent the genome adequately, they give o stable inheritance(Need repeated measures)
- They generally express late into the development of an organism. Hence their detection is dependent on the development stage of the organism.
- They usually exhibit dominance, sometimes they exhibit deleterious effects. They exhibit pleiotropy. They exhibit epistasis. They exhibit less polymorphism. They are highly influenced by the environmental factors.

29. Transformation

Transformation

In molecular biology, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane. Transformation occurs most commonly in bacteria and in some species occurs naturally. Transformation can also be effected by artificial means. Bacteria that are capable of being transformed, whether naturally or artificially, are called competent

Competent cells

Competent Cells are more likely to incorporate foreign DNA if their cell walls are altered so that DNA can pass through more easily. Such cells are said to be competent

Types of competence (there is two types of competence):

i. Natural competence
ii. Artificial competence

i. **Natural competence:** Bacteria are able to take up DNA from their environment by three ways; conjugation, transformation, and transduction. In transformation the DNA is directly entered to the cell. Uptake of transforming DNA requires the recipient cells to be in a specialized physiological state called competent state.

Artificial competence:

- It is a laboratory procedure by which cells are made permeable to DNA, with conditions that do not normally occur in nature. This procedure is comparatively easy and simple, and can be used in the genetic engineering of bacteria but in general transformation efficiency is low. There are two main methods for the preparation of competent cells They are **Calcium chloride method and Electroporation.** Transformation may also be used to describe the insertion of new genetic material into nonbacterial cells including animal and plant cells

History:

Transformation was first demonstrated in 1928 by British bacteriologist Frederick Griffith. Griffith discovered that a harmless strain of *Streptococcus pneumoniae* could be made virulent after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent.

In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They isolated DNA from a virulent strain of S. pneumoniae and using just this DNA was able to make a harmless strain virulent. They called this uptake and incorporation of DNA by bacteria "transformation." Transformation using electroporation was developed in the late 1980s, increasing the efficiency of in-vitro transformation and increasing the number of bacterial strains that could be transformed. Transformation of animal and plant cells was also investigated with the first transgenic mouse being created by injecting a gene for a rat growth hormone into a mouse embryo in 1982. In 1907 a bacterium that caused plant tumors, Agrobacterium tumefaciens, was discovered and in the early 1970s the tumor inducing agent was found to be a DNA plasmid called the Ti plasmid. By removing the genes in the plasmid that caused the cancer and adding in novel genes researchers were able to infect plants with A. tumefaciens and let the bacteria insert their chosen DNA into the genomes of the plants. Not all

plant cells are susceptible to infection by A. tumefaciens so other methods were developed including electroporation and micro-injection. Particle bombardment was made possible with the invention of the Biolistic Particle Delivery System (gene gun) by John Sanford in 1990.

30. Mechanisms of Transformation

Bacterial Transformation:

Bacterial transformation may be referred to as a stable genetic change brought about by the uptake of naked DNA (DNA without associated cells or proteins) and competence refers to the state of being able to take up exogenous DNA from the environment.

Two forms of competence exist:

1. Natural and
2. Artificial.

1. **Natural competence:** About 1% of bacterial species are capable of naturally taking up DNA under laboratory conditions; many more are able to take it up in their natural environments. Such bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s). DNA material can be transferred between different strains of bacteria, in a process called horizontal gene transfer.
2. **Artificial competence:** Artificial competence is induced by laboratory procedures and involves making the cell passively permeable to DNA by exposing it to conditions that do not normally occur in nature. Calcium chloride transformation is a method of promoting competence. Chilling cells in the presence of divalent cations such as Ca^{2+} (in $CaCl_2$) prepares the cell membrane to become permeable to plasmid DNA. The cells are incubated on ice with the DNA and then briefly heat shocked (e.g., 42°C for 30–120 seconds) thus allowing the DNA to enter the cells. This method works very well for circular plasmid DNA. An excellent preparation of competent cells will give ~10^8 colonies per microgram of plasmid. A poor preparation will be about 10^4/μg or less. Good, non-commercial preparations should give 10^5 to 10^6 transformants per microgram of plasmid. The method, however, usually does not work well for linear DNA, such as fragments of chromosomal DNA, probably because the cell's native exonuclease enzymes rapidly degrade linear DNA.

Interestingly, cells that are naturally competent are usually transformed more efficiently with linear DNA than with plasmid DNA.

Electroporation: Electroporation is another method of promoting competence. In the method the cells are briefly shocked with an electric field of 10-20 kV/cm that creates holes in the cell membrane through which the plasmid DNA enters. This method is ready to the uptake of large plasmid DNA. After the electric shock the holes are rapidly closed by the cell's membrane-repair mechanisms. The efficiency with which a competent culture can take up exogenous DNA and express its genes is known as transformation efficiency.

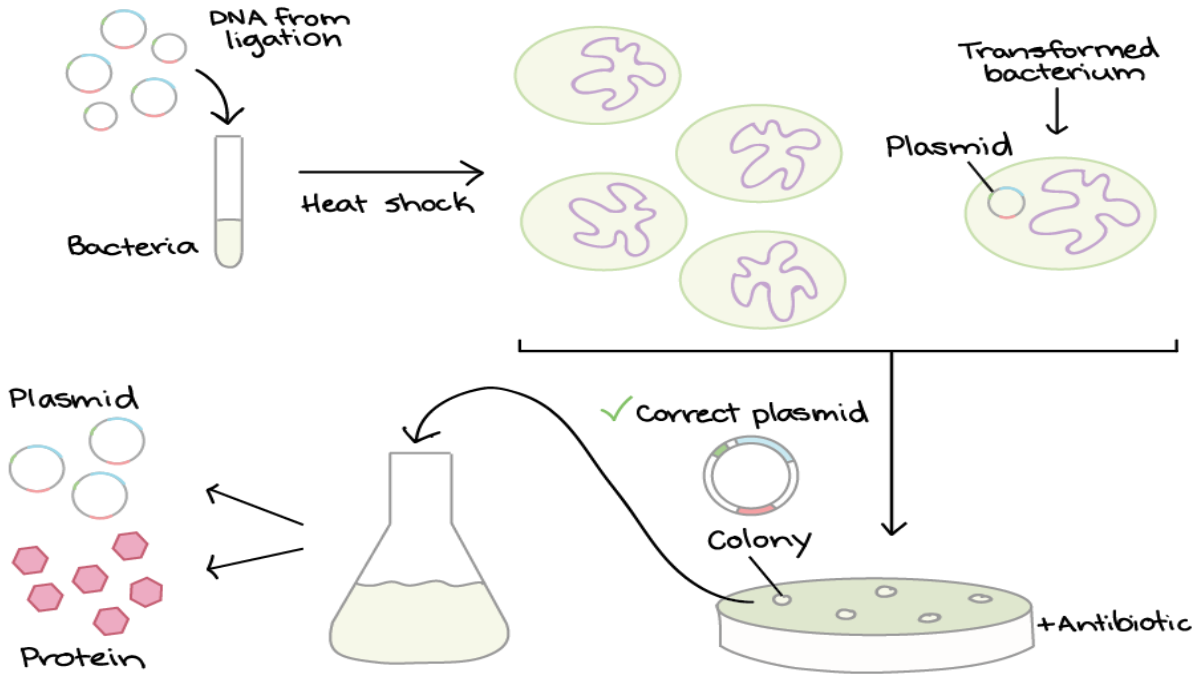
31. Bacterial transformation & selection

Bacteria can take up foreign DNA in a process called transformation. Transformation is a key step in DNA cloning. It occurs after restriction digest and ligation and transfers newly made plasmids to bacteria. After transformation, bacteria are selected on antibiotic plates. Bacteria with a plasmid are antibiotic-resistant, and each one will form a colony. Colonies with the right plasmid can be grown to make large cultures of identical bacteria, which are used to produce plasmid or make protein

DNA cloning

Transformation and selection of bacteria are key steps in DNA cloning. DNA cloning is the process of making many copies of a specific piece of DNA, such as a gene. The copies are often made in bacteria. In a typical cloning experiment, researchers first insert a piece of DNA, such as a gene, into a circular piece of DNA called a plasmid. This step uses restriction enzymes and DNA ligase and is called a ligation. After a ligation, the next step is to transfer the DNA into bacteria in a process called transformation. Then, we can use antibiotic selection and DNA analysis methods to identify bacteria that contain the plasmid we’re looking for.

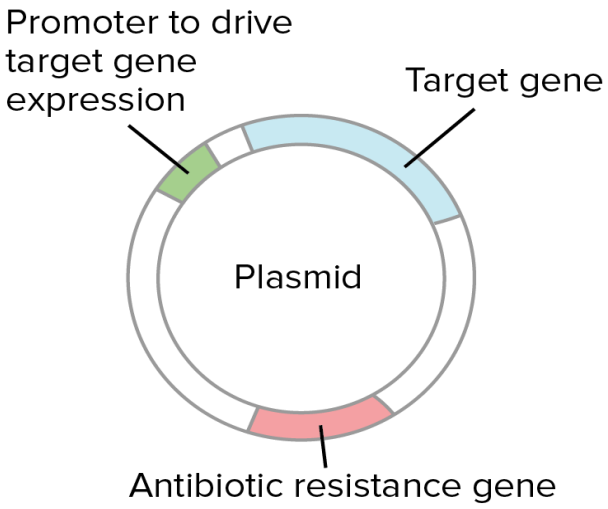
Steps of bacterial transformation and selection



Steps

Specially prepared bacteria are mixed with DNA (e.g., from a ligation). The bacteria are given a heat shock, which causes some of them to take up a plasmid. Plasmids used in cloning contain an antibiotic resistance gene. Thus, all of the bacteria are placed on an antibiotic plate to select for ones that took up a plasmid. Bacteria without a plasmid die. Each bacterium with a plasmid gives rise to a cluster of identical, plasmid-containing bacteria called a colony. Several colonies are checked to identify one with the right plasmid (e.g., by PCR or restriction digest).

A colony containing the right plasmid is grown in bulk and used for plasmid or protein production.



Why do we need to check colonies?

The bacteria that make colonies should all contain a plasmid (which provides antibiotic resistance). However, it's not necessarily the case that all of the plasmid-containing colonies will have the same plasmid.

How does that work?

When we cut and paste DNA, it's often possible for side products to form, in addition to the plasmid we intend to build. For instance, when we try to insert a gene into a plasmid using a particular restriction enzyme, we may get some cases where the plasmid closes back up (without taking in the gene), and other cases where the gene goes in backwards.

Why does it matter if a gene goes into a plasmid backwards?

In some cases, it doesn't. However, if we want to express the gene in bacteria to make a protein, the gene must point in the right direction relative to the **promoter**, or control sequence that drives gene expression. If the gene were backwards, the wrong strand of DNA would be transcribed and no protein would be made. Because of these possibilities, it's important to collect plasmid DNA from each colony and check to see if it matches the plasmid we were

trying to build. Restriction digests, PCR, and DNA sequencing are commonly used to analyze plasmid DNA from bacterial colonies.

32. Transformation in Plants

Gene Transfer is introduction of foreign genetic material, either DNA or RNA artificially or naturally into a cell. It is often also referred to as transformation and is one of the foundations of molecular biology. It is now possible to introduce and express DNA stably in nearly 150 different plant species. To achieve genetic transformation in plants, we need the construction of a vector (genetic vehicle)which transports the genes of interest, flanked by the necessary controlling sequences i.e. promoter, Terminator, Selectable marker and other genes that deliver the DNA into the host plant (Ex. vir genes of Agrobacterium)

Plant transformation

Plant transformation is a scientific approach whereby DNA from any organism is inserted into the genome of a species of interest. The inserted DNA is called a "transgene", and the resulting plant is said to be "transgenic". Transgenic plants are plants derived from cells in which genes (often of nonplant origin) have been stably introduced by transformation to give the plant a new and useful trait. Transgenic plants can be obtained after transformation of single cells and the subsequent regeneration into complete, fertile plants by tissue culture protocols. Transformed plant cells can be identified by their ability to grow on selective media containing an antibiotic or a herbicide as transformation vectors contain selection genes conferring such properties. Novel functions are expressed in transformed plant cells if the coding regions are surrounded by promoter and terminator regions that are recognized by the plant transcription machinery. The most preferred methods for plant transformation use either the particle gun or the natural transformation system of *Agrobacterium tumefaciens*, as they can cope with cells present in whole plants or tissues. *Agrobacterium tumefaciens* can be disarmed by deletion of the *onc*-genes that are naturally present between the 25-bp repeats of the T-DNA. Any gene introduced between these repeats is translocated into plant cells by *Agrobacterium tumefaciens.* Transformed cells with a single copy of the transgene usually show higher and more stable expression than multicopy lines, in which expression may suffer from posttranscriptional gene silencing. (T)-DNA integration in plant cells occurs at random sites in the genome by nonhomologous end-joining and related backup pathways.

Targeted integration of transgenes can be accomplished in plant cells by using either a site-specific recombination system (e.g. Cre-*lox*) or by homology-directed integration in combination with a site-specific nuclease (e.g. a zinc-finger nuclease). *Agrobacterium tumefaciens*-mediated

transformation can be applied not only in dicotyledonous plants, but also in monocots such as cereals and in yeasts and fungi. The T-DNA can be used as a mutagen causing insertion mutations. Libraries of *Arabidopsis thaliana* T-DNA transformants are in use now in mutant screens to identify insertion mutations in genes of interest (reverse genetics).

Table 49.1 Gene transfer (DNA delivery) methods in plants

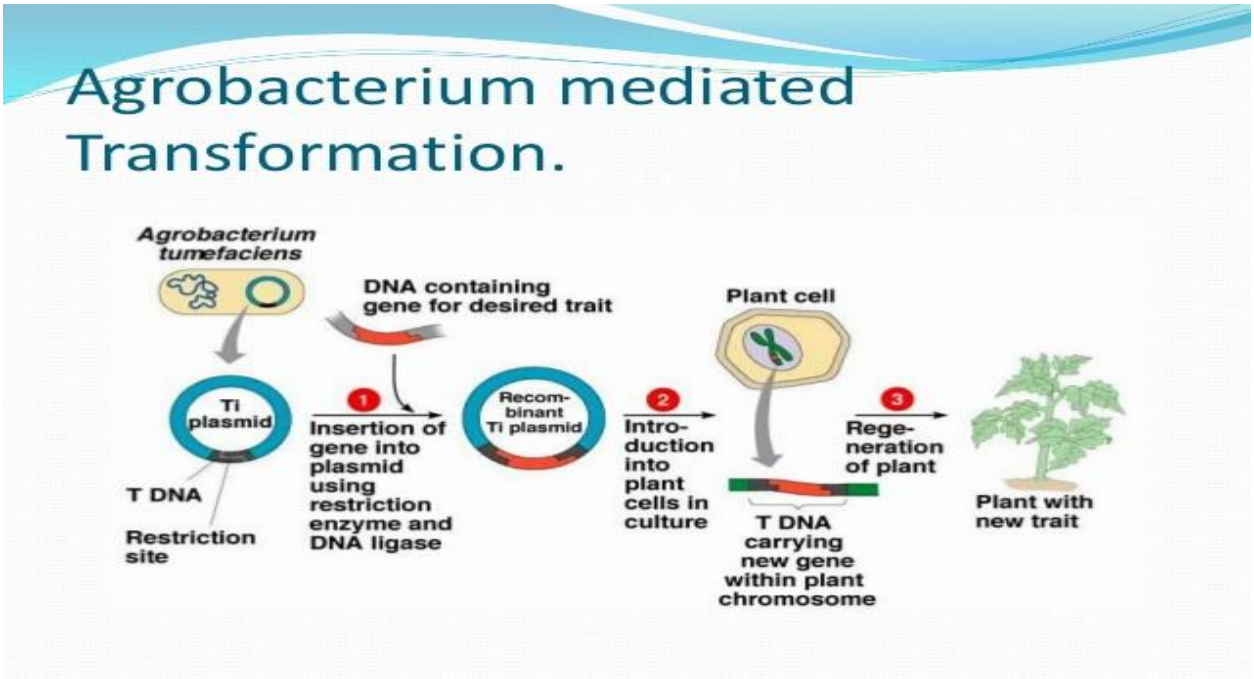
Method	*Salient features*
I. Vector–mediated gene transfer	
Agrobacterium (Ti plasmid)-mediated gene transfer	Very efficient, but limited to a selected group of plants
Plant viral vectors	Ineffective method, hence not widely used
II. Direct or vectorless DNA transfer	
(A) Physical methods	
Electroporation	Mostly confined to protoplasts that can be regenerated to viable plants. Many cereal crops developed.
Microprojectile (particle bombardment)	Very successful method used for a wide range of plants/ tissues. Risk of gene rearrangement high.
Microinjection	Limited use since only one cell can be microinjected at a time. Technical personnel should be highly skilled.
Liposome fusion	Confined to protoplasts that can be regenerated into viable whole plants.
Silicon carbide fibres	Requires regenerable cell suspensions. The fibres, however, require careful handling.
(B) Chemical methods	
Polyethylene glycol (PEG)-mediated	Confined to protoplasts. Regeneration of fertile plants is frequently problematical.
Diethylaminoethyl (DEAE) dextran-mediated	Does not result in stable transformants.

Vector-Mediated Gene Transfer:

Agrobacterium-Mediated Gene Transfer

Agrobacterium tumefaciens is a soil-borne, Gram-negative bacterium. It is rod shaped and motile, and belongs to the bacterial family of Rhizobiaceae. A. tumefaciens is a phytopathogen, and is treated as the nature's most effective plant genetic engineer.

Some workers consider this bacterium as the natural expert of inter-kingdom gene transfer. In fact, the major credit for the development of plant transformation techniques goes to the natural unique capability of A. tumefaciens. Thus, this bacterium is the most beloved by plant biotechnologists.



species of Agrobacterium:

i. A. tumefaciens that induces crown gall disease.

ii. A. rhizogenes that induces hairy root disease.

Crown Gall Disease and Ti Plasmid

Almost 100 years ago (1907), Smith and Townsend postulated that a bacterium was the causative agent of crown gall tumors, although its importance was recognized much later. As *A. tumefaciens* infects wounded or damaged plant tissues, in induces the formation of a plant tumor called crown gall. The entry of the bacterium into the plant tissues is facilitated by the release of certain phenolic compounds (acetosyringone, hydroxyacetosyringone) by the wounded sites. Crown gall symptoms include round, wart-like growths 2 inches or larger in diameter that appear at or just above the soil line, or on lower branches and stems. Plants with several galls may be unable to move water and nutrients up the trunk and become weakened, stunted and unproductive. Young plants can be killed by developing gall tissue. Crown gall formation occurs when the bacterium releases its Ti plasmid (tumor- inducing plasmid) into the plant cell cytoplasm. A fragment (segment) of Ti plasmid, referred to as T-DNA, is actually transferred from the bacterium into the host where it gets integrated into the plant cell chromosome (i.e. host genome). Thus, crown gall disease is a naturally evolved genetic engineering process.

The T-DNA carries genes that code for proteins involved in the biosynthesis of growth hormones (auxin and cytokinin) and novel plant metabolites namely opines, amino acid derivatives and agropines, sugar derivatives The growth hormones cause plant cells to

proliferate and form the gall while opines and agropines are utilized by *A. tumefaciens* as sources of carbon and energy. As such, opines and agropines are not normally part of the plant metabolism (neither produced nor metabolised). Thus, *A. tumefaciens* genetically transforms plant cells and creates a biosynthetic machinery to produce nutrients for its own use.

As the bacteria multiply and continue infection, grown gall develops which is a visible mass of the accumulated bacteria and plant material. Crown gall formation is the consequence of the transfer, integration and expression of genes of T-DNA (or Ti plasmid) of *A. tumefaciens* in the infected plant. The genetic transformation leads to the formation of crown gall tumors, which interfere with the normal growth of the plant. Several dicotyledonous plants (dicots) are affected by crown gall disease e.g. grapes, roses, stone-fruit trees.

33. Virus Mediated Gene Transfer

Viral transformation

Viral transformation is the change in growth, phenotype, or indefinite reproduction of cells caused by the introduction of inheritable material. Through this process, a virus causes harmful transformations of an in vivo cell or cell culture. Viral transformation can occur both naturally and medically. Natural transformations can include viral cancers, such as human papillomavirus (HPV) and T-cell Leukemia virus type I. Hepatitis B and C are also the result of natural viral transformation of the host cells. Viral transformation can also be induced for use in medical treatments. Cells that have been virally transformed can be differentiated from untransformed cells through a variety of growth, surface, and intracellular observations.

Transduction

Transduction is the process by which foreign DNA is introduced into a cell by a virus or viral vector.

Viral transformation (transduction)

Package the desired genetic material into a suitable plant virus and allow this modified virus to infect the plant. If the genetic material is DNA, it can recombine with the chromosomes to produce transformant cells. However genomes of most plant viruses consist of single stranded RNA which replicates in the cytoplasm of infected cell. For such genomes this method is a form of transfection and not a real transformation, since the inserted genes never reach the

nucleus of the cell and do not integrate into the host genome. The progeny of the infected plants is virus free and also free of the inserted gene.

Plant Viruses as Vectors

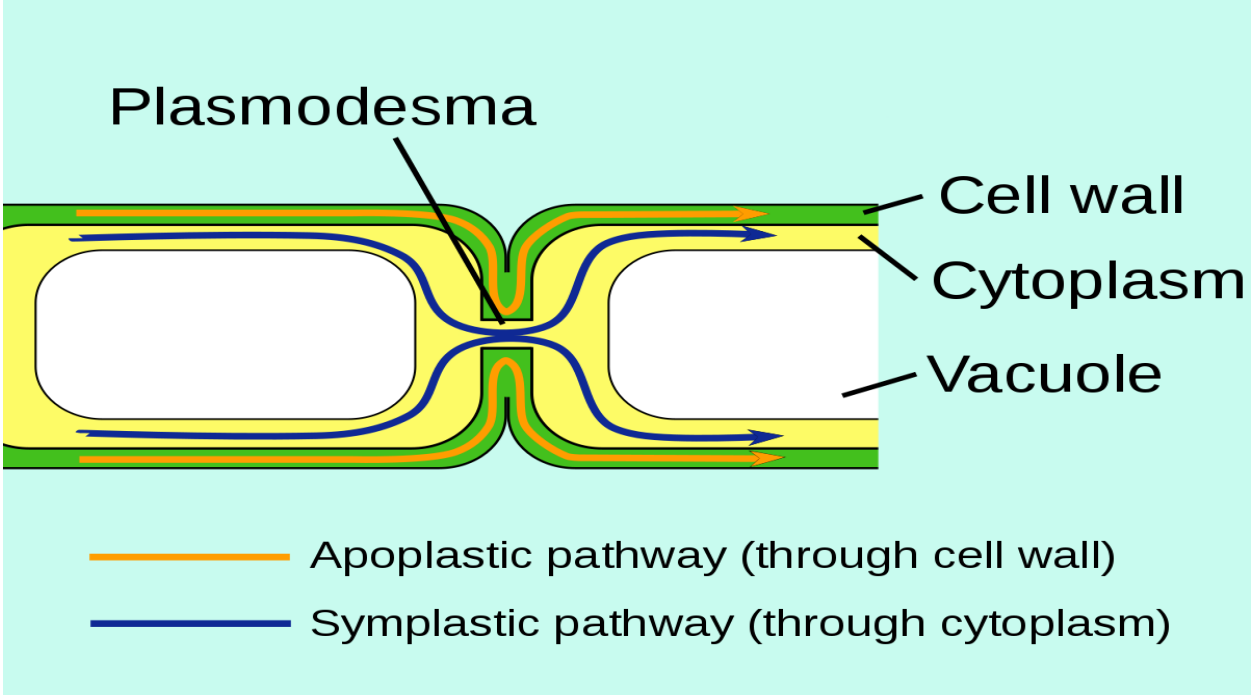
Plant viruses are considered as efficient gene transfer agents as they can infect the intact plants and amplify the transferred genes through viral genome replication. Viruses are natural vectors for genetic engineering. They can introduce the desirable gene(s) into almost all the plant cells since the viral infections are mostly systemic.

Plant viruses are non-integrative vectors:

The plant viruses do not integrate into the host genome in contrast to the vectors based on T-DNA of A. tumefaciens which are integrative. The viral genomes are suitably modified by introducing desired foreign genes. These recombinant viruses are transferred, multiplied and expressed in plant cells. They spread systemically within the host plant where the new genetic material is expressed.

Criteria for a plant virus vector

An ideal plant virus for its effective use in gene transfer is expected to posses the following characteristics. The virus must be capable of spreading from cell to cell through plasmodesmata. The viral genome should be able to replicate in the absence of viral coat protein and spread from cell to cell. This is desirable since the insertion of foreign DNA will make the viral genome too big to be packed. The recombinant viral genome must elicit little or no disease symptoms in the infected plants. The virus should have a broad host range. The virus with DNA genome is preferred since the genetic manipulations involve plant DNA.

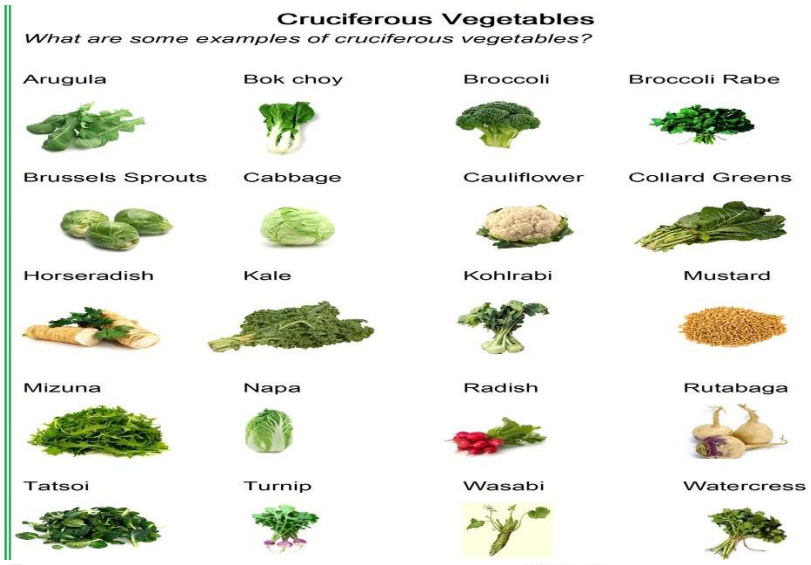


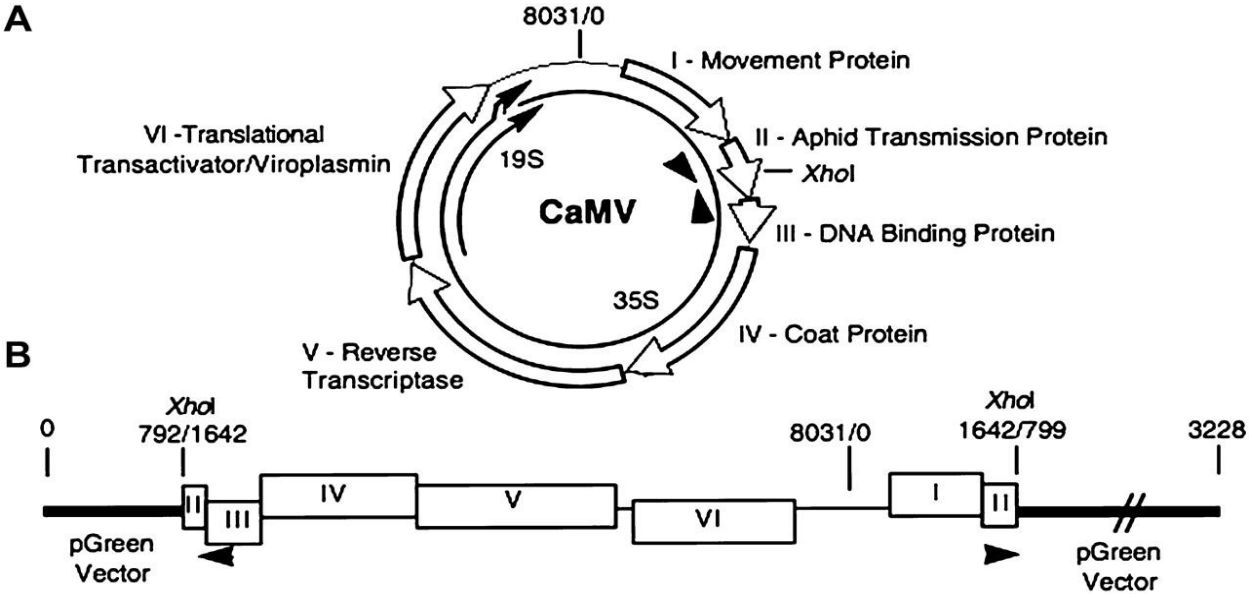
The three groups of viruses — caulimoviruses, Gemini viruses and RNA viruses that are used as vectors for gene transfer in plants are briefly described.

Caulimoviruses as Vectors:

The Caulimoviruses contain circular double- stranded DNA, and are spherical in shape. Caulimoviruses are widely distributed and are responsible for a number of economically important diseases in various crops. The caulimovirus group has around 15 viruses and among these cauliflower mosaic virus (CaMV) is the most important for gene transfer. The other caulimoviruses include carnation etched virus, dahlia mosaic virus, mirabilis mosaic virus and strawberry vein banding virus.

Cauliflower mosaic virus (CaMV): CaMV infects many plants (e.g. members of Cruciferae, Datura) and can be easily transmitted, even mechanically. Another attractive feature of CaMV is that the infection is systemic, and large quantities of viruses are found in infected cells. A diagrammatic view of the CaMV genetic map is depicted in Figure. The genome of CaMV consists of a 8 kb (8024 bp) relaxed but tightly packed circular DNA with six major and two minor coding regions. The genes II and VII are not essential for viral infection.





Use of CaMV in gene transfer:

For appropriate transmission of CaMV, the foreign DNA must be encapsulated in viral protein. Further, the newly inserted foreign DNA must not interfere with the native assembly of the virus. CaMV genome does not contain any non-coding regions wherein foreign DNA can be inserted. It is fortunate that two genes namely gene II and gene VII have no essential functions for the virus. It is therefore possible to replace one of them and insert the desired foreign gene. Gene II of CaMV has been successfully replaced with a bacterial gene encoding dihydrofolate reductase that provides resistance to methotrexate. When the chimeric CaMV was transmitted to turnip plants, they were systemically infected and the plants developed resistance to methotrexate.

Limitations of CaMV as a vector

CaMV vector has a limited capacity for insertion of foreign genes. Infective capacity of CaMV is lost if more than a few hundred nucleotides are introduced. Helper viruses cannot be used since the foreign DNA gets expelled and wild-type viruses are produced.

34. Virus Mediated Gene Transfer 2

Gemini Viruses as Vectors

The Gemini viruses are so named because they have geminate (Gemini literally means heavenly twins) morphological particles i.e. twin and paired capsid structures. These viruses are characterized by possessing one or two single-stranded circular DNAs (ss DNA). On replications, ss DNA forms an intermediate double-stranded DNA. The Gemini viruses can infect a wide range of crop plants (monocotyledons and dicotyledons) which attract plant biotechnologists to employ these viruses for gene transfer. Curly top virus (CTV) and maize streak virus (MSV) and bean golden mosaic virus (BGMV) are among the important Gemini viruses. It has been observed that a large number of replicative forms of a Gemini virus genome accumulate inside the nuclei of infected cells. The single-stranded genomic DNA replicates in the nucleus to form a double-stranded intermediate. Gemini virus vectors can be used to deliver, amplify and express foreign genes in several plants/ explants (protoplasts, cultured cells). However, the serious drawback in employing Gemini viruses as vectors is that it is very difficult to introduce purified viral DNA into the plants. An alternate arrangement is to take the help of Agrobacterium and carry out gene transfer.

RNA Plant Viruses as Vectors

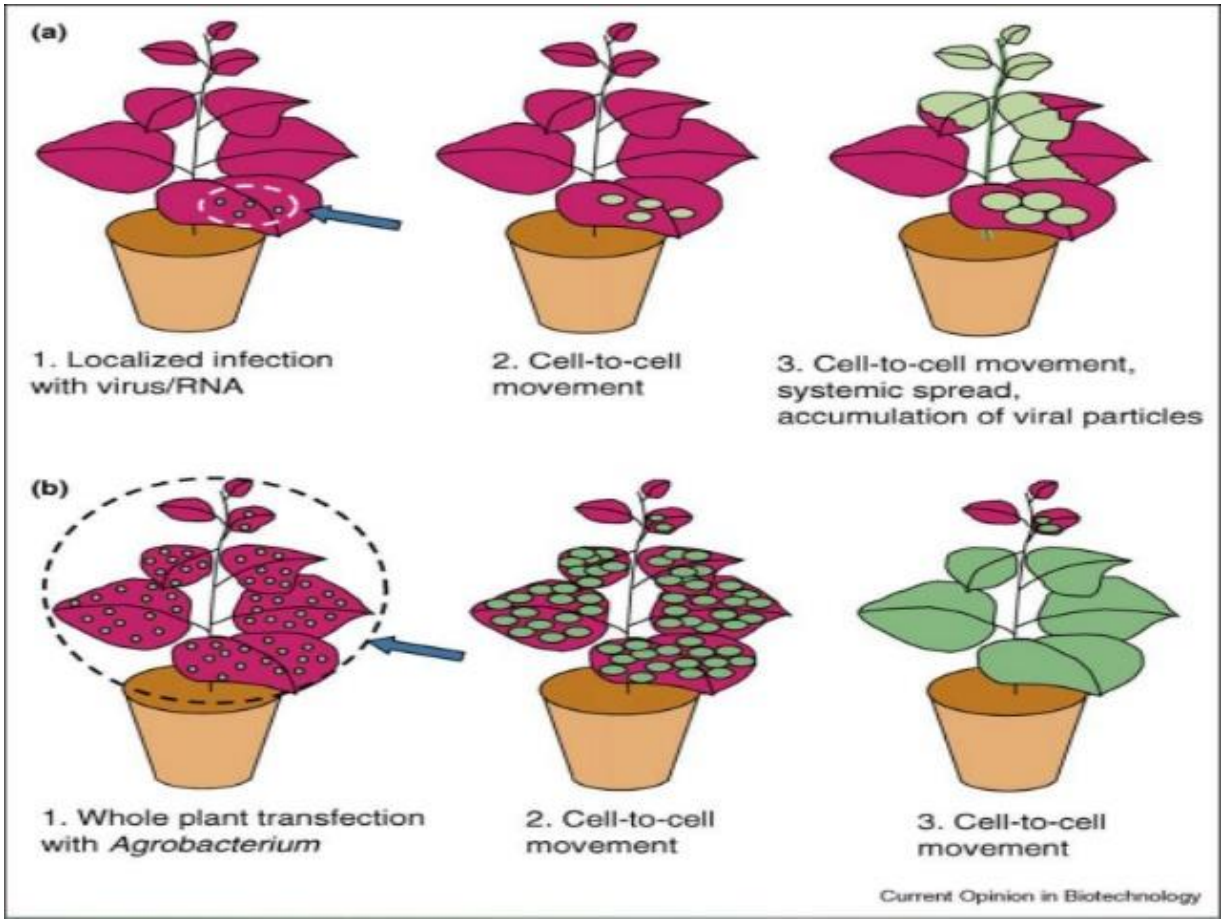
There are mainly two type's single-stranded RNA viruses:

Mono-partite viruses:

These viruses are usually large and contain undivided genomes for all the genetic information e.g. tobacco mosaic virus (TMV).

Multipartite viruses:

The genome in these viruses is divided into small RNAs which may be in the same particle or different particles, e.g. brome mosaic virus (BMV). HMV contains four RNAs divided between three particles. Plant RNA viruses, in general, are characterized by high level of gene expression, good efficiency to infect cells and spread to different tissues. But the major limitation to use them as vectors is the difficulty of joining RNA molecules in vitro.



Use of cDNA for gene transfer:

Complementary DNA (cDNA) copies of RNA viruses are prepared in vitro. The cDNA so generated can be used as a vector for gene transfer in plants. This approach is tedious and slow or complicated and therefore inefficient. However, some success has been reported. A gene sequence encoding chloramphenicol resistance (enzyme- chloramphenicol acetyltransferase) has been inserted into brome mosaic virus genome. This gene expression, however, has been confined to protoplasts.

Limitations of Viral Vectors in Gene Transfer

The ultimate objective of gene transfer is to transmit the desired genes to subsequent generations. With virus vectors, this is not possible unless the virus is seed-transmitted. However, in case of vegetatively propagated plants, transmission of desired traits can be done e.g. potatoes. Even in these plants, there is always a risk for the transferred gene to be lost anytime. For the reasons referred above, plant biotechnologists prefer to insert the desired genes of interest into a plant chromosome.

35. Vectorless or direct gene transfer

In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant cell without the help of a vector. The methods used for direct gene transfer in plants are

1. Chemical method
2. Physical method

- **Chemical mediated gene transfe**r

1. Chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts.
2. Calcium phosphate is also used to transfer DNA into cultured cells.

Liposome mediated gene transfer or Lipofection

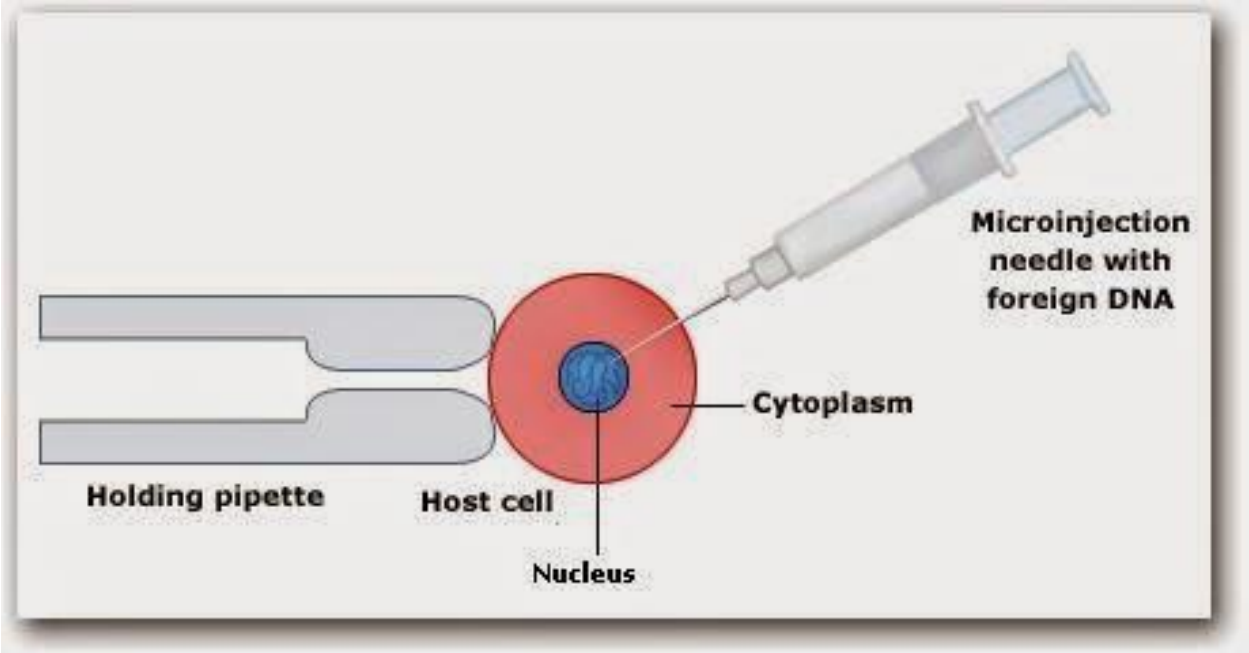
Liposomes are circular lipid molecules with an aqueous interior that can carry nucleic acids. Liposomes encapsulate the DNA fragments and then adher to the cell membranes and fuse with them to transfer DNA fragments. Thus, the DNA enters the cell and then to the nucleus. Lipofection is a very efficient technique used to transfer genes in bacterial, animal and plant cells.

Silicon carbide method

In this method, fibres of organic material like silicon carbide are used for gene transfer. These fibres, when mixed with plasmid DNA and plant tissue or cells, help in penetration of the foreign DNA into the plant tissue.

Microinjection

where the DNA is directly injected into plant protoplasts or cells (specifically into the nucleus or cytoplasm) using fine tipped (0.5 - 1.0 micrometerdiameter) glass needle or micropipette. This method of gene transfer is used to introduce DNA into large cells such as oocytes, eggs, and the cells of early embryo.



Electroporation

Involves a pulse of high voltage applied to protoplasts/cells/ tissues to make transient (temporary) pores in the plasma membrane which facilitates the uptake of foreign DNA. The cells are placed in a solution containing DNA and subjected to electrical shocks to cause holes in the membranes. The foreign DNA fragments enter through the holes into the cytoplasm and then to nucleus.

Particle gun/Particle bombardment

In this method, the foreign DNA containing the genes to be transferred is coated onto the surface of minute gold or tungsten particles (1-3 micrometers) and bombarded onto the target tissue or cells using a particle gun (also called as gene gun/shot gun/microprojectile gun).The microprojectile bombardment method was initially named as biolistics by its inventor Sanford (1988). Two types of plant tissue are commonly used for particle bombardment- Primary explants and the proliferating embryonic tissues.

Transformation

This method is used for introducing foreign DNA into bacterial cells e.g. E. Coli. The transformation frequency (the fraction of cell population that can be transferred) is very good in this method. E.g. the uptake of plasmid DNA by E. coli is carried out in ice cold CaCl2 (0-50C) followed by heat shock treatment at 37-450C for about 90 sec. The transformation efficiency refers to the number of transformants per microgram of added DNA. The CaCl2 breaks the cell wall at certain regions and binds the DNA to the cell surface.

Conjuction

It is a natural microbial recombination process and is used as a method for gene transfer. In conjuction, two live bacteria come together and the single stranded DNA is transferred via cytoplasmic bridges from the donor bacteria to the recipient bacteria.

Selection of transformed cells from untransformed cells

The selection of transformed plant cells from untransformed cells is an important step in the plant genetic engineering. For this, a marker gene (e.g. for antibiotic resistance) is introduced into the plant along with the transgene followed by the selection of an appropriate selection medium (containing the antibiotic). The segregation and stability of the transgene integration and expression in the subsequent generations can be studied by genetic and molecular analyses (Northern, Southern, Western blot, PCR).

36. Electroporation

Electroporation or electro-permeabilization is the process of applying electrical field to a living cell for a brief duration of time in order to create microscopic pores in the plasma membrane called electro-pores. This technique is used for transferring the recombinant DNA molecule into wide range of hosts starting from bacteria to plant (plant protoplasts) and animal cells

Principle:

The phospholipid molecules of the plasma membrane are not static. When we apply electric field to them their kinetic energy increases resulting in the increase in the membrane permeability at certain points. This is exactly where we see the formation of electro-pores. The recombinant DNA can pass through these transient pores before they close.

Procedure

In this process cells are mixed with the recombinant DNA and the mixture is placed in a small chamber with electrodes connected to a specialized power supply. Then a brief electric impulse is discharged across the electrodes, which makes pores (holes) in the plasma membrane. These pores remain for some time and are again resealed themselves. Recombinant DNA enters the cell which are removed and plated in fresh selective medium. The process of selection is then applied to isolate cells carrying recombinant DNA.

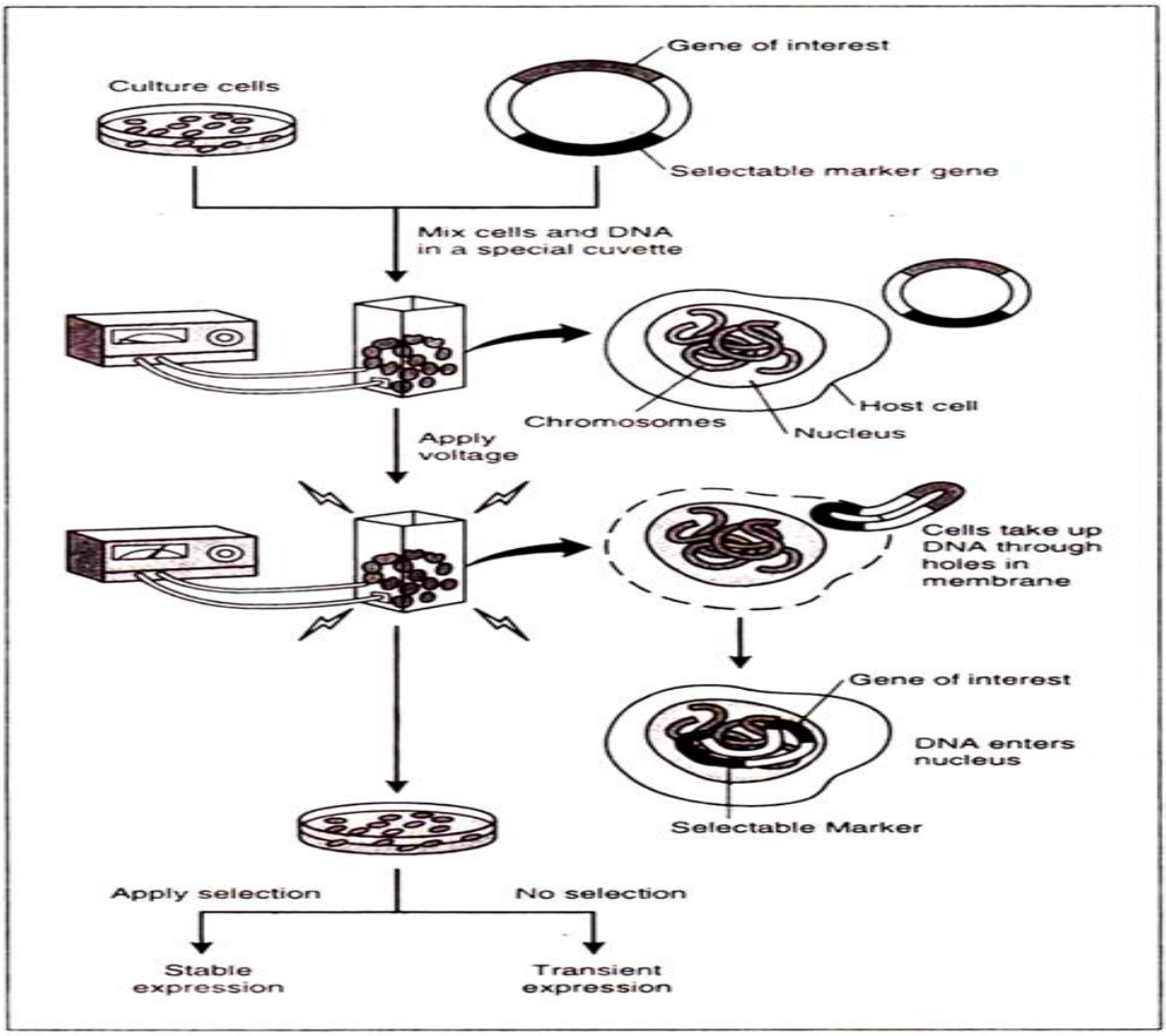
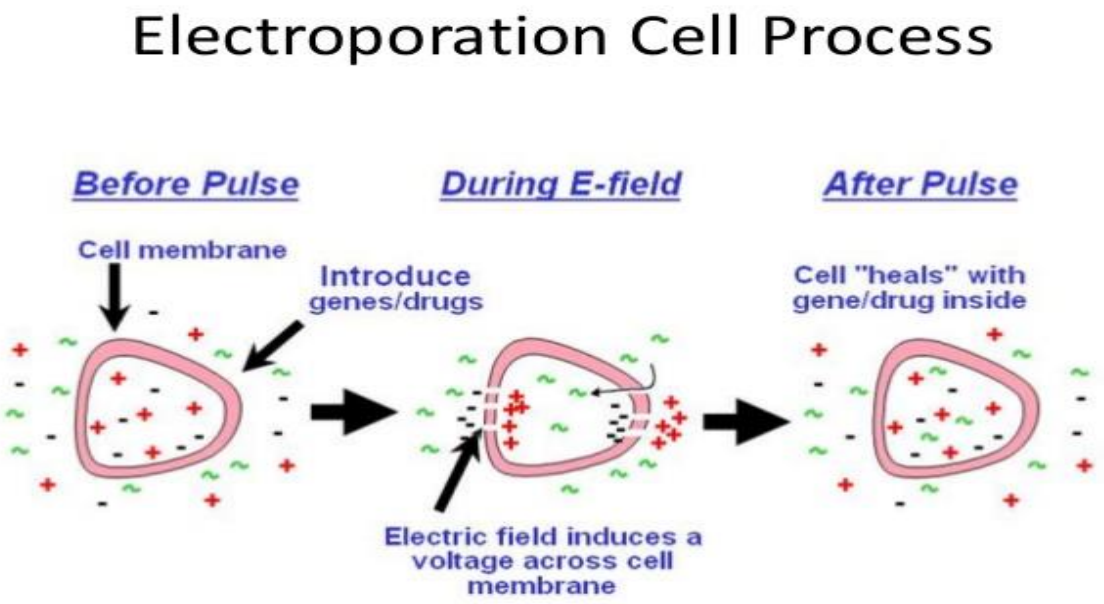


Fig. 5.6: Gene transfer by electroporation



Advantages of electroporation:

1. This technique is simple, convenient and rapid, besides being cost-effective.
2. The transformed cells are at the same physiological state after electroporation.
3. Efficiency of transformation can be improved by optimising the electrical field strength, and addition of spermidine.

Limitations of electroporation:

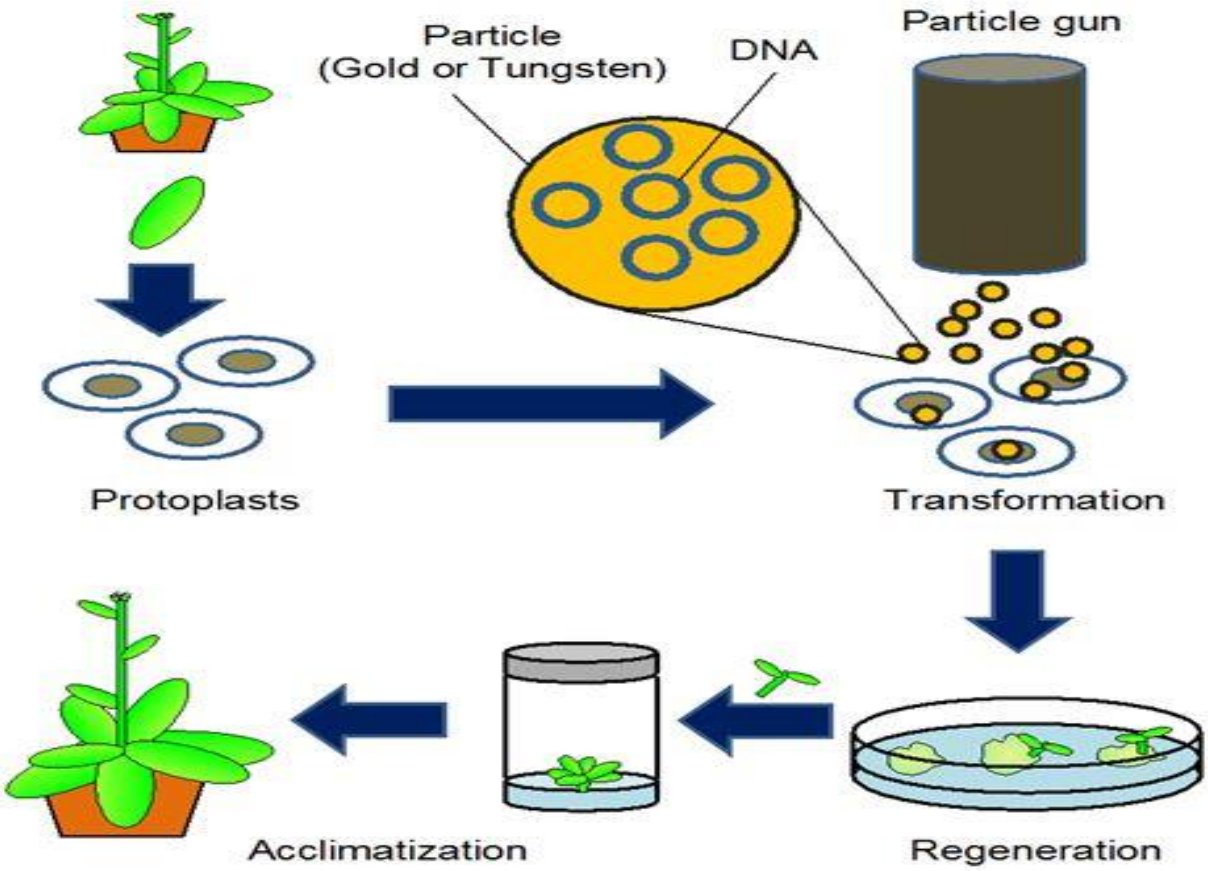
1. Under normal conditions, the amount of DNA delivered into plant cells is very low.
2. Efficiency of electroporation is highly variable depending on the plant material and the treatment conditions.
3. Regeneration of plants is not very easy, particularly when protoplasts are used.

37. Particle Bombardment (Biolistics)

Biolistics is a method where cells are physically impregnated with nucleic acids or other biological molecules. A biolistic particle delivery system is a device for plant transformation where cells are bombarded with heavy metal particles coated with DNA/RNA. This technique was invented by John Stanford in 1984 for introduction of DNA into cells by physical means to avoid the host-range restrictions of *Agrobacterium.Agrobacterium*-mediated geneti

transformation system works well for dicotyledonous plants but has low efficiency for monocots. Biolistic particle delivery system provides an effective and versatile way to transform almost all type of cells. It has been proven to be a successful alternative for creating transgenic organisms inprokaryotes, mammalian and plant species.

In this process, construct having gene of interest is coated on the surface oftiny particles of gold or tungsten (0.6 – 1 mm in size). Prior to coating, DNA is precipitated with calcium chloride, spermidine and polyethylene glycol. These coated microparticles are loaded on to the macrocarrier and accelerated to high speed by using pressurized helium gas. Plant cell suspensions, callus cultures, or tissues could be used as the target of thesemicroprojectiles. As the microprojectiles penetrate the plant cell walls and membranes to enter the cells, coated DNA is released from its surface and incorporated into the plant's genome. In biolistics, use of binary vectors with T-DNA border sequences is not required. This method is especially important for monocots, for which efficiency of other transformation methods is not satisfactory. A wide range of tissues such as apical and floral meristems, embryos, seedlings, leaves, cultured cells and floral tissues could be used as target in this method.



A number of parameters should be carefully considered before using particle bombardment.

These can be classified under three categories

Physical parameters

Nature, chemical and physical properties of the metal particles utilized to carry the foreign DNA. The nature and preparation of DNA,binding of DNA on the particles and target tissues.

Environmental parameters

Variables such as temperature, photoperiod and humidity of donor plants, explants, and bombarded tissues affect physiology of tissues and influence receptiveness of the target tissue.

Biological parameters

Choice and nature of explants, pre- and post bombardment culture conditions, osmotic pre- and post-treatment of explants.

Factors affecting bombardment

Several attempts are made to study the various factors, and optimize the system of particle bombardment for its most efficient use.

Some of the important parameters are described.

Nature of micro particles:

Inert metals such as tungsten, gold and platinum are used as micro particles to carry DNA.

These particles with relatively higher mass will have a better chance to move fast when bombarded and penetrate the tissues.

Nature of tissues/cells:

The target cells that are capable of undergoing division are suitable for transformation.

Some more details on the choice of plant material used in bombardment are already given.

Amount of DNA

The transformation may be low when too little DNA is used. On the other hand, too much DNA may result is high copy number and rearrangement of transgenes. Therefore, the quantity of DNA used should be balanced. Recently, some workers have started using the chemical aminosiloxane to coat the micro particles with low quantities of DNA adequate enough to achieve high efficiency of transformation.

Advantages of particle bombardment over Agrobacterium-mediated DNA transfer

This system is species independent and can been used successfully for a wide range of organisms. Many species which are recalcitrant to other direct transfer methods or are not ready to Agrobacterium-mediated transformation have been transformed by this technique. Introduced DNA does not need sequences necessary for T-DNA replication and transfer as complex interaction between bacterium and plant tissue does not take place. Transformation of organelle DNA (mitochondria and chloroplasts) has also been achieved by this method. Multiple genes can be introduced in a single plant. Particles can be coated with DNA/RNA/siRNA/large fragments of nucleic acids.

Limitations of particle bombardment method:

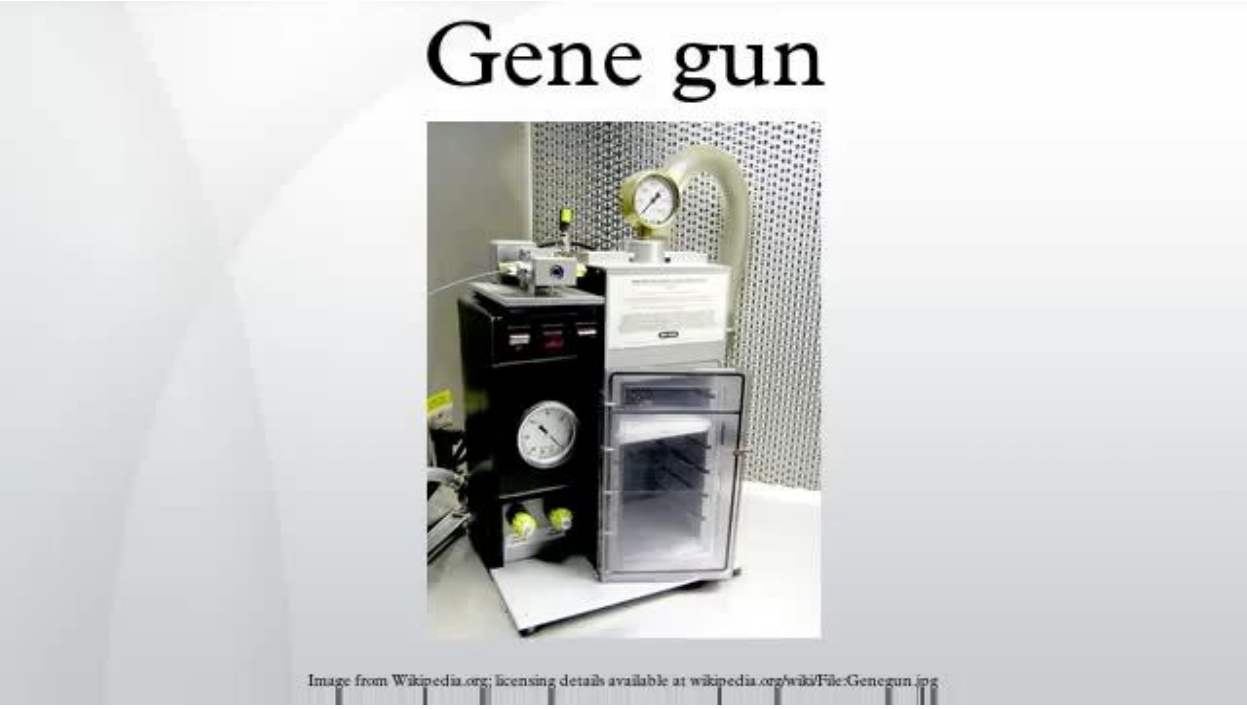
- Limited regeneration capacity of tissue being bombarded
- Efficiency of stable integration of DNA.
- Insertion of multiple copies of the gene
- Integration of rearranged and/or truncated DNA sequences
- Damage to the cellular tissue.
- Specialized and expensive equipment’s are required

38. Particle Bombardment (Biolistics) 2

Gene Delivery System:

This system has many names and also Known as Particle Bombardment, Biolistics, Microprojectile bombardment, Particle acceleration, Particle inflow gun, Gene gun. A gene gun or a biolistic particle delivery system, originally designed for plant transformation, is a device for delivering exogenous DNA (transgenes) to cells. Using a gene gun directly shoots a piece of DNA into the recipient plant tissue.

Tungsten or gold beads are coated in the gene of interest and fired through a stopping screen, accelerated by Helium, into the plant tissue. The particles pass through the plant cells, leaving the DNA inside.



Advantage:

This method can be use to transform all plant species.

No binary vector is required.

Transformation protocol is relatively simple.

Disadvantage:

Difficulty in obtaining single copy transgenic events.

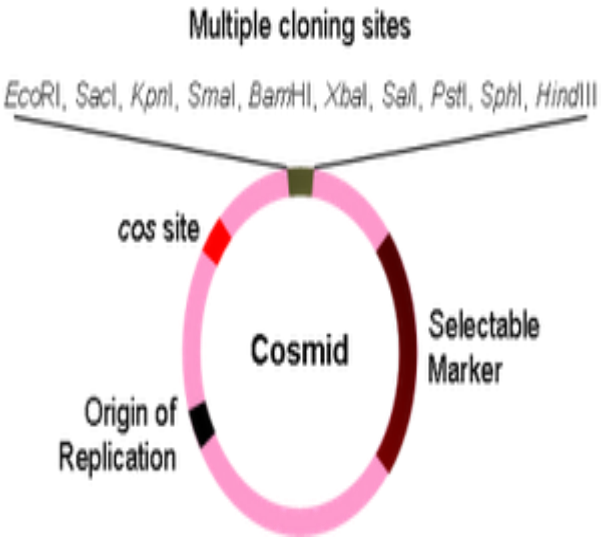
High cost of the equipment and microcarriers.

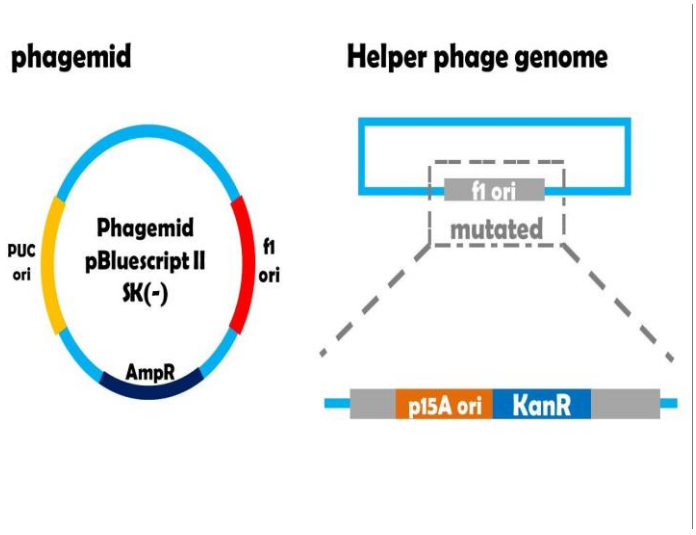
Intracellular target is random (cytoplasm, nucleus, vacuole, plastid, etc.).

Transfer DNA is not protected.

39. Microinjection

The process of using a fine glass micropipette to manually inject transgene at microscopic or borderline macroscopic level is known as microinjection. The transgene, in the form of plasmids, cosmids, phage, YACs, or PCR products, can be circular or linear and need not be physically linked for injection





Microinjection involves direct mechanical introduction of DNA intothe nucleus or cytoplasm using a glass micro capillary injection pipette. The protoplasts are immobilized in low melting

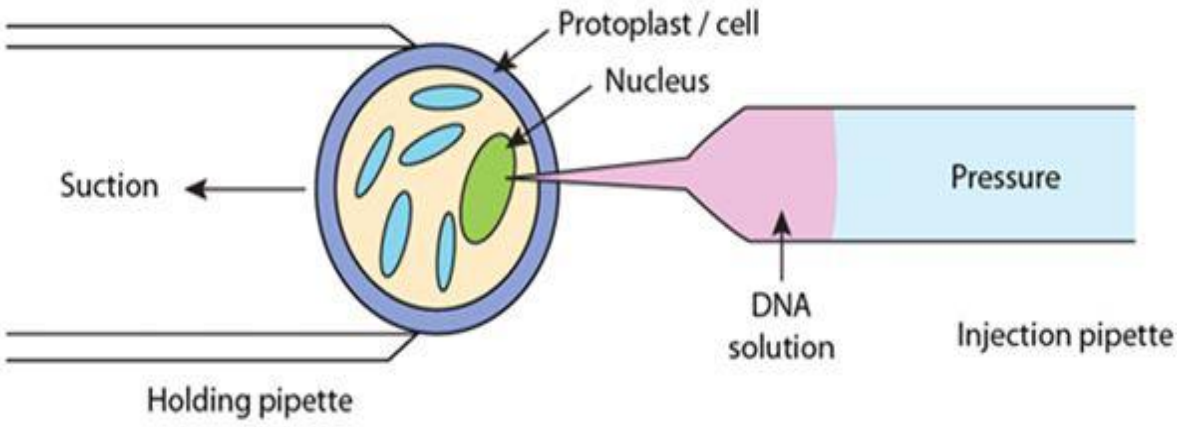
agar, while working under a microscope, using a holding pipette and suction force. DNA is then directly injected into the cytoplasm or the nucleus.

The injected cells are then cultured *invitro* and regenerated into plants. Successful examples of this process has been shown in rapeseed, tobacco and various other plants. Stable transformants can be achieved through this method but it requires technical expertise and is a time consuming process.

Also, microinjection has achieved only limited success in plant transformation due to the thick cell walls of plants and a lack of availability of a single-cell-to-plant regeneration system in most plant species.

In this technique a traditional compound microscope (around 200X magnification) or an inverted microscope (around 200x magnification) or a dissecting stereomicroscope (around 40-50x)is used.

Under the microscope target cell is positioned and cell membrane and nuclear envelope are penetrated with the help of two micromanipulators. One micromanipulator holds the pipette and another holds the micro capillary needle.



There are two types of microinjection systems; constant flow system and pulsed flow system

In the **constant flow** system the amount of sample injected is determined by the duration for which needle remains in the cell. The constant flow system is relatively simple and inexpensive but outdated.

The **pulsed flow** system has greater control over the volume of substance delivered, needle placement and movement and has better precision. This technique results in less damage to the receiving cell, however, the components of this system are quite expensive.

40. Chemical Gene Transfer Methods

There are two types of chemical methods used for plant transformation

1. Polyethylene glycol (PEG)-mediated transfer
2. DEAE Dextran-Mediated transfer

Polyethylene glycol (PEG)-mediated transfer

Polyethylene glycol (PEG), in the presence of divalent cations (using Ca^{2+}), destabilizes the plasma membrane of protoplasts and renders it permeable to naked DNA.

In this way, the DNA enters nucleus of the protoplasts and gets integrated with the genome.

The procedure involves the isolation of protoplasts and their suspension, addition of plasmid DNA, followed by a slow addition of 40% PEG-4000 (w/v) dissolved in mannitol and calcium nitrate solution. As this mixture is incubated, protoplasts get transformed.

Advantages of PEG-mediated transformation:

- A large number of protoplasts can be simultaneously transformed.
- This technique can be successfully used for a wide range of plant species.

Limitations of PEG-mediated transformation:

- The DNA is susceptible for degradation and rearrangement.
- Random integration of foreign DNA into genome may result in undesirable traits.
- Regeneration of plants from transformed protoplasts is a difficult task.

(DEAE) Dextran Mediated transfer

This method was initially reported by Vaheri and Pagano in 1965 for enhancing the viral infectivity of cell but later adapted as a method for plasmid DNA transfer. Diethyl aminoethyl dextran (DEAE-dextran) is a soluble poly cationic carbohydrate that promotes interactions between DNA and endo cytotic machinery of the cell.

In this method, the negatively charged DNA and positively charged DEAE – dextran form aggregates through electrostatic interaction and form apolyplex. A slight excess of DEAE – dextran in mixture results in net positive charge in the DEAE – dextran/ DNA complex formed. These complexes, when added to the cells, bind to the negatively charged plasma membrane and get internalized through endocytosis. Complexed DNA delivery with DEAE-dextran can be improved by osmotic shock using DMSO or glycerol.

Several parameters such as number of cells, polymer concentration, transfected DNA concentration and duration of transfection should be optimized for a given cell line

Advantages

This method is Simple and inexpensive, more sensitive and it can be applied to a wide range of cell types and Can be used for transient transfection.

Disadvantages

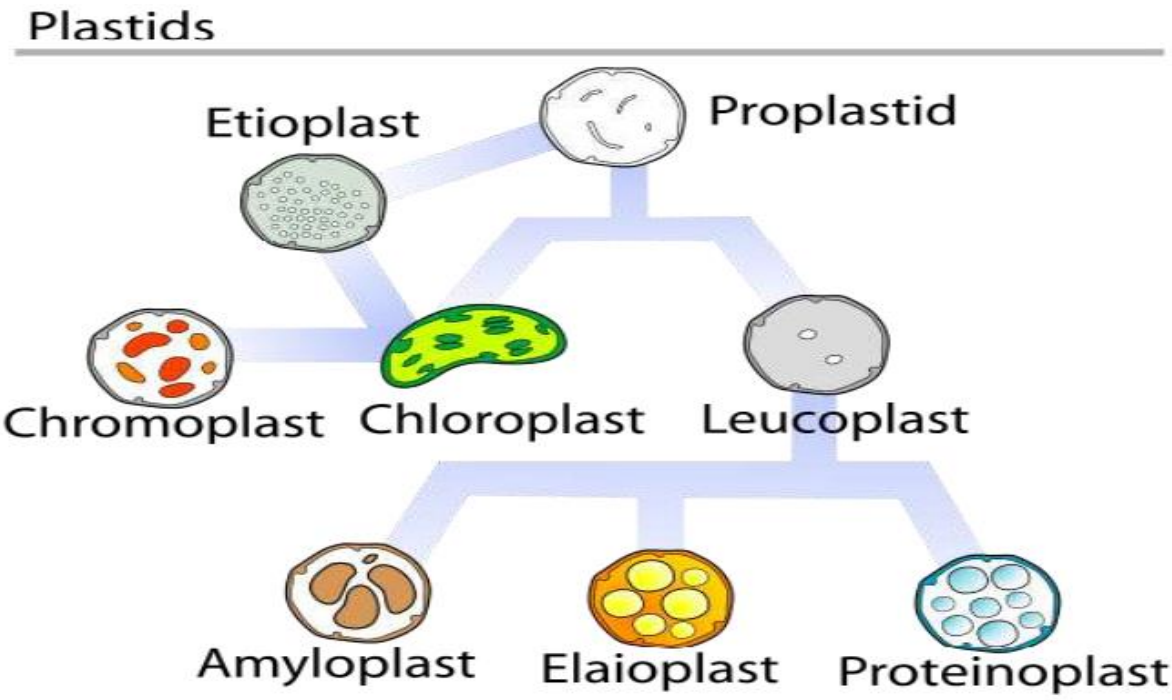
DEAE is a toxic to cells at high concentrations. Transfection efficiency varies with cell type. It can only be used for transient transfection but not for stable transfection. Typically produces less than 10% delivery in primary cells.

41. Plastid transformation

Plastid

Plastids are the major organelle of plant and algal cells. These are the site of manufacture and storage of important chemical compounds. It has circular, dsDNA copies and it replicates autonomously of the cell. Plastids are thought to have been originated from endosymbiotic bacteria and plastid genes show maternal inheritance.

Derived from proplastids in meristem



Plastids Have diverse functions

- Chloroplasts – green plastids – for photosynthesis
- Chromoplasts – coloured plastids – for pigment synthesis and storage
- Gerontoplasts – control dismantling of photosynthetic apparatus during senescence
- Leucoplasts – colourless plastids – monoterpene synthesis
- Leucoplasts include amyloplasts (starch), elaioplasts (fats), proteinoplasts (proteins) and tannosomes (tannins)

Comparison of the nuclear and plastid genomes of angiosperm

	Nuclear genome	Plastid genome
Chromosomes	Two copies of each of many chromosomes; the number of chromosomes per diploid cell is species -specific	~60 copies of a single circular chromosome per plastid ~50–60 chloroplasts per cell
Genes per chromosome	Could be thousands	~120–150
Arrangement and operons transcription of genes	Each gene is separate (individually transcribed)	Many genes are in (transcribed together)

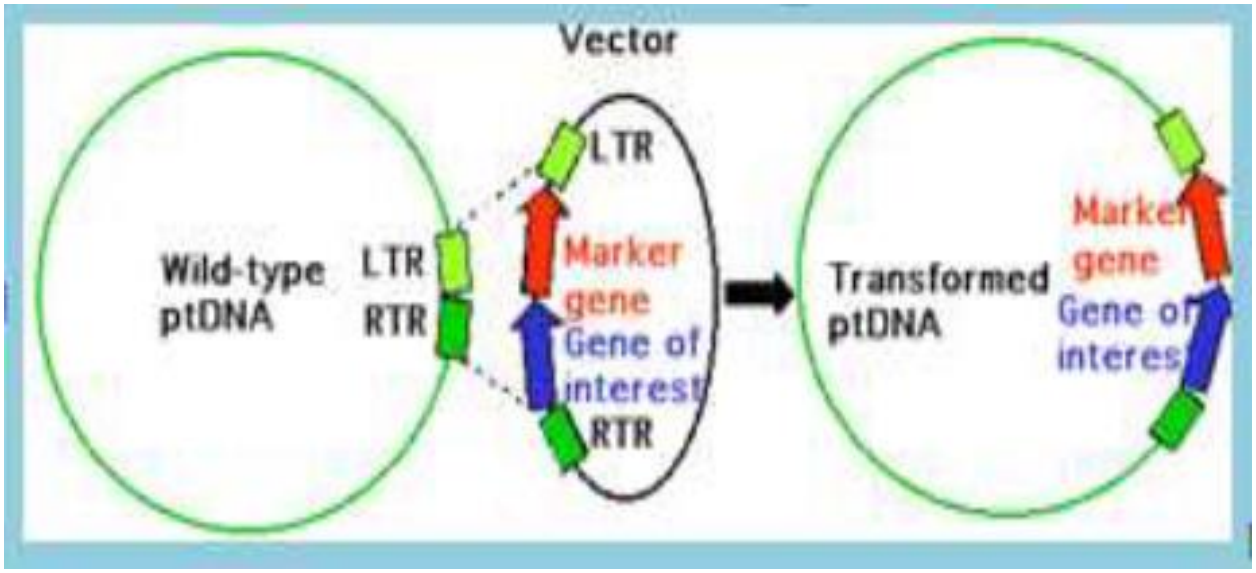
Why plastid is used for transformation?

Due to the High protein expression levels and absence of epigenetic effects. Plastids have uniparental inheritance is commercially favored and easy transgene stacking in operons. Since plastids are maternally inherited, they aren't transmitted by pollen these are biologically safe.

Difficulty in delivering foreign DNA through double membrane of the plastid. The enormous copy number (polyploidy) of the plastid genome. The desired genetic modification must be in each copy of plastid genome in each cell. Failure to achieve homoplasmy results in rapid somatic segregation and genetic instability. Repeated rounds of selection and regeneration are required.

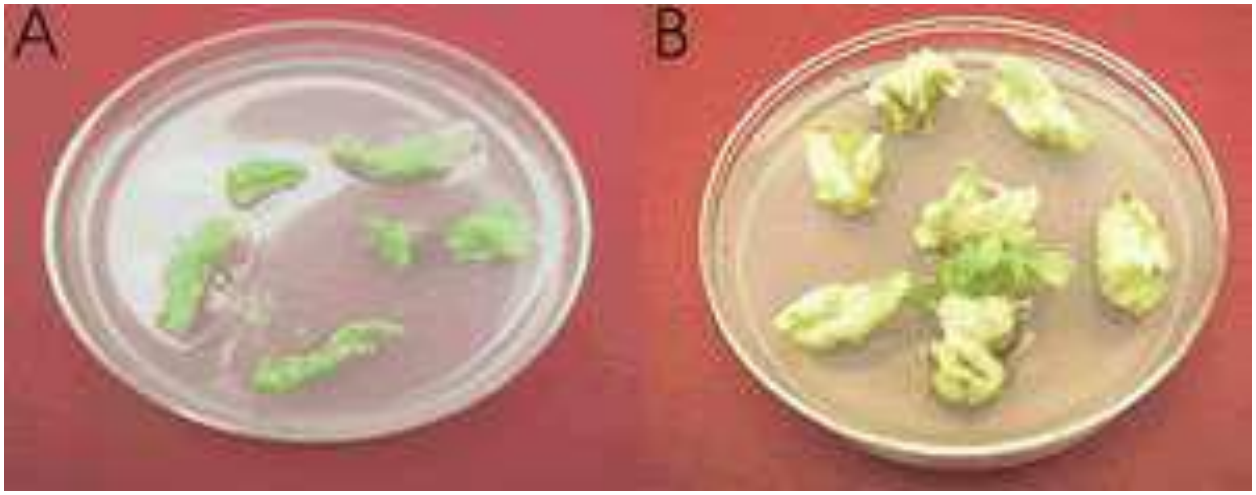
Chloroplast transformation requirements

For chloroplast transformation chloroplast specific expression vector is needed and a method for DNA delivery through a double membrane of the chloroplast. An efficient selection for the transplastome is required.



DNA delivery into plastids

Successful methods include biolistic and polyethylene glycol-mediated transfer. Biolistic is preferred as it is less time-consuming and demanding. Integration of foreign DNA into plastid genome occurs via homologous recombination. Homologous recombination operates in plastids at a high efficiency.



Transformation of the chloroplast genome by bombarding tobacco leaves with micro projectiles coated with DNA. Following bombardment, leaf discs are placed onto antibiotic containing medium (panel A). Transgenic plants are regenerated from the transformed tissue that is able to develop green chloroplasts (panel B).

42. Methods of chloroplast transformation

The following points highlight the top five methods of chloroplast transformation in higher plants.

The methods are:

1. Vectors for Plastid Transformation

2. Engineering for High-Level Protein Production

3. Biolistic Transfer

4. PEG Mediated Transformation

5. Galistan Expansion Femtosyringe Method.

Vectors for Plastid Transformation:

Vectors used for plastid transformation utilize left (LTR) and right (RTR) targeting regions to direct inserting of transgene into the plastid region. Some of the commonly used plasmid transformation vectors are plasmid repeat vector (pRV) and vector pRB94 and pRB95. Expression vector for chloroplast transformation contains two-open reading frame under the control of chloroplast-specific promoter and termination signal.

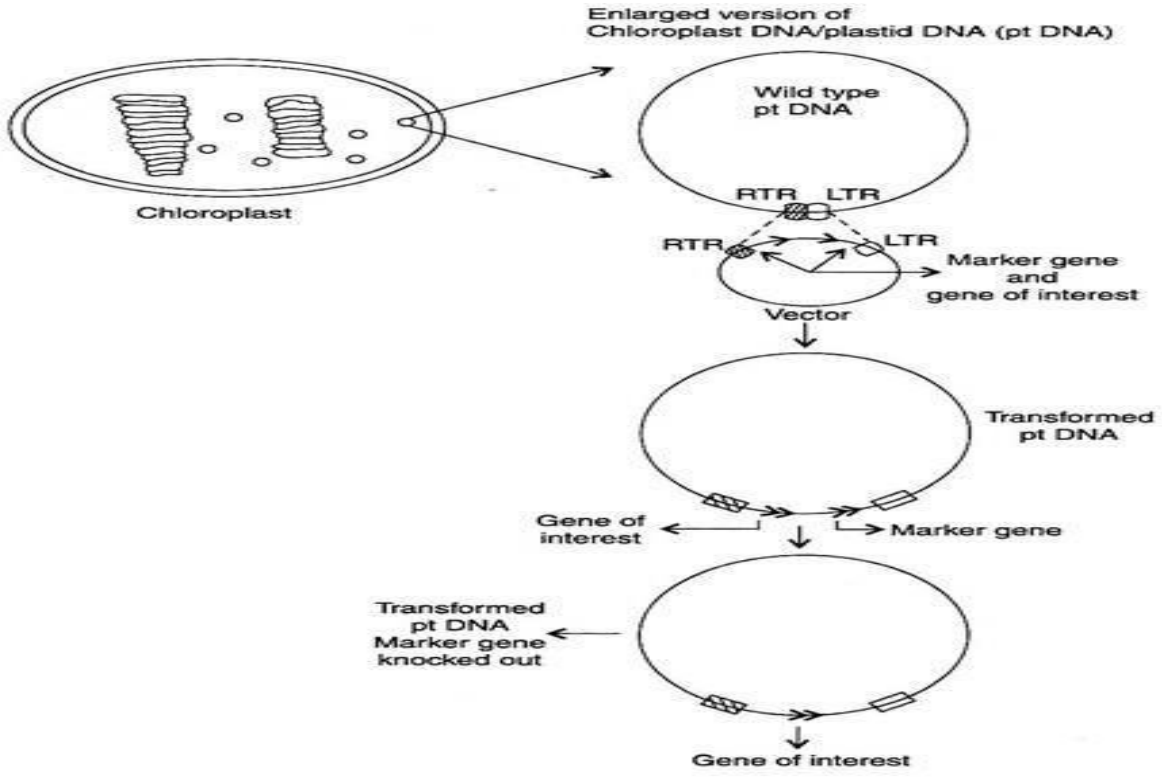


Fig. 19.1 Transformation of plastid DNA by recombination events

Presence of homologous sequence facilitate two recombination events which consequently responsible for the insertion of marker gene and genome of interest into the LRT and RTR regions of the plastid. Some of the well-known plastid transformation vectors are plasmid repeat vector, and other is pRB95. Some of the expression system facilitated the expression of ribosome binding site region inserted at intergenic regions allowing production of target protein from polycistronic mRNA transcript

Engineering for High-Level Protein Production:

Strategies for production of high amount of recombinant proteins have been adopted like utilization of strong promoter, and stable mRNA transcript determined by 5′ untranslated (UTR) and 3′ untranslated (UTR) of the transgene. Considering these facts several plastid expression vectors are designed to contain 5′ regulatory region PL casette and 3′ regulatory region (T casette), strong sigma 70-type PEP promoter of the rRNA operon promoter (prnn).

The 3′ UTR regulatory sequence of mRNA include RNA stem loop structure, which acts as a inefficient transcription terminator. Most 3′ UTR T casettes are derived from PbSA, rbcL and rps 16 genes. The 5′ UTR (PL casette) region play important role in translation efficiency. In addition, careful optimization of transgene codons, despite its prokaryotic nature of expression system, resulted in high protein production.

Biolistic Transfer: Biolystic is one of the efficient approaches for plastid transformation.

Effective penetration and high transfer frequency are some of the plus points of biolystic method. There are number of bacteria and viruses are known to infect chloroplast. It's envelop is made up of double membrane and actually considered that chloroplast transformation was considered to be virtually impossible.

However, invention of biolistic gene gun technology paved the pathway of direct delivery of target gene into the living cell. It is fortunate that DNA is deposited in a chloroplast and successfully integrated into the chloroplast genome.

PEG Mediated Transformation:

Polyethylene glycol is widely used in transformation work. Despite its efficiency, PEG mediated transformation is far behind than the biolistic approach. Foreign DNA is taken by protoplast in presence of PEG and transported by unknown process from cytoplasm into the chloroplast and finally integrated into the genome.

Galistan Expansion Femtosyringe Method:

The existing microinjection method in which recipent cells damaged by the release of cellular contents into the needle after injection have raised fresh look into the designing of novel approach for chloroplast transformation of wide range of species. A novel galinstan

femtosyringe method designed for chloroplast transformation involves microinjection of foreign DNA into chloroplast

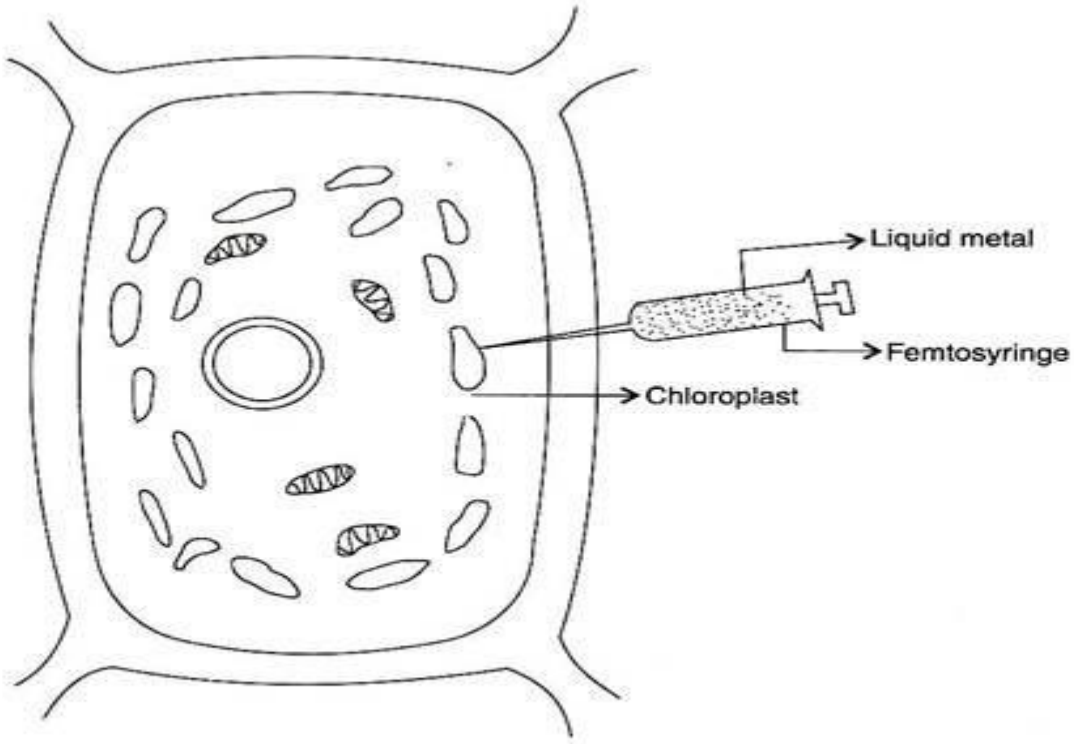
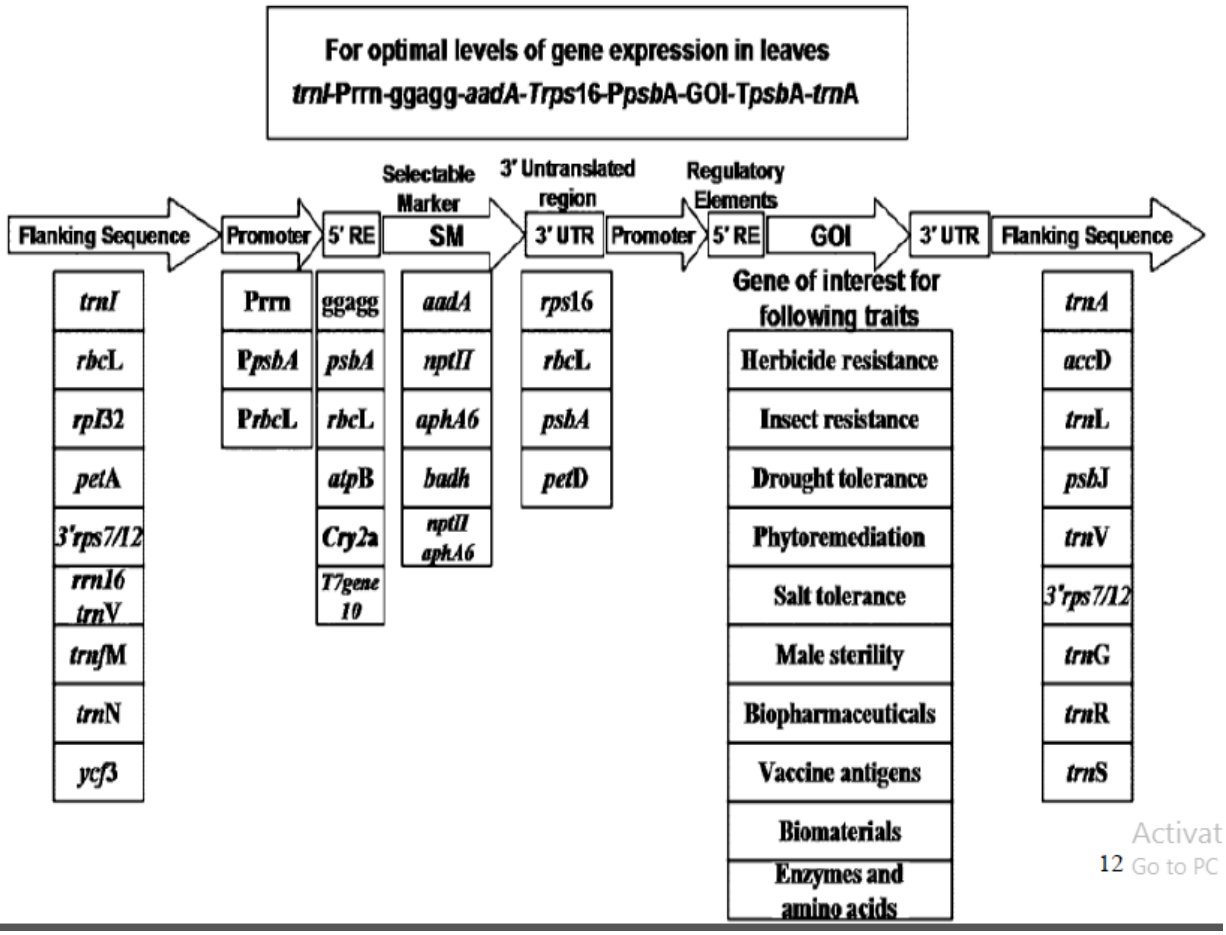


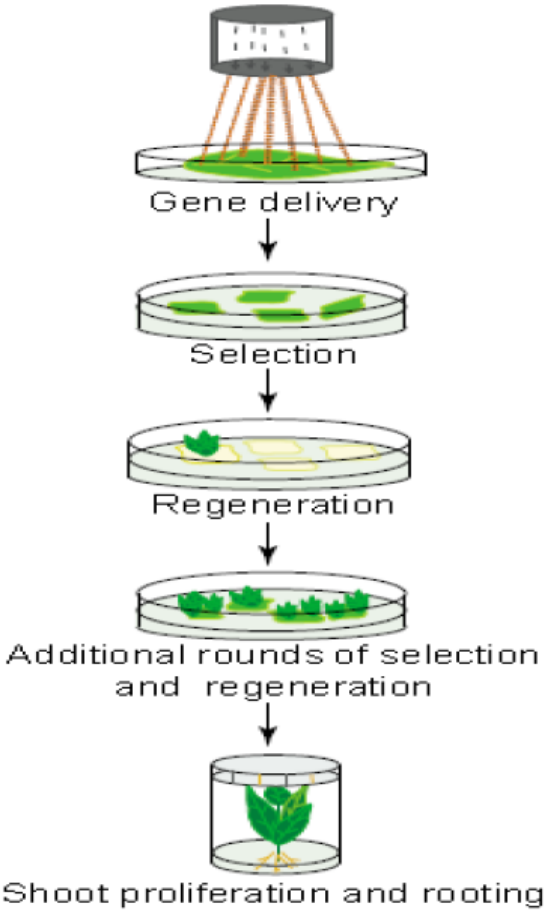
Fig. 19.3 Galistan femtosyringe method of plastid transformation (After Daniel et al 1999)

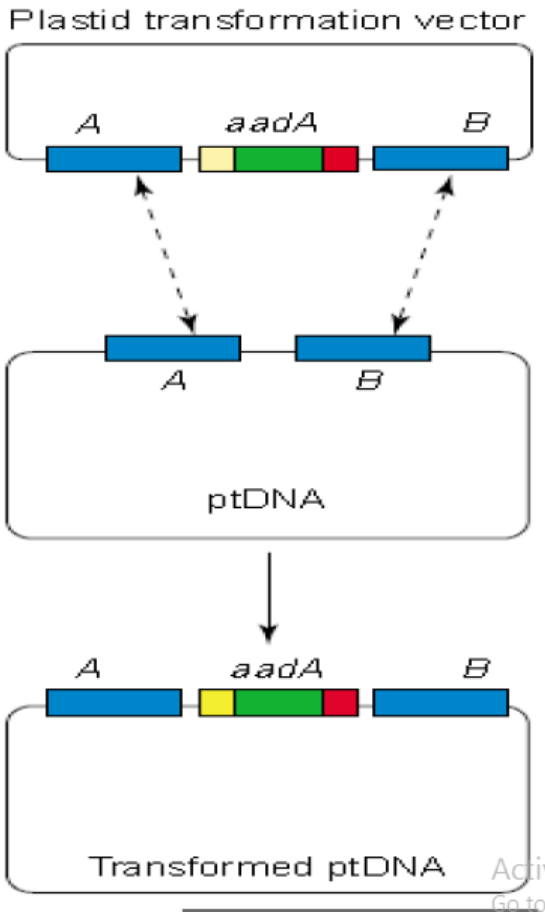
The heat induced expansion of a liquid metal within a glass syringe forces the foreign DNA through a minute capillary top with a diameter of approximately 0.1 µm. The liquid metals employed in the specialized femtosyringe are generally galinstan, an alloy of gallium, indium and tin.

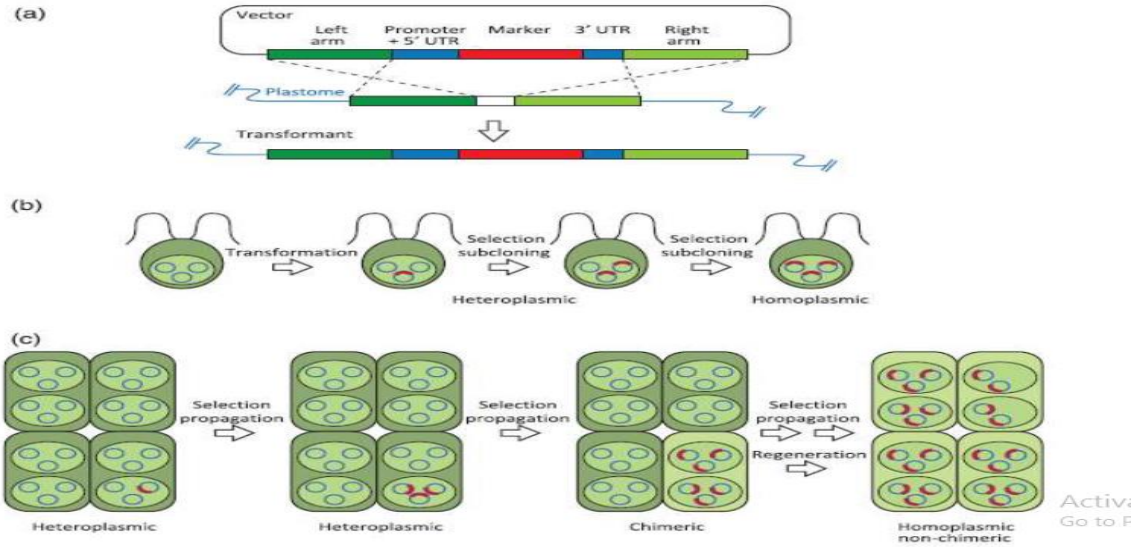
43. Molecular biology of Chloroplast Transformation

Stable transformation system depends on integration of the transforming DNA into the plastid genome by homologous recombination. Sequence to be introduced into the plastid genome must flanked on both side by region of homology with the chloroplast genome. Primary transplastomic event results hetroplasmic cells. Hetroplasmy is unstable so it will resolve into homoplasmy.









Marker removal

- Recombination between directly repeated sequences excises the intervening DNA sequence and one copy of the direct repeat.

- The breakage and joining of DNA strands involved in recombination can be mediated by the native homologous recombination machinery present in plastids.

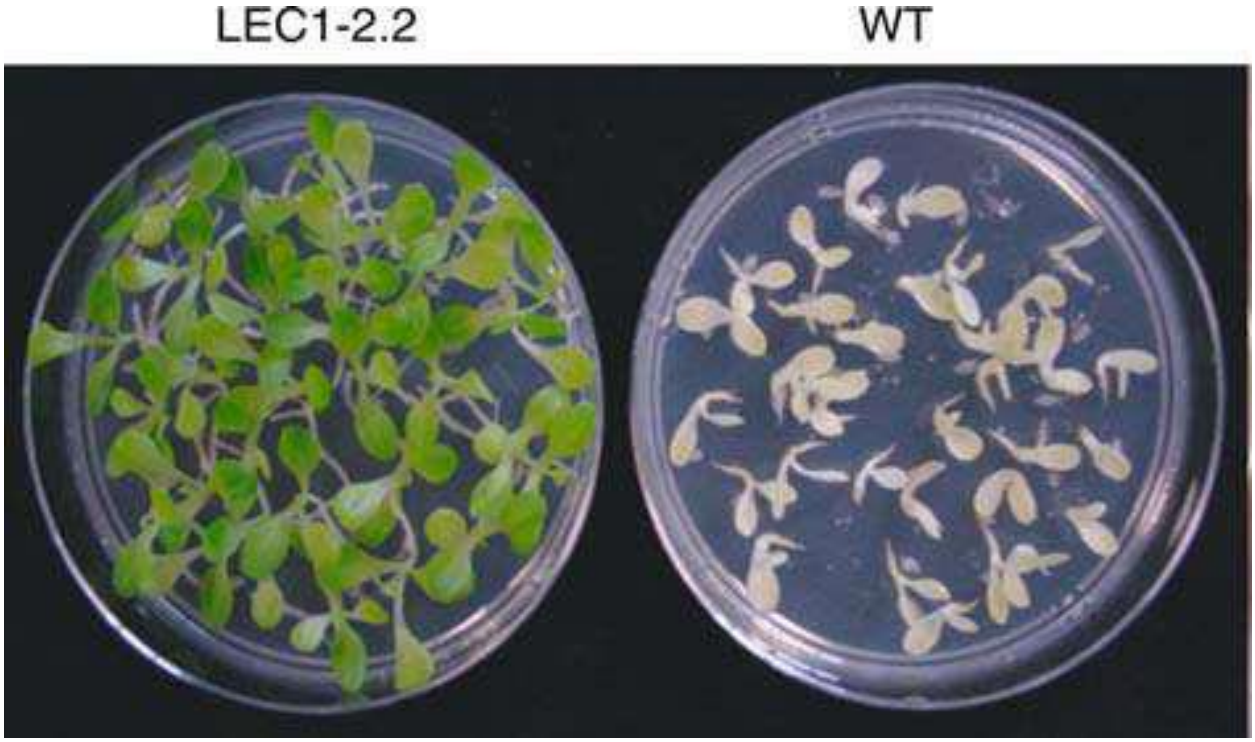
Case Study – *Lactuca sativa*

1. Protoplast isolation

- Lettuce seeds were sterilized and sown on MS medium with 2% sucrose
- Shoot tips from leaves obtained were transferred to MS medium with 3% sucrose
- The leaves were cut into pieces and incubated in PG solution, followed by enzyme solution consisting of 1% cellulase and 0.25% macerozyme
- Protoplast suspension was filtered through nylon mesh
- Protoplasts were collected at surface after centrifugation at 70g for 8min

2.Transformation and culture

- 10µl transforming DNA and 0.6ml PEG solution was added to protoplast suspension and incubated at 25ºC for 10min
- Protoplasts were mixed with 1:1 solution of B5 and 2% agarose to a density of 3.6 X 104 protoplasts per ml
- The suspension was plated onto Petri dishes and cultured at 25ºC in the dark
- Selection was initiated on the 7th day by fresh medium containing spectinomycin dihydrochloride



- 100% of spectinomycin-resistant lettuce cell lines were true plastid transformants
- A limitation was the high frequency of polyploid cell lines

Analyses

- PCR – specific primers were used to assess the presence of *aadA* gene in resistant cell lines
- Immunoblot analysis – using HRP-conjugated secondary antibodies
- Southern and Northern blots were performed to look for target genes and their transcripts

Production of human therapeutic proteins

Why lettuce is favoured over tobacco?

- Most of the plant is leaf tissue and this tissue contains the greatest number of plastids per cell
- Unlike tobacco, lettuce has no toxic alkaloids that need to be removed - low purification and downstream processing costs
- Lettuce is a relevant human foodstuff that can be consumed without cooking

Year	Milestone	DNA delivery	Approach	Selection	Reference
1988	*Chlamydomonas reinhardtii* 1st stable plastid transformation	Biolistic	Homologous targeting	Photosynthetic competence	Boynton & Gillham (Science, 240)
1990	*Nicotiana tabacum* 1st stable plastid transformation	Biolistic	Homologous targeting	Spectinomycin (rrn16)	Svab et al (PNAS, 87)
1993	*Nicotiana tabacum* 1st high level foreign protein (2.5% GUS)	PEG	Homologous targeting	Spectinomycin Kanamycin	Golds et al (Biotech. 11) O'Neill et al (Plant J. 3)
1995	*Nicotiana tabacum* New agronomic trait: *B. thruingiensis* Marker gene elimination: co-transformation	Biolistic	Homologous targeting	Spectinomycin	McBride et al (Biotech. 13) Carrer and Maliga (Biotech. 13)
1998	*Arabidopsis thaliana* 1st stable plastid transformation	Biolistic	Homologous targeting	Spectinomycin	Sikdar et al (Plant Cell Rep. 18)
1999	*Solanum tuberosum* (potato) 1st stable plastid transformation *Oryza sativa* (rice) 1st stable plastid transformation	Biolistic	Homologous targeting	Spectinomycin	Sidorov et al (Plant J. 19) Khan and Maliga (Nat. Biotech. 17)

Year	Milestone	DNA delivery	Approach	Selection	Reference
2000	*Nicotiano tabacum* 1st human protein expression	Biolistic	Homologous targeting	Spectinomycin	Staub et al (Nat. Biotech. 18)
2001	*Lycopersicon esculentum* (tomato) 1st foreign protein in fruit Marker gene elimination: CRE-lox New agronomic traits: glyphosate tolerance and PPT resistance	Biolistic	Homologous targeting	Spectinomycin	Ruf et al(Nat. Biotech. 19) Corneille et al (Plant J. 19) Ye et al (Plant J. 25) Lutz et al (Plant Physiol. 125)
2002	*Porphyridium* sp. 1st stable plastid transformation	Biolistic	Homologous targeting	Spectinomycin	Lapidot et al (Plant Physiol. 129)
2003	*Chlamydomonas reinhardtii* : Foot-and-mouth disease virus VP1 protein expression *Brassicacea* (oil seeds) 1st stable plastid transformation Phytoremediation: Mercury	Biolistic	Homologous targeting	Spectinomycin	Sun et al (Biotechnol Lett. 25) Skarjinskaia et al (Transgenic Res. 12) Ruiz et al (Plant Physiol. 132)
2004	*Gossypium hirsutum* (cotton) 1st stable plastid transformation *Glycin max* (soybean) 1st stable plastid transformation *Linum usitatissimum* L. (flax): PHB polymer expression	Biolistic	Homologous targeting	aph A-6 npt II Spectinomycin	Kumar et al (PMB. 56) Dufourmantel et al (PMB. 55) Wrobel et al (J. Biotech. 107)

44. Pesticide

Pesticides are substances that are meant to control pests, including weeds. Any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs. Substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport

Classification

Pesticides can be classified by target organism.

Type of pesticide	Target pest group
Algicides or algaecides	Algae
Avicides	Birds
Bactericides	Bacteria
Fungicides	Fungi and oomycetes
Herbicides	Plant
Insecticides	Insects
Miticides or acaricides	Mites
Molluscicides	Snails
Nematicides	Nematodes
Rodenticides	Rodents
Virucides	Viruses

- Algaecides are used for killing and/or slowing the growth of algae.
- Antimicrobials control germs and microbes such as bacteria and viruses.
- Biopesticides are made of living things, come from living things, or they are found in nature.
- Desiccants are used to dry up living plant tissues.
- Defoliants cause plants to drop their leaves.

- Disinfectants control germs and microbes such as bacteria and viruses.
- Fungicides are used to control fungal problems like molds, mildew, and rust.
- Herbicides kill or inhibit the growth of unwanted plants, aka weeds.
- Illegal and Counterfeit Pesticides are imported or sold illegally.

- Insecticides are used to control insects.
- Insect Growth Regulators disrupt the growth and reproduction of insects.
- Miticides control mites that feed on plants and animals. Mites are not insects, exactly.
- Molluscicides are designed to control slugs, snails and other molluscs.
- Mothballs are insecticides used to kill fabric pests by fumigation in sealed containers.
- Natural and Biological Pesticides control pests using things found in nature, or man-made versions of things found in nature.
- Ovicides are used to control eggs of insects and mites.
- Pheromones are biologically active chemicals used to attract insects or disrupt their mating behavior. The ratio of chemicals in the mixture is often species-specific.
- Repellents are designed to repel unwanted pests, often by taste or smell.
- Rodenticides are used to kills rodents like mice, rats, and gophers.

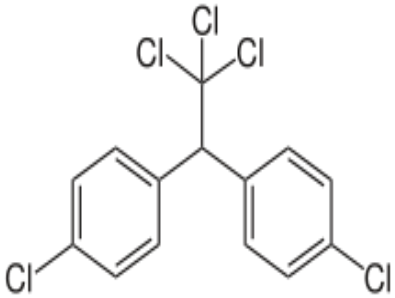
45. Uses of pesticides

Pesticides are used to control organisms that are considered to be harmful, for example, they are used to kill mosquitoes that can transmit potentially deadly diseases like West Nile virus, yellow fever, and malaria. They can also kill bees, wasps or ants that can cause allergic reactions. Insecticides can protect animals from illnesses that can be caused by parasites such as fleas Pesticides can prevent sickness in humans that could be caused by moldy food or diseased produce. Herbicides can be used to clear roadside weeds, trees, and brush. They can also kill invasive weeds that may cause environmental damage. Herbicides are commonly applied in ponds and lakes to control algae and plants such as water grasses that can interfere with activities like swimming and fishing and cause the water to look or smell unpleasant. Uncontrolled pests such as termites and mold can damage structures such as houses. Pesticides are used in grocery stores and food storage facilities to manage rodents and insects that infest food such as grain. Each use of a pesticide carries some associated risk. Proper pesticide use decreases these associated risks to a level deemed acceptable by pesticide regulatory agencies. DDT, sprayed on the walls of houses, is an organochlorine that has been used to fight malaria since the 1950s. Recent policy statements by the World Health Organization have given stronger support to this approach.

However, DDT and other organochlorine pesticides have been banned in most countries worldwide because of their persistence in the environment and human toxicity. DDT use is not always effective, as resistance to DDT was identified in Africa as early as 1955, and by 1972 nineteen species of mosquito worldwide were resistant to DDT

Dichlorodiphenyltrichloroethane, commonly known as DDT, is a colorless, tasteless, and almost odorless crystalline chemical compound, an organochlorine, originally developed as an insecticide, and ultimately becoming infamous for its environmental impacts. First synthesized in 1874, DDT's insecticidal action was discovered by the Swiss chemist Paul Hermann Müller in

1939. DDT was used in the second half of World War II to control malaria and typhus among civilians and troops.



Benefits

Pesticides can save farmers' money by preventing crop losses to insects and other pests; in the U.S., farmers get an estimated fourfold return on money they spend on pesticides. One study found that not using pesticides reduced crop yields by about 10%.

Another study, conducted in 1999, found that a ban on pesticides in the United States may result in a rise of food prices, loss of jobs, and an increase in world hunger. There are two levels of benefits for pesticide use, primary and secondary. Primary benefits are direct gains from the use of pesticides and secondary benefits are effects that are more long-term.

Primary benefits

- Controlling pests and plant disease vectors
- Improved crop/livestock yields
- Improved crop/livestock quality
- Invasive species controlled

Controlling human/livestock disease vectors and nuisance organisms

- Human lives saved and disease reduced. Diseases controlled include malaria,with millions of lives having been saved or enhanced with the use of DDT alone.
- Animal lives saved and disease reduced
- Controlling organisms that harm other human activities and structures
- Drivers view unobstructed
- Tree/brush/leaf hazards prevented
- Wooden structures protected

46. Effects of pesticides

Health effects

Pesticides may cause acute and delayed health effects in people who are exposed. Pesticide exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer.

A 2007 systematic review found that "most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure" and thus concluded that cosmetic use of pesticides should be decreased. Non-Hodgkin lymphoma is a cancer that starts in white blood cells called lymphocytes, which are part of the body's immune system. leukemia involves the production of abnormal white blood cells -- the cells responsible for fighting infection. There is substantial evidence of associations between organophosphate insecticide exposures and neurobehavioral alterations. Limited evidence also exists for other negative outcomes from pesticide exposure including neurological, birth defects, and fetal death.

Environmental effects

Widespread use of pesticides in agriculture has experts worried due to their long-term environmental damage. Some pesticides can stick around for years, posing a very real threat to the ecological system and hence human health. Excessive and careless use of pesticides can contaminate water sources and soil, make fruits and vegetables less nutritious, and reduce biodiversity. Some pesticides have also been linked to the dramatic reduction in the number of bees across the world, posing a huge threat to agriculture and food security, given that bees pollinate more than 70% of all crops.

Economics

In one study, the human health and environmental costs due to pesticides in the United States was estimated to be $9.6 billion: offset by about $40 billion in increased agricultural production.

Additional costs include the registration process and the cost of purchasing pesticides: which are typically borne by agrichemical companies and farmers respectively. The registration process can take several years to complete (there are 70 different types of field test) and can cost $50–70 million for a single pesticide.

At the beginning of the 21st century, the United States spent approximately $10 billion on pesticides annually.

47. Effects of pesticides on human health

- Experts broadly classify the effects of pesticides as topical or systemic.
- Topical reactions are usually limited to areas of the body that have come in direct contact with a pesticide.
- Inflammation of the skin (dermatitis) such as a rash or blisters is usually the most common topical symptom.
- Other topical reactions may include sneezing, wheezing, and coughing, usually triggered by petroleum distillates that many pesticides contain.
- Severe pesticide poisoning can cause seizures, change in heart rate, and sometimes even coma and death

Short-Term Effects of Pesticides

- Short-term pesticide poisoning or acute toxicity from pesticides is usually the result of a single and brief exposure to a pesticide.
- This kind of poisoning can happen due to exposure via the skin, inhalation, through the eyes, or orally.
- Symptoms of acute toxicity can become apparent instantly or take as long as 48 hours
- Short-term effects of pesticides can manifest as:
- Nausea and vomiting
- Diarrhea
- Loss of consciousness
- Seizures
- Coughing and sore throat
- Extreme weakness

Long-Term Effects Of Pesticides

- While continual, low-dose exposure to pesticides don't usually show immediate effects, they cause serious harm to human health in the long term.

- Repeated exposure to pesticides, even in small doses, has been linked to a number of diseases such as cancer, Parkinson's, Alzheimer's, sterility, and developmental disorders.
- Chronic exposure to pesticides can also lead to genetic changes and serious nerve disorders.
- Some studies have even linked pesticides to asthma, ADHD, depression, and anxiety

- Some pesticides contain chemicals that may be endocrine disruptors. These types of pesticides can be especially damaging because they interfere with our hormones and hormonal balance.
- Over a period of time, even low concentrations of these chemicals can cause obesity, diabetes, thyroid tumors, decreased fertility, uterus abnormalities, and early puberty.
- Lastly, pesticides are also known to cause neurological issues such as loss of memory and coordination, visual impairment, mood instability, and reduced motor skills

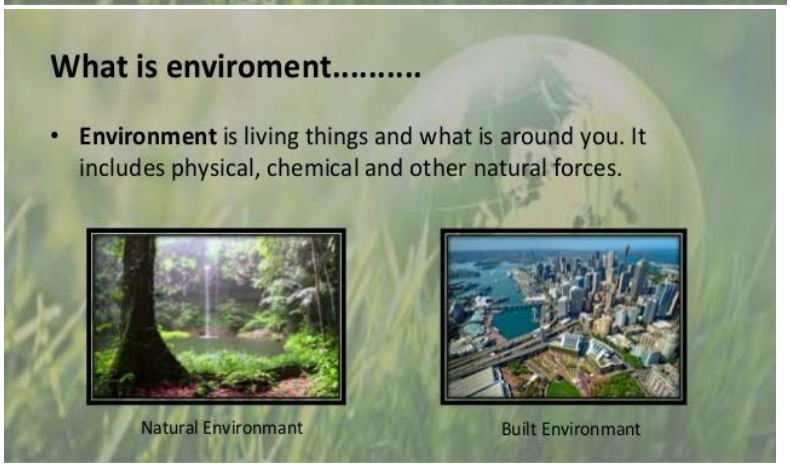
Effects Of Pesticides On Pregnant Women

- Exposure to pesticides and pesticide residue can lower fertility in women.
- A Harvard study found that women who ate more than two servings of fruits or vegetables with high pesticide residue each day were 18% less likely to become pregnant and 26% less likely to have a live birth compared to women with lower exposure

Effects Of Pesticides On Kids

Children are especially susceptible to harmful effects of pesticides. They can easily become exposed to pesticides (via inhalation or skin contact) in schools, daycare, playgrounds, hospitals, and any other public areas, no matter how careful you are. Kids' bodies are smaller and still growing, they take more breaths per minute, and they also eat and drink more relative to their weight – all factors that make them more likely to absorb pesticides and residue. Their little kidneys and liver also cannot eliminate pesticides from their bloodstream as effectively as an adult's.

48. Effects of pesticides on environment



Although each pesticide is meant to kill a certain pest, a very large percentage of pesticides reach a destination other than their target. Pesticides easily contaminate the air, ground and water when they run off from fields, escape storage tanks, are not discarded properly, and especially when they are sprayed aerially.

Water

Pesticides can be found in rain, ground water, streams, rivers, lakes and oceans. There are four major ways that pesticides can reach the water:

- it can drift outside of the area of where it was sprayed,
- it can leach through the soil,
- it can be carried as runoff,
- or it may be spilled accidentally.

Soil

The use of pesticides decreases the general biodiversity in the soil. Soil quality is higher without chemicals and this allows for higher water retention, necessary for plants to grow.

Plants

Nitrogen fixation, which is necessary for the growth of many large plants, is hindered by pesticides that can be found in soil. This can lead to a large decline in crop yields. Application of pesticides to crops that are in bloom can kill honeybees, which act as pollinators. This also decreases crop pollination and reproduction.

Animals

Animals may be poisoned by pesticide residues that remain on food after spraying.

An application of pesticides in an area can eliminate food sources that certain types of animals need, causing the animals to relocate, change their diet, or starve.

Poisoning from pesticides can even make its way up the food chain; for example, birds can be harmed when they eat insects and worms that have consumed pesticides.

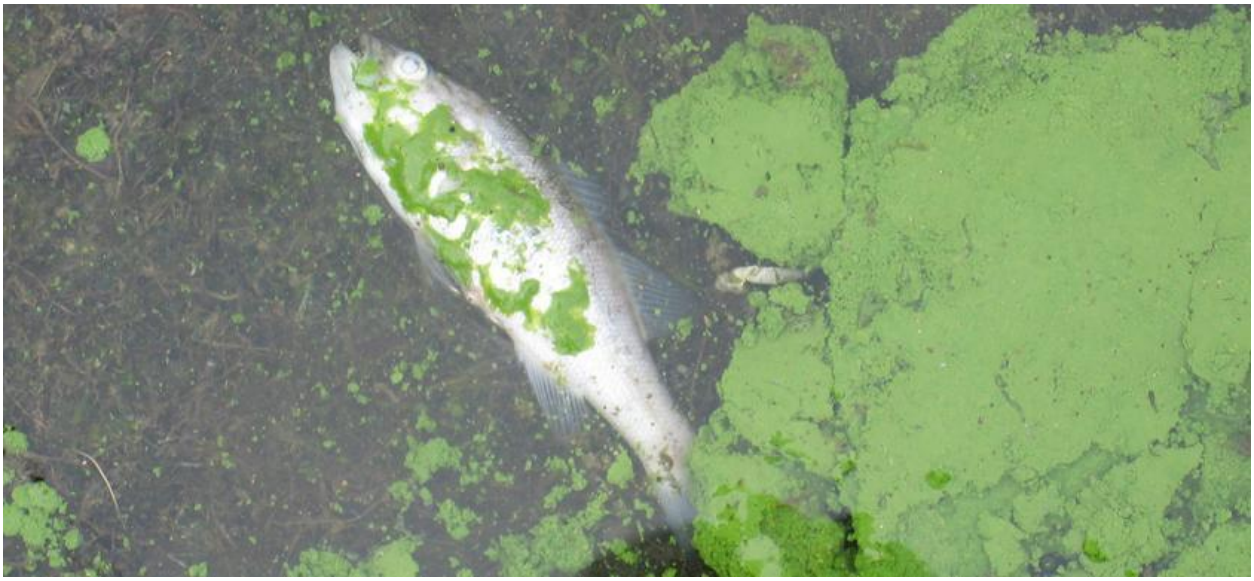
Birds

Birds are being harmed by pesticide use. Rachel Carson's book Silent Spring discusses the loss of several bird species due to accumulation of pesticides in their tissues. Types of

fungicides used in farming are only slightly toxic to birds and mammals, but may kill off earthworms, which can in turn reduce populations of the birds and mammals that feed on them

Aquatic Life

Fish and other aquatic biota may be harmed by pesticide-contaminated water. Application of herbicides to bodies of water can cause plants to die, diminishing the water's oxygen and suffocating the fish. Repeated exposure of some pesticides can cause physiological and behavioural changes in fish that reduce populations, such as abandonment of nests, decreased immunity to disease, and increased failure to avoid predators. Additionally, as some pesticides come in granular form, birds and other wildlife may eat the granules, mistaking them for grains of food. A few granules of a pesticide are enough to kill a small bird. Herbicides may also endanger bird populations by reducing their habitat.



Negative effects of pesticides

- Are carried on the wind
- Leaves residue on produce
- Remains inside produce and animals through bio accumulation
- Runs off into open water which contaminates public water supply as well as fish and other seafood.
- Pesticides can enter the body through the skin, eyes, mouth, and nose.
- Fetuses can suffer from exposure and develop behavioral problems as well as growth issues
- Babies can develop lower cognitive scores and fewer nerve cells and can have a lower birth rate as well as being born prematurely.

- Toxins from pesticides can stay in the body and build up in the liver
- There are many possible reactions that include fatigue, skin irritations, nausea, vomiting, breathing problems, brain disorders, blood disorders, liver and kidney damage, reproductive damage, cancer, and in some cases, death.

49. Signs and symptoms by pesticide poisoning

How do pesticides enter our bodies?

Pesticides can enter your body during mixing, applying, or clean-up operations. There are generally three ways a chemical or material can enter the body through the skin (dermal) through the lungs (inhalation) by mouth (ingestion).

Dermal (absorption through skin or eyes)

In most work situations, absorption through the skin is the most common route of pesticide exposure. People can be exposed to a splash or mist when mixing, loading or applying the pesticide. Skin contact can also occur when you touch a piece of equipment, protective clothing, or surface that has pesticide residue on it. Pesticides can also be absorbed through your eyes. In addition, pesticides, can cause injuries to the eye itself.

Inhalation (through the lungs)

Inhalation may occur when working near powders, airborne droplets (mists) or vapors. The hazard from low-pressure applications is fairly low because most of the droplets are too large to remain in the air. Applying a pesticide with high pressure, ultra-low volume, or fogging equipment can increase the hazard because the droplets are smaller and they can be carried in the air for considerable distances. Pesticides with a high inhalation hazard will be labelled with directions to use a respirator.

Ingestion (by mouth)

While ingestion (by mouth) is a less common way to be exposed, it can result in the most severe poisonings. There are numerous reports of people accidentally drinking a pesticide that has been put into an unlabeled bottle or beverage cup/container (including soft drink cans or bottles). Workers who handle pesticides may also unintentionally ingest the substance when eating or smoking if they have not washed their hands first.

Types of pesticide poisoning:

There are two types of pesticide poisoning:

Acute poisoning

This happens when someone has been exposed to a high dose of pesticide. This could occur when the pesticide is being mixed, for example, or if a hose breaks drenching the person or bystanders with liquid pesticide solution. Another example might be accidental ingestion of a pesticide, such as a child swallowing the chemical.

Chronic poisoning

This results from a person being exposed to a small amount of pesticide on many occasions over a long period of time. Chronic poisoning may happen when the operator repeatedly uses pesticide improperly, especially if they do not wear protective clothing and equipment or wears protective clothing which is not clean or is worn out, like wearing cracked or torn gloves.

Symptoms of pesticide poisoning

Symptoms of mild poisoning includes headache, sweating, diarrhea irritation of nose and throat eye irritation, nausea, fatigue, changes of mood, skin irritation, insomnia, loss of appetite, thirst, weakness, restlessness, dizziness, sore joints and nervousness.

Symptoms of severe poisoning

Symptoms of severe poisoning includes vomiting, convulsions, loss of reflexes, unconsciousness, inability to breathe, fever muscle twitching, thirst, constriction of eye pupils (eye pupils become small), increased rate of breathing

50. Treatment for Pesticide Poisoning

When pesticides are released into the air, we breathe them in through our nose and mouth. Once in the lungs, the pesticides quickly enter the blood and spread poison through the whole body. Because some pesticides have no smell, it is often hard to know if they are in the air. The most common forms of air-borne pesticides are fumigants, aerosols, foggers, smoke bombs, pest strips, sprays, and residues from spraying. You can also inhale pesticide dust in a storage area, when it is being used in an enclosed area, such as a greenhouse, or when it is being transported to the fields. Pesticide dust in the air can travel miles to pollute an area far from where it was used. It is easy for pesticide dust to get into houses.

If you think you have breathed in pesticides, get away from the pesticides right away! Do not wait until you feel worse.

Treatment

If you or someone else breathes in pesticides: Get the person away from the area where she breathed in the poison, especially if it is an enclosed area. Get fresh air. Loosen clothing to make breathing easier. Sit with head and shoulders raised.

If the person is unconscious, lay her on her side and watch her to make sure there is nothing blocking her breathing. If the person is not breathing, quickly do mouth-to-mouth breathing. Seek medical help. Take the pesticide label or name of the pesticide with you.

51. Treatment for Pesticide Poisoning 2

Like other toxic chemicals, pesticides can poison people in different ways:

They can poison through the skin and eyes, through the mouth (by swallowing) or through the air (by breathing). Each kind of poisoning needs a different kind of treatment.

When pesticides get on the skin

Most pesticide poisonings are from pesticides being absorbed through the skin. This can happen when they spill while being moved, when they splash during mixing, during spraying, or when you touch crops that have just been sprayed. Pesticides can also get on your skin through your clothes, or when you wash clothes with pesticides on them.

Rashes and irritation are the first signs of poisoning through the skin. Because skin problems may be caused by other things, such as a reaction to plants, insect bites, infections, or allergies, it can be hard to know if the problem is caused by pesticides.

Talk to other workers to find out if the crop you are working with causes this kind of reaction. If you work with pesticides and get any unexpected skin rashes, it is safest to treat them as if they are caused by pesticides.

Treatment

If you or someone else gets pesticides on the body:

Quickly remove any clothing the pesticides spilled onto. Wash the pesticides off the skin as soon as possible with soap and cool water. If it got into the eye, rinse the eye with clean water for 15 minutes.

If the skin is burned from pesticides:

Rinse well with cool water. Do not remove anything stuck to the burn. Do not apply lotions, fats, or butter. Do not break blisters. Do not remove loose skin. Cover the area with a sterile dressing, if available.

When pesticides are swallowed

People can swallow pesticides by eating, drinking, or smoking cigarettes in the fields while working with pesticides, or by drinking water polluted with pesticides.

Children can drink or eat pesticides, especially if pesticides are stored in containers also used to hold food, or left in the open or low to the ground.

Treatment

When someone swallow's pesticides:

If the person is unconscious, lay her on her side and make sure she is breathing. If the person is not breathing, quickly do mouth-to-mouth breathing. Mouth-to-mouth breathing can also expose you to the pesticide, so cover your mouth with a pocket mask, a piece of cloth, or thick plastic wrap with a hole cut in the middle, before you start mouth-to-mouth breathing. Find the pesticide package and read the label right away. The label will tell you if you should make the person vomit up the poison or not. If the person can drink, give her lots of clean water.

Seek medical help. If it is available, always take the pesticide label or name with you.

Do not vomit if the label says not to. Never vomit after swallowing a pesticide that contains gasoline, kerosene, xylene, or other petroleum-based liquids. This will make the problem worse. Never make the person vomit or drink if she is unconscious, confused, or shaking badly. If you are sure vomiting is OK, give the person: a glass of very salty water or 2 tablespoons of pounded strong-tasting edible plant (such as celery, basil, or another local herb) followed by 1

or 2 glasses of warm water. Keep the person moving around. This can help her vomit sooner. After vomiting, activated or powdered charcoal can help absorb any poison still in the stomach

52. Approaches to pesticide poisoning

When effectively applied, pesticides can kill or control pests, including weeds, insects, fungi, bacteria, and rodents. Chemical pest control has contributed to dramatic increases in yields for most major fruit and vegetable crops

Pesticide poisoning

A pesticide poisoning occurs when chemicals intended to control a pest affect non-target organisms such as humans, wildlife, or bees

Types of pesticide poisoning.

Humans may be harmed by pesticides in two ways: they may be poisoned or injured.

Pesticide poisoning is caused by pesticides that harm internal organs or other systems inside the body. Pesticide-related injuries usually are caused by pesticides that are external irritants.

Hazard

Hazard is the risk of harmful effects from pesticides. Hazard depends on both the toxicity of the pesticide and your exposure.

Hazard = Toxicity x Exposure

Exposure

When a pesticide contacts a surface or organism, that contact is called a pesticide exposure.

For humans, a pesticide exposure means getting pesticides in or on the body. The toxic effect of a pesticide exposure depends on how much pesticide is involved and how long it remains there.

Types of Exposures

- Pesticides contact your body in four main ways:
- Oral exposure (when you swallow a pesticide),

- Inhalation exposure (when you breathe in a
- pesticide),
- Ocular – (through the eyes), or
- Dermal (through the skin)

Strategies to exposure/poison

- Diagnosis
- Prevention
- Treatment

53. Herbicides

A herbicide is a chemical substance used to control or manipulate undesirable vegetation, especially weeds. Herbicides are extensively used in gardening, farming, and landscape turf management. Herbicides tend to have wide-ranging effects on non-target species (other than those the pesticide is meant to control or kill). Herbicides, also commonly known as weedkillers, are chemical substances used to control unwanted plants. Modern herbicides are often synthetic mimics of natural plant hormones which interfere with growth of the target plants. The term organic herbicide has come to mean herbicides intended for organic farming. Some plants also produce their own natural herbicides, such as the genus Juglans (walnuts), or the tree of heaven; such action of natural herbicides, and other related chemical interactions, is called allelopathy.

Herbicides are classified into two categories

selective and non-selective.

Selective herbicides kill specific unwanted plants while leaving desirable vegetation relatively unharmed.

Non-selective herbicides (total weed killers) kill all or most plant species.

Methods of application

A herbicide can be applied directly to the plant, applied to the soil, or sprayed onto the foliage. Herbicides are applied before, during, or after crop planting in row-crop farming to maximize crop production by diminishing the development of unwanted plants. Herbicides are also applied in ponds and lakes to control aquatic plants, in forests to prepare logged areas for replanting, and to golf courses, lawns, parks, and other areas to clear out unwanted vegetation.

Herbicide Application time

Herbicides generally are applied at different times, depending upon the emergence time of the weeds and upon the type of fruit plants. Herbicides that are applied at specific times include the following:

Preplant herbicides are used before the crop is planted to control germinating weed seeds, and are usually mixed into the top 2 to 3 inches of soil. No preplant herbicides are labeled for fruit plants.

Preemergence herbicides are used after the crop has been planted, but before the weeds or crop emerges. Restrictions on the age of plants to be treated must be followed.

Postemergence herbicides are used after the crop and/or weeds have emerged from the soil surface and are growing. The most common of these is Round-Up®, which can be purchased without a pesticide license.

Herbicides usually are more effective when temperatures before application have favored uniform germination and rapid weed growth. Rapidly growing weeds are easiest to kill. High temperatures at the time of application also tend to increase the activity of the herbicide but also increase the possibility of crop injury. Moderate temperatures between 70 and 85°F are the most favorable for spraying. Wind can also be a factor in herbicide application. It can cause improper distribution over the weeds, reducing herbicide effectiveness while increasing the danger of drift onto desirable plants. Fewer problems occur if sprays are used when the wind velocity is low and the wind is blowing away from desirable plants.

Maximum Yield explains Herbicide

Besides selective and non-selective classifications, a herbicide can also be categorized according to three other characteristics:

Persistence - How long the herbicide remains potent

Mechanism of action that how does it works. Means of uptake - How the plants will absorb it (e.g., through the roots, aboveground foliage, etc.)

Mode of action

A herbicide's effectiveness is strongly influenced by its toxic mode of action and the application method. Herbicides can act by inhibiting a plant's amino acid production, growth, photosynthesis, cell division, or by mimicking natural auxin hormones to cause deformities.

Most modern herbicides are synthetic mimics of a natural plant's hormones that obstruct the target plant's growth. Some plants such as the tree of heaven and juglans (walnuts) produce their own natural herbicide. Organic herbicides are useful and are commonly used in organic gardens, but they are less effective and more costly than synthetic herbicides because they based on natural materials. For difficult cases, a combination of several herbicides is recommended when dealing with herbicide resistance.

First herbicides

Although research into chemical herbicides began in the early 20th century, the first major breakthrough was the result of research conducted in both the UK and the US during the Second World War into the potential use of herbicides in war. The first modern herbicide, 2,4-D, was first discovered and synthesized by W. G. Templeman at Imperial Chemical Industries. In 1940, he showed that "Growth substances applied appropriately would kill certain broad-leaved weeds in cereals without harming the crops." By 1941, his team succeeded in synthesizing the chemical. In the same year, Pokorny in the US achieved this as well.

54. Types of Herbicides

The different types of herbicides are all designed to kill plant tissue. However, they accomplish it by two basic methods. They are known as Contact Herbicides and Systemic Herbicides.

Contact herbicides: Contact is a word that means the chemical in that specific type of herbicide will kill the parts of the plant it contacts. For broadleaf weeds this means it will kill the above ground leafy part of the plants. It will not directly kill the below ground plants parts, such as roots, bulbs, tubers, or rhizomes. Contact herbicides are popular because they work quickly by killing the tissue in as fast as one day. Some herbicides will combine contact with systemic chemicals for a faster effect. New Round Up Weed and Grass Killer has combined both for faster control. For some plants, killing the above ground portions will not be enough to wipe out the plant completely. Most plants will regrow plant tissue and the herbicide will need to be reapplied. However, each time the plant has to use energy to start growth again will weaken the plant and eventually kill it.

Systemic Herbicide

For systemic types of herbicides, the word "Systemic" means the plant absorbs through the leaves or stems and transports it internally throughout the plant. The chemical travels with the sap so it usually doesn't have the quick "knockdown" effect. The greatest benefit of a systemic type of herbicide is that it will kill the entire plant, roots and all. The speed of chemical movement in the plant is largely dependent on soil and air temperature.

A chemical sprayed in early spring may take a couple weeks longer to work than the same chemical sprayed in mid-summer. The speed of kill is also dependent on the "mode of action" of the chemical (how the chemical works inside the plant).

Five types of herbicides:

Broad spectrum - these work on a wide variety of plants.

Selective - these work on a narrow range of plants.

Contact - these kill plant tissue at or near the point of contact with it (they do not spread around the plant). Therefore, they require even coverage in their application.

Systemic - these move through the plant tissues via the plant's circulation system, and these can be injected into the plant.

Residual - these can be applied to the soil in order to kill weeds by root uptake. They remain active in the ground for a certain length of time, and can control germinating seedlings.

55. Effect of Herbicides on People

Herbicides are poisonous chemicals that are used to kill unwanted plants, and are considered to be a type of pesticide. They are frequently used around the home and farm and represent a serious health hazard to adults, but they are especially hazardous to children and pets.

Types

Herbicides are commonly found as liquids or powders, and are sometimes premixed into fertilizer products.

Herbicides are classified according to the types of plants that they affect.

Broad-spectrum herbicides will kill any plant on which they are applied, while selective herbicides are designed to target only certain types of plants.

Contact herbicides affect only the part of a plant that the chemical touches, while systemic herbicides are designed to be drawn up into the plant through its roots or absorbed through its leaves and stems.

Systemic herbicides kill the entire plant. Although many modern herbicides are less toxic than their predecessors, they are still poisons and should always be handled with caution.

Skin Irritations and Allergic Reactions

According to the University of Missouri, herbicides are designed to be toxic to plants but in general are not highly toxic to mammals. Skin irritations are some of the most common effects when a person comes into contact with herbicides, and are most likely to happen on exposed areas, such as the hands and forearms. Some chemicals may burn the skin and should be washed off immediately with cold water. The Department of Veterans Affairs confirms that chloracne, a form of acne, is associated with exposure to Agent Orange. It can be mild or severe, and last up to several years. In severe cases, the skin may thicken and flake off. During the Vietnam War, the U.S. military sprayed millions of gallons of the herbicide Agent Orange between 1961 and 1971, in an attempt to defoliate trees in the jungle and deprive the enemy of food and cover.

Cancers

The Department of Veterans Affairs acknowledges that the herbicide Agent Orange is responsible for a wide range of health problems in Vietnam veterans, including several types of cancer. The National Academy of Sciences concluded that there was a positive correlation between the incidence of Hodgkin’s disease, a cancer of the lymph system, and exposure to

the herbicide Agent Orange. The VA also notes that non-Hodgkin's lymphoma is also associated with exposure to the defoliant. Herbicides are also suspected as causes of prostate cancer, cancers of the lungs and bronchial tubes, and cancers of the larynx and trachea.

Nervous System Disorders

Some herbicides can cause nervous system disorders, such as peripheral neuropathy.

The early symptoms of this disease include numbness and tingling in the toes and fingers, gradually spreading to include the hands and feet. Pain may be present, as well as muscle weakness and sensitivity to touch. Acute peripheral neuropathy occurs within a few weeks of being exposed. The VA cites peripheral neuropathy as another symptom of exposure to Agent Orange.

Effects on Children

Children and infants are at a higher risk for illnesses from herbicides than adults. According to the EPA, because children are still developing, their immune systems are less able to protect them from damage from herbicides. Children are also more likely to play in areas that expose them to chemicals, such as rolling on the floor or lawn. Mild exposure can result in complaints of dizziness and nausea, but herbicides may also cause neurological and developmental damage to children.

Pets

Pets can be poisoned by herbicides by coming into contact with the chemicals when they are outside, but herbicides kept in the home may also be a problem if they are stored where pets can get to them. Pets can ingest herbicides by chewing on plants or toys that have been contaminated, or when they lick themselves after coming into contact with the chemical. Animals that bring herbicides into the house may spread the chemicals around the home and leave residue on furniture and carpets.

56. Herbicide Resistance

Weeds are unwanted & useless plants that grow along with the crop plants. Weeds compete with the crops for light & nutrients, besides harboring various pathogens. So it is estimated that the worlds crop yield is reduced by 10 – 15 % due to the presence of weeds. To tackle the problem of weeds , modern agriculture has developed a wide range of weed killers (herbicides). Herbicides are broad spectrum as they can kill wide range of weeds.

An ideal herbicide is to posses the following characters:

- Capable of killing weeds with out affecting crop plants
- Not toxic to animals & microorganisms
- Rapidly trans located with in the target plant
- Rapidly degraded in the soil

- Commercially available herbicides is that they can not discriminate weeds from the crop plants .
- For this reason , crops are also affected by herbicides hence the need to develop herbicide resistance plants

Herbicide resistance

Herbicide resistance is the inherited ability of an individual plant to survive a herbicide application that would kill a normal population of the same species. Herbicide resistance does not equate to poor performance of a herbicide. Resistant weeds can often survive application of herbicide at rates that are much greater than the recommended rate. Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In contrast, tolerance can be defined as the inherent ability of a plant to survive a herbicide treatment at a normal use rate. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering. Resistance may occur in plants by random and infrequent mutations; no evidence has been presented to demonstrate herbicide-induced mutation. Through selection, where the herbicide is the selection pressure, susceptible plants are killed while herbicide resistant plants survive to reproduce without competition from susceptible plants. Thus, the appearance of herbicide resistance in a field is an example of rapid weed evolution."

Factors Leading to the Development of Herbicide Resistance

Because weeds contain a tremendous amount of genetic variation that allows them to survive under a variety of environmental conditions the development of a resistant species is brought about through selection pressure imposed by the continuous use of an herbicide. Factors that can lead to or accelerate the development of herbicide resistance include weed characteristics, Herbicide characteristics and cultural practices.

Weed characteristics

- Annual growth habit.
- High seed production.
- Relatively rapid turnover of the seed bank due to high percentage of seed germination each year (i.e., little seed dormancy).
- Several reproductive generations per growing season.
- Extreme susceptibility to a particular herbicide.
- High frequency of resistant gene(s), (e.g. Lolium rigidum).

Herbicide characteristics

- Herbicide characteristics which lead to rapid development of herbicide resistance in weed biotypes include:

- A single site of action
- Broad spectrum control.
- Long residual activity in the soil.

Cultural practices

Cultural practices can also increase the selective pressure for the development of herbicide resistant biotypes. In general, complete reliance on herbicides for weed control can greatly enhance the occurrence of herbicide resistant weeds.

Other factors include:

- Shift away from multi crop rotations towards mono cropping.
- Little or no cultivation or tillage for weed control or no elimination of weeds that escape herbicide control.
- Continuous or repeated use of a single herbicide or several herbicides that have the same mode of action.
- High herbicide use rate relative to the amount needed for weed control.
- Orchard and vineyard systems.
- Roadsides.

57. Mechanisms of Herbicide Resistance

For a herbicide to reach its active site in the plant, it must be taken into the plant and moved in lethal concentrations to the site where it has activity. Once it reaches the target site, it must be able to bind to the active site and stop that particular pathway. For a plant to be resistant, there must be a change that will allow it to avoid one or more of these steps. Theoretically, there could be a change in any one of these necessary steps beginning with uptake of the herbicide into the plant.

Potential mechanisms that could be responsible for resistance

Target-site mutation – there is a change in the binding site that prevents the herbicide from binding or interacting.

Metabolism – the herbicide is modified into a nontoxic molecule before it reaches the target site.

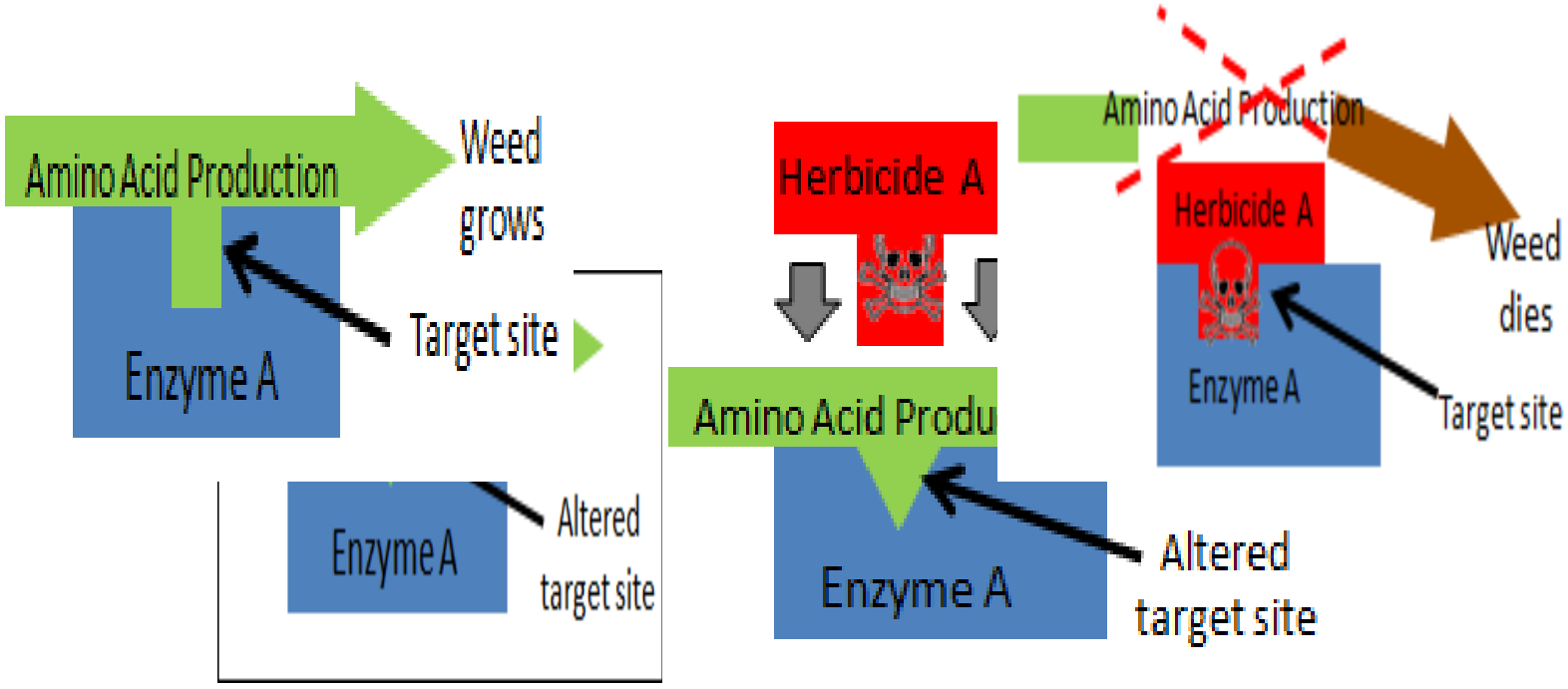
Sequestration – the herbicide is physically removed from the target site.

Reduced uptake – the herbicide is not taken up in lethal quantities.

Reduced translocation – the herbicide is not transported to the site in the plant where it has activity.

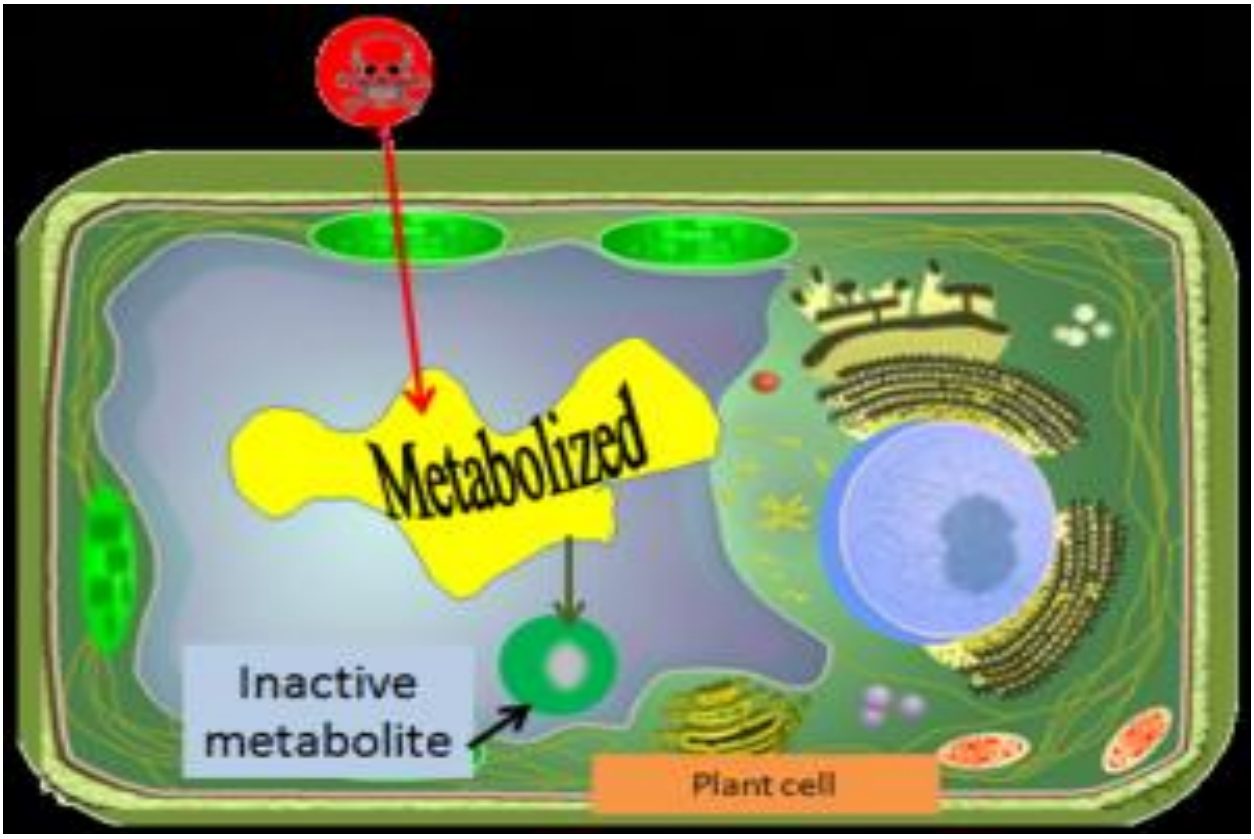
Altered target site

- An herbicide has a specific site (target site of action) where it acts to disrupt a particular plant process or function (mode of action).
- If this target site is somewhat altered, the herbicide no longer binds to the site of action and is unable to exert its phytotoxic effect.
- This is the most common mechanism of herbicide resistance.



Enhanced metabolism:

Metabolism within the plant is one mechanism a plant uses to detoxify a foreign compound such as an herbicide. A weed with the ability to quickly degrade an herbicide can potentially inactivate it before it can reach its site of action within the plant.



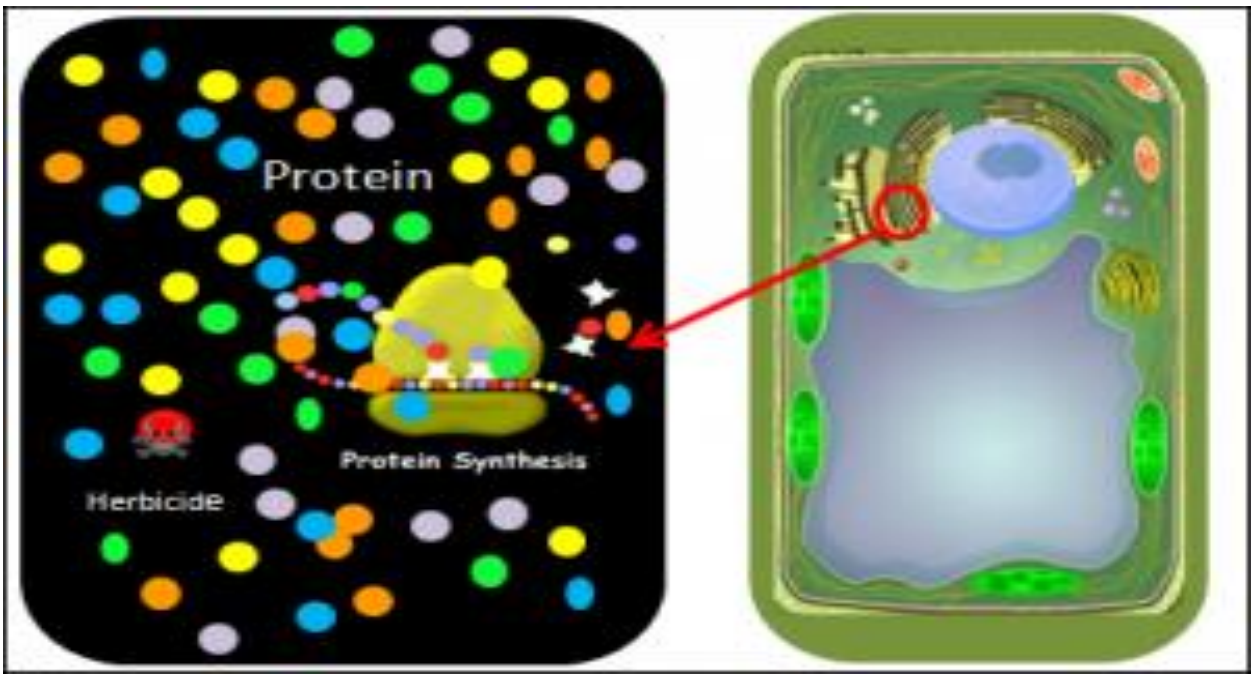
Compartmentalization or sequestration

Some plants are capable of restricting the movement of foreign compounds (herbicides) within their cells or tissues to prevent the compounds from causing harmful effects.

In this case, an herbicide may be inactivated either through binding (such as to a plant sugar molecule) or removed from metabolically active regions of the cell to inactive regions, the cell wall, for example, where it exerts no effect.

Over-expression of the target protein:

If the target protein, on which the herbicide acts, can be produced in large quantities by the plant, then the effect of the herbicide becomes insignificant.



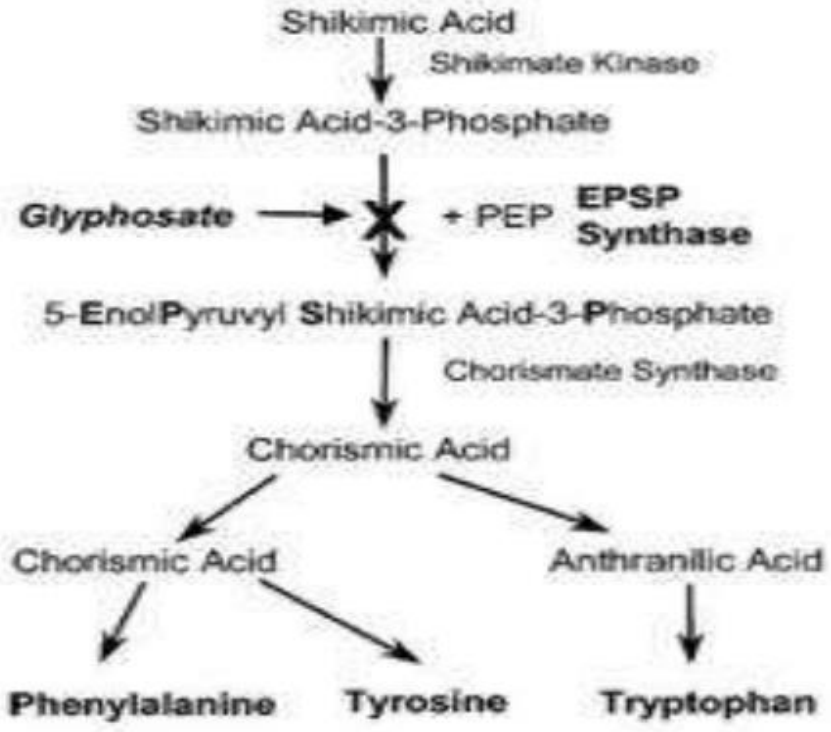
58. Mechanisms of Herbicide Resistance 2

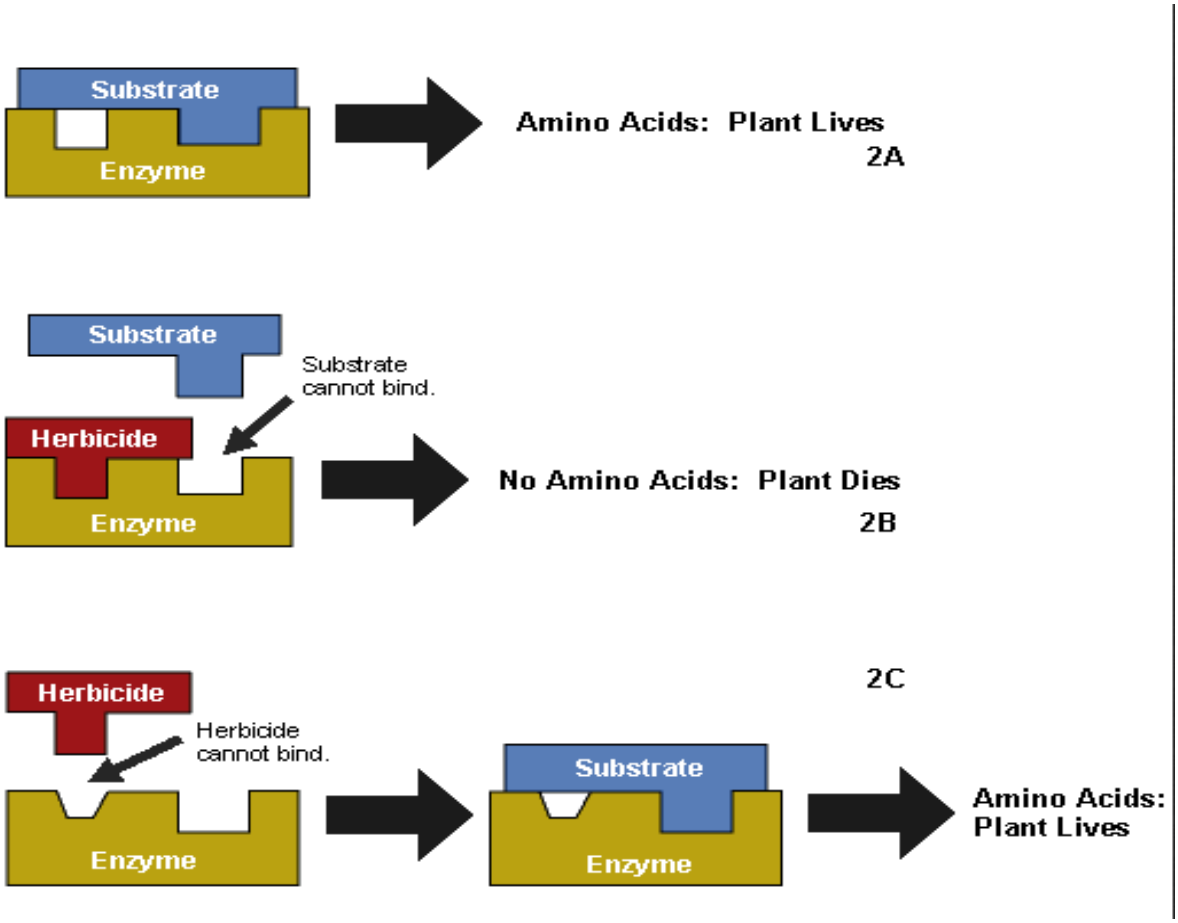
Glyphosate

It is a broad spectrum herbicide , effective against 76 of worlds worst 78 weeds. Less toxic to animals , is rapidly degraded & short life span. The American company (Monsanto) market it as round up .

Mechanism of Glyphosphate action

Capable of killing the plants in low concentration. Rapidly transported to growing tissues. It is competitive inhibitor of EPSPS (a key enzyme shikimic acid path way


Shikimic Acid
Shikimate Kinase
Shikimic Acid-3-Phosphate
Glyphosate
+ PEP
EPSP Synthase
5-EnolPyruvyl Shikimic Acid-3-Phosphate
Chorismate Synthase
Chorismic Acid
Chorismic Acid
Anthranilic Acid
Phenylalanine
Tyrosine
Tryptophan



Shikimic acid pathway results in the formation of amino acids, phenols, metabolites.

Glyphosate binds with EPSPS & blocks metabolism. Thus biosynthesis of aa & other products is inhibited. So cell division & plant growth is blocked. Shikimic acid pathway doesn't occur in animals.

So it is not toxic to animals

Advantages of using herbicides

- Broad spectrum of weeds controlled
- Reduced crop injury
- Reduced herbicide carryover
- New mode of action for resistance management
- Crop management flexibility and simplicity
- Use of herbicides that are more environmentally friendly

Disadvantages of herbicides

- Mammalian toxicity
- Eco toxicity

- Weeds become super weeds
- Reduced crop yield
- Creates soil and air pollution
- Herbicides also damage the Crop plants along with weed

Herbicide resistant Crops/Plants

- A number of biological manipulations involved in genetic engineering are in use to develop herbicide resistance plant
- Over expression of EPSPS gene
- Use of mutant EPSPS gene
- Detoxification of herbicide by a foreign gene

59. Herbicide Resistance Crops/Plants

How to produce herbicide resistant crops?

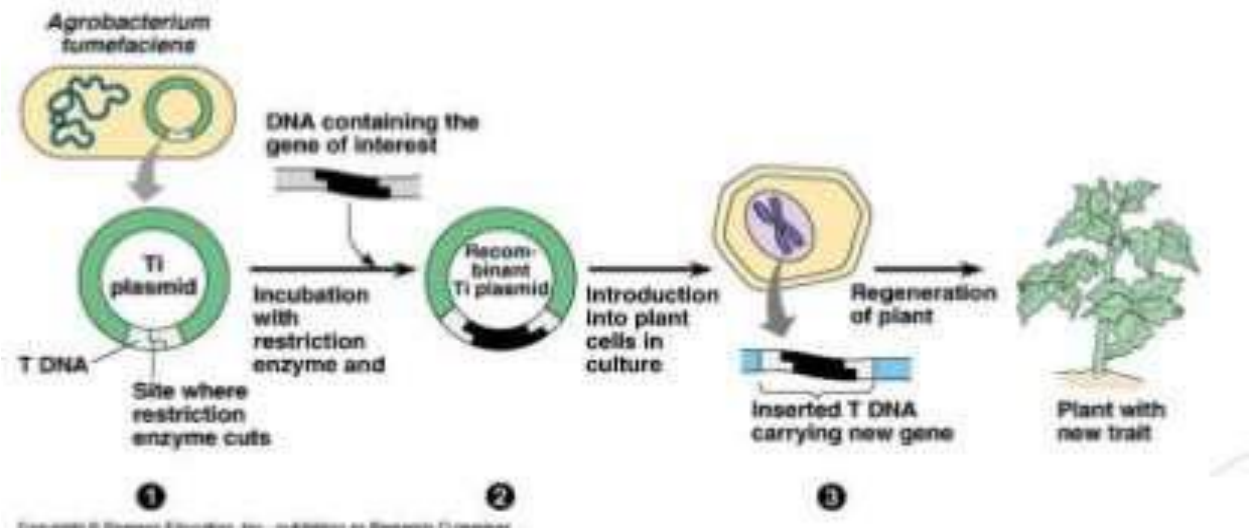
A number of biological manipulations involved in genetic engineering are in use to develop herbicide resistance plant by Over expression of EPSPS gene, Use of mutant EPSPS gene, Detoxification of herbicide by a foreign gene.

Glyphosphate resistance in crop/plants

1.Over expression of EPSPS gene

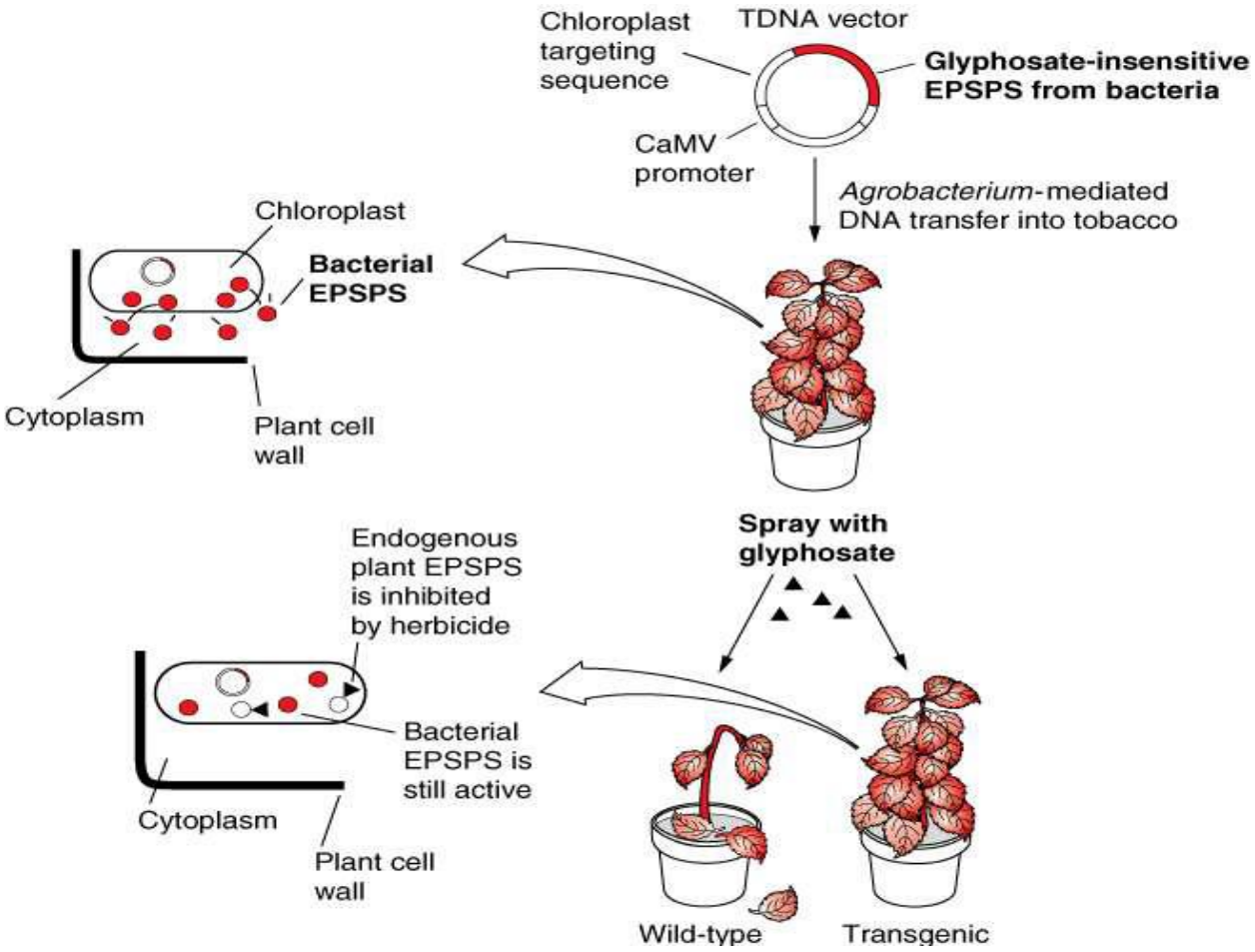
An over expression gene of EPSPS was detected in petunia. Gene from petunia was isolated & introduced in to other plants. The transgenic plants can tolerate glyphosphate 2 -4 times higher than that required to kill wild type weed plants

Transfer of petunia gene into bacterium and then into plant



2.Use of mutant EPSPS

EPSPS mutant gene resistant to glyphosphate was found in s. typhimurium it was found that single base substitution (C to T) change in amino acid from proline to serine. This enzyme cannot bind to glyphosphate using agrobacterium as vector mutant EPSPS was introduced in to tobacco plants but this is failed. It was later known that shikimic acid Pathway occurs in chloroplast, mutant EPSPS was produced in cytoplasm. This gene is not capable of transported to chloroplast. Later years mutant EPSPS gene was tagged with chloroplast specific transit EPSPS enzyme that freely enter chloroplast & confer resistance against herbicide



3.Detoxification of glyphosphate

The soil microorganisms possess enzymes glyphosphate oxidase that converts to glyphosate to glyoxylate .

That gene was isolated from ochrobactrum anthropy & was introduced in to crop plants e.g: oil seed rape

glyphosate glyphosate oxidaseglyoxylate + amp

Use of combine strategy

High resistance is acquired when the above 3 strategies combine together by this approach mutant, detoxification, over expression genes were employed in the same organism thus provides resistance.

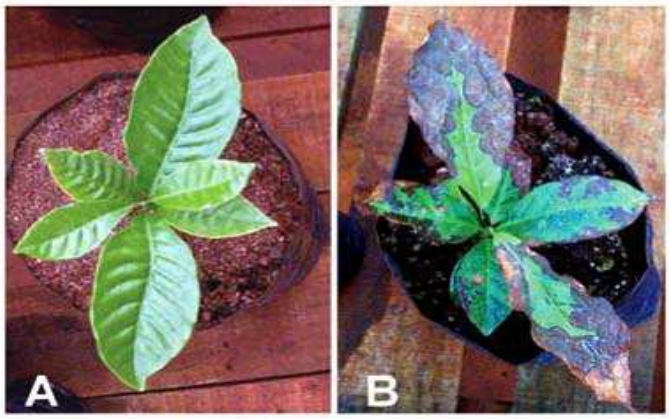
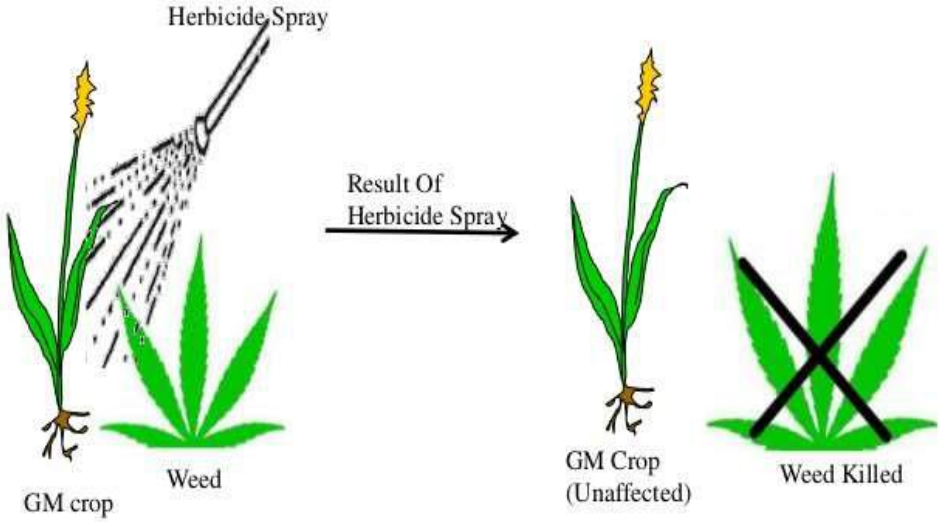


Figure 1. Herbicide tolerant coffee plant (A) and non-transformed plant (B), one week after spraying with ammonium glufosinate at 200 mg.L^{-1} (Ribas et al., 2006).

Herbicide Tolerance

- Over 63% of Gm crops grown globally have herbicide tolerance traits.
- Herbicide tolerance is achieved through the introduction of a gene from a bacterium conveying resistance to some herbicides. In situations where weed pressure is high, the use of such crops has resulted in a reduction in the quantity of the herbicides used.



60. Level of Herbicide Resistance

The level of herbicide resistance in weeds varies by weed biology and resistance mechanism. In some cases, resistance occurs when the species survives application of a labeled rate, while in other cases, the species can survive up to 1000 times the labeled rate. (1X equals the labeled rate.) This is important in terms of being able to identify herbicide resistance in the field.

Herbicide Resistance Characteristics

- Low-Level Resistance
- High-Level Resistance

Low-Level Resistance

- A continuum of plant responses from slightly injured to nearly dead
- The majority of plants display an intermediate response
- Susceptible plants will be present in the population, especially when herbicide resistance is determined early

High-Level Resistance

- Plants are slightly injured to uninjured
- Few plants have an intermediate response
- Susceptible plants can be present in the population

Low-Level Resistance

- A continuum of plant responses from slightly injured to nearly dead
- The majority of plants display an intermediate response
- Susceptible plants will be present in the population, especially when herbicide resistance is determined early

High-Level Resistance

Plants are slightly injured to uninjured

Few plants have an intermediate response

Susceptible plants can be present in the population

Herbicide Resistance Types

- Single Herbicide Resistance
- Cross Herbicide Resistance
- Multiple Herbicide Resistance
- Cross Resistance

Single Herbicide Resistance

Resistant to only one herbicide

Cross Herbicide Resistance

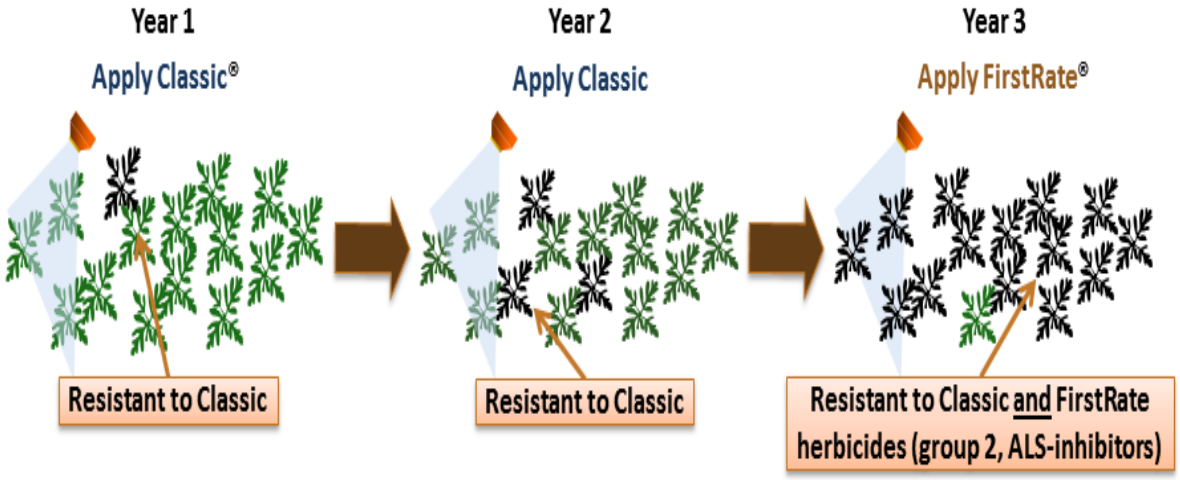
Resistant to two or more herbicide families with same mechanism of action

Single resistance mechanism

Multiple Herbicide Resistance

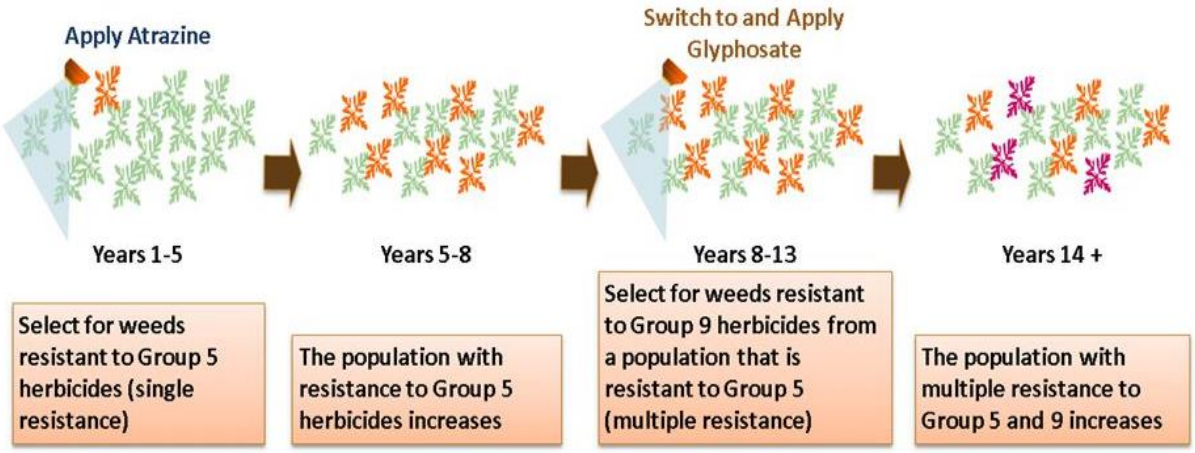
Resistant to two or more herbicides with different mechanisms of action. May be the result of two or more different resistance mechanisms

Cross Resistance: same mechanism of action



Multiple Resistance

Multiple resistance can occur following repeated applications of a single herbicide and selection for herbicide-resistant biotypes followed by repeated applications of another herbicide and selection for herbicide-resistant biotypes.



61. Management strategies for avoiding and managing herbicide resistant weeds

The North Central Weed Science Society (NCWSS) Herbicide Resistance Committee has developed the following list of strategies for avoiding and managing problems with herbicide resistant weed biotypes. Keep in mind that reliance upon any one strategy is not likely to be effective. The crop producer must use the following strategies in carefully selected combinations if herbicide resistant weed problems are to be avoided or properly managed.

Uses

Use herbicides only when necessary. Where available, herbicide applications should be based on economic thresholds. Continued development of effective economic threshold models should be helpful.

Rotate herbicides

Rotate herbicides (sites of action). Do not make more than two consecutive applications of herbicides with the same site of action to the same field unless other effective control practices are also included in the management system. Two consecutive applications could be single annual applications for two years, or two split applications in one year.

Application of herbicides

Apply herbicides in tank-mixed, prepackaged, or sequential mixtures that include multiple sites of action. Both herbicides, however, must have substantial activity against potentially resistant weeds for this strategy to be effective. Remember that in the past, weeds that were selected for herbicide resistance often were not the primary target species. It may be expensive to apply herbicide combinations that duplicate a wide spectrum of weed control activity. Many of the more economical herbicide combinations may not be adequate.

Rotate crops

Rotate crops, particularly those with different life cycles (e.g. winter annuals such as winter wheat, perennials such as alfalfa, summer annuals such as corn or soybeans).

At the same time, remember not to use herbicides with the same site of action in these different crops against the same weed unless other effective control practices are also included in the management system.

Planting new plants

Planting new herbicide resistant crop varieties should not result in more than two consecutive applications of herbicides with the same site of action against the same weed unless other effective control practices are also included in the management system.

Scout fields

Scout fields regularly and identify weeds present. Respond quickly to changes in weed populations to restrict spread of weeds that may have been selected for resistance

Cleaning

Clean tillage and harvest equipment before moving from fields infested with resistant weeds to those that are not.

Encourage

Encourage railroads, public utilities, highway departments and similar organizations that use total vegetation control programs should be encouraged to use vegetation management systems that do not lead to selection of herbicide resistant weeds. Resistant weeds from total vegetation control areas frequently spread to cropland. Chemical companies, state and federal agencies, and farm organizations can all help in this effort.

62. Plant stress and its types

"Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant." OR

"Stress could also be defined as significant deviation of the optimal condition of life."

Types of Stress

There are many factors that limit plants growth, development and productivity, mainly these factors or stresses are of two distinct types termed as:

•Biotic stresses

•A-biotic stresses

A-biotic Stresses

Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way

Types of A-biotic Stresses

There are several A-biotic factors that lead to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity.

Water/ drought stress, Heat stress, Cold stress, Light stress, Wind stress, Salinity stress, Heavy metals

water stresses:

There are two types of water stresses

1. Drought

2. Flooding / Water logging

Drought

In case of drought:

Reduced availability of water for vital cellular function. Reduced Turgor pressure. Osmotic pressure. Stomata closure, reduced carbon dioxide supply and slower the rate of biochemical reactions during prolonged periods of dehydration

Flooding / Water Logging

In case of Flooding / Water Logging the soil become saturated with water & thus the oxygen content decreased in the soil. This also leads to the deficiency of oxygen also in plant cell & tissue. Many physiological functions are disturbed by this oxygen deficiency. Cell lyses & rupturing of cells also accrue due to access of water. FR13A rice-type has high level flooding tolerance.

Temperature Stress

Heat Stress

Cold Stress

Effects of Heat Stress

Dehydration. Yellowing of plants and leaves; chlorophyll deteriorates. Withered leaves, Sunburn; reddish-purple tint or freckles on leaves and pseudo bulbs, leaf tip and root may turn brown. Alteration of gene expression is the major cause of heat stress. Leathery leaves; damage on cellular level. In response to high temperature all organisms, including plants, synthesize a set of proteins called as heat shock proteins (HSPs) The induction of HSPs at permissive

temperatures have been associated with the acquisition of thermo-tolerance to withstand the stress

Effects of Cold Stress

Cold stress decreases membrane fluidity. It alters the lipid composition of membrane. Freezing causes ice to form in a plant cell wall and inter-cellular spaces. Metabolism retarded. Delayed energy dissipation leading to radical formation and oxidative stress. Chilling causes protoplast volume shrinkage upon extra-cellular ice formation. Low temperature limitations have been overcome by the identification of cold-tolerant genes for applications in genetically transformed crops.

63. Plant stress and its types 2

Light Stress

A plant is under light stress when it is unable to quench light energy; it is receiving either by way of photochemical or non-photochemical processes. Light stress leads to photo-inhibition; the reduction in capacity for photosynthesis, inhibition is primarily in photo system-ll reaction centre. High light also decreases Leafy-area, Seed size, Yield.

Plants have evolved protective and response mechanisms against photo- damage. ROS can be utilized as a signaling molecule for response against light stress as well as other A-biotic and biotic stresses.

Wind Stress

About 50% of crop yield in Pakistan is effected by the wind stress.Due to following reasons: Storm & high pressure winds cause the falling of flowers which decrease the crops yield. Increase transpiration rate. Hot wind also have retarding growth effects on plants. Wounds or wilting caused by winds give sites for the virus or bacterial infections.

Salt Stress

Salt stress is one of the major A-biotic stresses. All carbohydrates, fatty acids and protein content were adversely affected due to salinity effect. Salt stress induces the synthesis of abscisic acid which closes stomata when transported to guard cells, therefore, photosynthesis declines and photo inhibition and oxidative stress occurs. Some physiological damages also occur due to salt stress that are

- Water deficiency
- Ion cyto-toxicity
- Osmotic stress

The accumulation of Na+ and Cl- ions in the cells is very toxic in terms that these ions can influence the enzymatic action.

Heavy Metal Stress

- Heavy metals
- Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers
- Cu, Zn, Ni, Co, Cs, Hg, Cr, etc.
- Essentials heavy metals:
- Required for all kinds of plants. Cu – Photosynthesis
- Beneficial heavy metals:
- Required for specific plant groups. Ni – Activation of Enzymes
- Non-Essentials heavy metals:
- Not required & accumulated in plants body due to there weak uptake control mechanism.
- Example: Cd, Hg, Cr etc.
- Source of entrance:
- In terrestrial system metals ions enter in the plant through root hairs & in aquatic system through foliage either cracks on the cuticle or stomata plasmolema.
- Stressed cause by heavy metals:
- Elevated concentration of both essentials & non-essentials heavy metals in the soil can lead to the toxicity symptoms to the plant growth.
- It can also disturb the photosynthetic system.

64. Biotic Stress

Biotic stress is stress that occurs as a result of damage done to an organism by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. The most prominent biotic stress that the plants experience is due to disease outbreaks. Damages lead by insect interference is also gaining a pick position regarding the biotic stress of plants. A plant Disease may be defined as the series of invisible and visible responses of plant cells or tissues to a pathogenic microorganism or an environmental factor that results in adverse changes in form, function & integrity of plants and may lead to partial impairment or death of the plant or its parts

- Plant affected by a disease is known as Host, while the organism that produces the disease is termed as Pathogen.
- Some diseases along with their pathogen concern are undermentioned:

Names of disease	Pathogens
Late blight of potato	*Phytophthora infestens*
Black rust of wheat	*Puccinia graminis f.sp. trtici*
Damping off seedling	*Pythium spp.*
White rust of Crucifers	*Albugo candida*
Citrus cancer	*Xanthomonas campestris pv.citri*
Crown gall of vascular plants	*Agrobacterium tumefacience*
Mosaic of Tomato & Tobacco	*Tobacco mosaic virus*
Yellow vein mosaic of Vindi	*Yellow Vein Mosaic Virus/YVMV*
Papaya mosaic disease	*Papaya Distortion Ringspot Virus*
Ear Cockle of Wheat	*Anguina tritici*

General losses due to disease:

i. Killing of Plants
ii. Killing of brunches
iii. General stunting
iv. Damage to the leaf tissues
v. Damage to the reproductive organ including fruits and seeds.

Besides the pathogenic attacks, insects also lead certain major damages into economically important plants.

eg : Cotton is attacked by more than 160 species of harmful insects.

Viruses

Viruses are submicroscopic entities capable of causing disease. They are a piece of nucleic acid (genetic material) surrounded by a protein coat. Plant viruses are made up of two

components – a protein coat and the nucleic acid center. The nucleic acid is the infectious component of a virus. Viruses are obligate parasites, meaning that they must be within living tissue before they can reproduce themselves. In general, viruses are seldom lethal to plants, but do severely affect the host both in quantity, quality and longevity. Symptoms may often be very characteristic for a specific virus on a specific host. Symptoms along with other criteria are used to identify virus diseases. An advanced array of symptoms can be recognized today as expressions of viral diseases in plants.

Symptoms

Symptoms vary with the virus involved, the species of plant infected, and the environmental conditions. In some cases, such as virus disease of geraniums, certain environmental conditions bring out symptoms while other conditions mask or hide symptoms.

Symptoms associated with virus infection are reduced growth resulting in stunting, mosaic pattern of light and dark green (or yellow and green) on the leaves, malformation of leaves or growing points, yellow streaking of leaves (especially monocots) yellow spotting on leaves, ring-spots or line patterns on leaves, cup-shaped leaves, uniform yellowing, bronzing, or reddening of foliage, flower color breaking, distinct yellowing only of veins, crinkling or curling of margins of leaves. Some of these would include abnormal leaf color, abnormal vein patterns of leaves, mottling in leaves

Figure 1. Upper leaves (rose mosaic virus) compared with iron chlorosis in lower leaves. Courtesy Thomas Lee, Texas Agricultural Extension Service - 1995.

Figure 2. St. Augustine Decline and Sugarcane Mosaic virus infected St. Augustine grass.

Courtesy Thomas Lee, Texas Agricultural Extension Service - 1995.

- spotting patterns in leaves, and abnormal leaf shape
- There are also abnormalities of flower color, fruit size, shape and color

Spread

Viruses can be spread from plant to plant by several means. Some of these would include transmission from the parent plant to an offspring through the genetic structure of the plants. Other ways in which viruses can be transmitted are through vegetative propagation, grafting and budding, seed transmission and mechanical spread by insects and man.

65. Biotic stress II

Bacteria

Bacteria are amongst the microbes which benefit as well as harm the plants. Pathogenic bacteria which belong to the genera like Xanthomonas, Erwinia, etc. are responsible for most of the diseases caused to plants. The bacterial diseases in plants are of concern to the farmers. This is because reduction in the yield of crops affect the entire economy based on agriculture.. There are many different types of diseases caused by bacteria in plants. The blights, leaf spots and other such diseases affect growth of plants.

Blight Disease

This disease is marked by the chlorosis of different plant tissues. Flowers, leaves, and twigs of the affected plants produce less amount of chlorophyll and thereafter browning takes place. The blight disease can also cause death of plants. Fire blight is one such disease found in pear and apple plants; Erwinia amylovora is the bacteria that causes fire blight. In this disease, the affected areas of plants become sunken and appear to be scorched; the surface of these plants blackens; cracks are also observed on the surface. To prevent the fire blight disease from spreading further, chemicals like terramycin can be used.

Brown Spot Disease

The causal organism of this disease is the bacterium called *Pseudomonas synringae*.

Signs observed in this disease include the following: gray spots, cracking of leaves, brownish yellow leaf borders, etc.

Hot and humid climate is favorable for the development of this disease. A white colored substance oozes out of the affected plants and spreads on the surface of fruits. Reduction in crop yield is observed in the brown sport. This disease mainly affects vegetable and ornamental plants

Crown Gall Disease

This disease is soil-borne and affects a variety of plants including roses, apples, cherries, raspberries, etc.

The disease is named so because of the galls (abnormal outgrowths) formed at crowns (i.e. the place where stem and roots intersect) of these plants. *Agrobacterium tumefaciens* is the causal organism of this disease. The gall disease is treated or controlled with the use of both chemical and biological methods. A chemical called Gallex can be painted on galls for the purpose of treatment. The biological method of control is that of inoculating the cuttings and new transplants with bacteria which compete for growth with the pathogenic ones. This method automatically reduces the number of disease causing bacteria.

66. Biotic stress III

Fungi

Fungi are one of the living organisms that can cause plant disease and are the cause of about eighty-five percent of all plant diseases. More than 100,000 species of fungi have been classified and include molds, mildews, and mushrooms. Most are beneficial or benign, with only about eight percent of fungal species causing plant diseases. Unlike plants, fungi do not have chlorophyll and cannot photosynthesize. Instead they must rely on other living things for sustenance.

Fungal Diseases of Plants

BROWN PATCH

It is a turfgrass disease caused by different species of the Rhizoctonia fungus. Patches of brown and yellow color appear on the lawn in irregular shapes. It does not affect the roots and crown of the grass, so it is also known as Foliar disease.

CANKER

- There are some fungal infections that affect the roots and barks of the plants. One such fungi is canker fungi. It is found on woody trees and is notorious for causing localized damage to the barks of trees.

CLEMATIS WILT

It is caused by a fungus (Phoma clematidina), which makes an entry into the plant body through cuts and wounds created by insects. Generally, it appears on large-flowered hybrid plants in brown- and black-colored patches.

DOWNY MILDEW

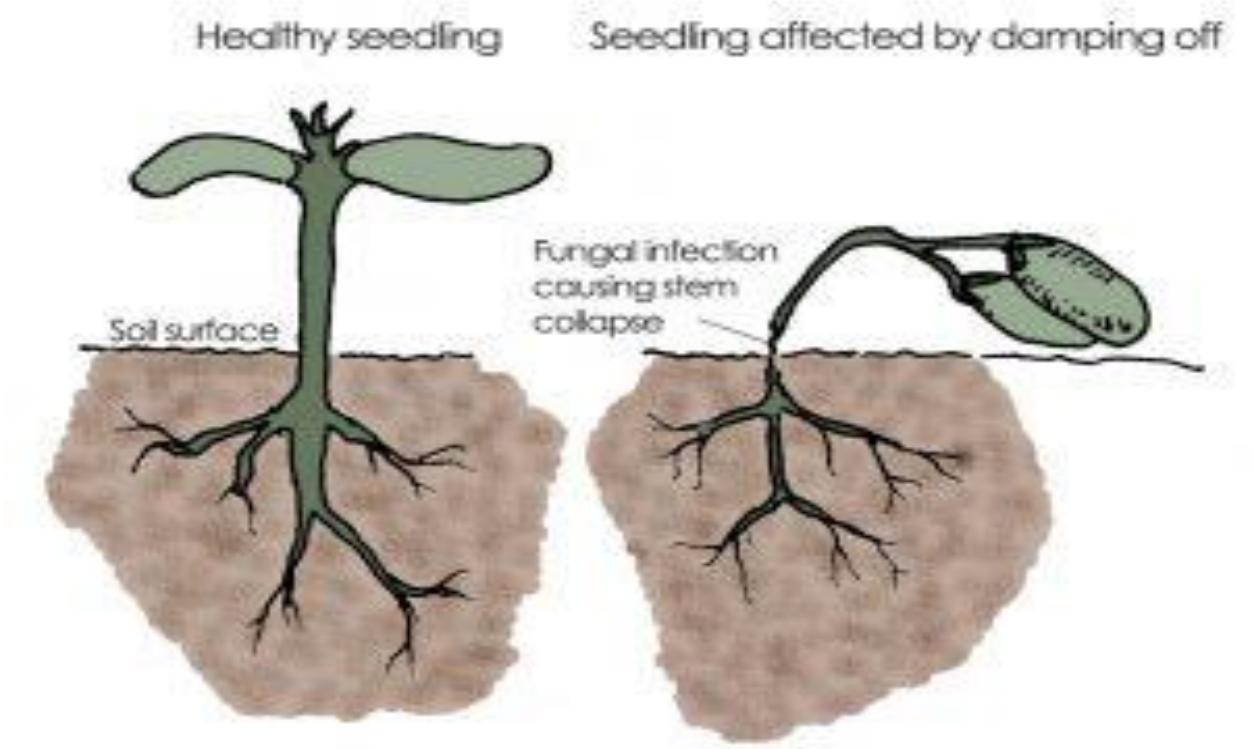
This disease is caused by Peronosporaceae which affects a number of plants. It can be identified when discolored blotches appear on the leaves; a mold-like growth also develops on the plants. It affects the growth and strength of the plant.

CLUB ROOT

This infection is found in cabbages, turnips, and radishes which is caused by the Plasmodiophora brassicae fungus. The color of the leaves changes to bluish-green, and the roots if pulled out appear clubbed and are easily broken.

DAMPING OFF

This is a disease caused by different fungi -- Pythium and Phytophthora being some of them. It infects the seed and decays it, thus affecting the growth and vigor of the whole plant.



Fungal disease	Factors conducive to spread	Crops affected	Symptoms
White blister/White rust (*Albugo candida*)	Optimum conditions for disease development are 3-4 hours in mild temperatures (6- 24?C).	Brassicas (including Asian leafy brassicas).	White blisters and swellings on leaves and heads of affected plants; blisters consist of masses of white dust-like spores; up to 100% losses have been reported.
Downy mildews (individual species damage particular crop families)	High humidity, leaf wetness and cool to mild temperatures (10-16 °C).	Wide host range including onions; peas; lettuce; celery; spinach; kale; herbs; cucurbits; brassicas; Asian leafy brassicas.	Symptoms usually begin with yellowish leaf spots which then turn brown; downy growth appears on underside of leaves.
Powdery mildews (some species are restricted to particular crops or crop families)	Moderate temperatures (20-25?C); relatively dry conditions (unlike downy mildews).	Wide host range and very common, especially in greenhouse crops: cucumber; melons; pumpkin; zucchini;parsnip; beetroot; potato; herbs; peas; bitter melon;tomato; capsicum; Brussels sprouts; cabbage; swedes.	Small, white, powdery patches on most above-ground surfaces; usually observed first on undersides of leaves but eventually cover both surfaces; affected leaves become yellow, then brown and papery and die.

Clubroot (*Plasmodiophora brassicae*)	Warm weather; acidic soil (pH less than 7); high soil moisture.	Brassicas (including Asian leafy brassicas).	Plants are yellow and stunted and may wilt in hotter parts of the day; large malformed 'clubbed' roots which prevent the uptake of water and nutrients, reducing the potential yield of the crop.
Pythium species	Cold, wet soil conditions; known as water moulds, they enter untreated water supplies; water supplies for irrigation and hydroponics should be tested regularly.	Many vegetable crops in including cucurbits; brassicas; lettuce.	May kill seedlings, which die before they emerge or soon after emergence; plant collapse.
Sclerotinia rots (*S. sclerotiorum*and *S. minor*) – a range of common names are used	Windy, cool, humid weather; wet soil; survival structures known as sclerotia remain viable in soil for long periods (10-15 years).	Most vegetable crops.	Water-soaked rotting of stems, leaves, and sometimes fruit; followed by a fluffy, white and cottony fungal growth which contain hard black pebble-like sclerotia.

Sclerotium rots (*Sclerotium rolfsii* and *S. cepivorum*)	*S. rolfsii* – Warm, moist conditions. *S. cepivorum* – Development is favoured by cool soil conditions (14-19?C) and low moisture.	*S. rolfsii* – Wide host range including: beans; beets; carrot; potato; tomato; capsicum; cucurbits.*S. cepivorum* – only affects onions, garlic and related Alliums (shallots; spring onions; leeks).	*S. rolfsii* – Lower stem and root rots; coarse threads of white fungal growth surround the diseased areas; small brown fungal resting bodies. *S. cepivorum*– Yellowing and wilting; fluffy fungal growth containing black sclerotia forms at the bases of bulbs.
Fusarium wilts and rots (Various Fusarium species including *F. solani* and *F. oxysporum*)	Warm to hot weather.	Wide host range including: brassicas; carrots; cucurbits;onions; spring onions; potato; tomato; herbs; peas; beans.	Causes severe root and crown rots or wilt diseases by attacking roots and basal stems; cucurbit fruit and potato tubers can be affected in storage.
Botrytis rots – for example Grey mould (*Botrytis cinerea*)	Cool, wet weather.	Celery; lettuce; beans; brassicas; cucumber; capsicum; tomato.	Softening of plant tissues in the presence of grey fungal growth.
Anthracnose (*Colletotrichum spp.* except for in lettuce – *Microdochium panattonianum*)	Cool, wet conditions.	Wide range of crops including: lettuce; celery; beans; cucurbits; tomato, capsicum; potato; globe artichoke.	Typical symptoms begin with sunken and water-soaked spots appearing on leaves, stems and/or fruit.

Rhizoctonia rots (*Rhizoctonia solani*) – range of common names, e.g. Bottom rot (lettuce) and Wire stem (Brassicas)	Warm, humid weather; can survive for long periods in the soil in the absence of a host plant.	Wide host range including: lettuce; potato; brassicas;beans; peas; beets; carrots; capsicum; tomato; cucurbits.	Range of symptoms depending on the crop being grown but can affect roots, leaves, stems, tubers and fruit; plants wilt and may collapse and die.
Damping-off (Pythium, Rhizoctonia, Phytophthora, Fusarium or Aphanomyces)	Occurs under cold, wet soil conditions; shore flies and fungus gnats can spread Pythium and Fusarium.	Many vegetable crops including: leafy vegetables;brassicas; carrots; beetroot; cucurbits, eggplant; tomato;coriander; spring onions; beans	Young seedlings have necrotic stems or roots; seedlings die or show a reduction in growth.
Cavity spot (Pythium sulcatum)	Growing carrots after carrots; acidic soil; not harvesting carrots as soon as they reach marketable size.	Carrots.	Cavity spots are small elliptical lesions often surrounded by a yellow halo.

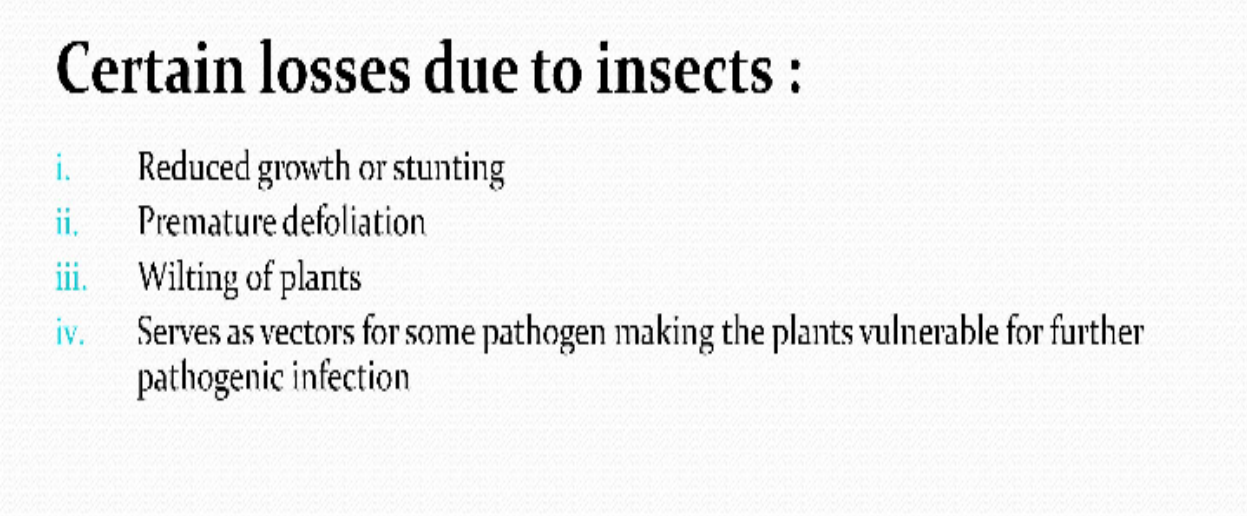
Tuber diseases (Various species)		Potato and sweetpotato.	Potato tubers may be infected with superficial skin diseases, such as common scabs, powdery scab, and Rhizoctonia. Sweetpotatoes may be infected by scurf.
Rusts (several species, e.g. *Puccinia sorghi*– sweet corn; *Uromyces appendiculatus*– beans; *Puccinia allii* – spring onions).	Wind can spread spores great distances; favoured by low rainfall, 100% relative humidity and cool to mild temperatures.	Sweet corn; beans; onions; spring onions; beets; celery; silverbeet; endive.	Small, red or reddish-brown pustules that form on the underside of the leaves and sometimes on the pods as well; dusty reddish-brown spores released from pustules (may be black in cold weather).
Black root rot (Different species on different vegetable crops)	Cool soil temperatures; high soil moisture.	Lettuce; beans; cucurbits.	Blackening of roots; stunted plants; plants may die.

Insects

Some insects can cause serious damage if left untreated. Boring insects, such as the clearwing moth, ambrosia beetle and other species of beetles, bore into trees to construct galleries for reproduction. Although one may only see minor amounts of sawdust and frass coming from the trunk of a tree, inside the tree these insects may be doing irreversible damage.

Beetle galleries can be very extensive. The damage is caused because these beetles bore into the phloem layer of the tree, through the cambium, and into the xylem.

When this happens the tree is effectively girdled, usually dies and may fail catastrophically. Sucking insects such as aphids, scale and whitefly, penetrate leaf surfaces with their mouthparts to feed on phloem. Problems arise if disease or virus is vectored.



67. Physiological alteration in host plants due to biotic stress

Water and mineral absorption and translocation

Plant Pathogen such as fungi that cause Damping off and root rot diseases, bacteria, most Nematodes and some viruses extensively damage roots, which directly affects the functioning of roots resulting in reduced absorption of water and minerals from the soil. Water & mineral absorption is also reduced by some vascular pathogen that inhibit root hair formation.

Physical blockage in xylem vessels lead to prevention water & mineral translocation . Eg. Agrobacterium tumefaciens [Crown gall disease] Plasmodiophora brassicae [Clubroot disaese]

Transpiration

Rate of transpiration increases in plants after certain disaese infection [leaf rust, Apple scab, Downy Mildew]. This is because, these disaeses result in destrucion of protection devices in plants like cuticle & epidermis, increase permeability of leaf cells and lead to the dysfunction of stomata resulting in uncontrolled loss of water and loss of turgor to which wilting of leaves follows.

Photosynthesis

Photosynthesis is tremendously decrease after pathogenic infection. This is carried out by Reduction in photosynthetic area :

1.Plant diseases

Plant diseases that attack green aerial tissues [like leaf spot, blights] render harmful effects by destructing photosynthetic leaf tissue or defoliation, which result in lessening the photosynthetic

area thereby reducing the photosynthesis. eg. Complete defoliation of potato leaves after infected by blight disaese

ii. Reduction in Chlorophyll content

Chlorosis is one of the most common symptoms of plant disaese and refers to the destruction or inhibition of chlorophyll per chloroplast subsequent to infection and usually occurs in young growing leaves. ' It is resulted by viral infection. Eg. Tobacco mosaic disaese, vein chlorosis of Vindi etc. It is carried out by the enzyme chlorophyllase secreted after disaese infection in leaves that causes conversion of chlorophyll to chlorophyllide and phytol.

iii. Effect of Toxins

In some fungal and bacterial disaeses Photosynthesis is reduced because of toxins such as tabtoxins and tentoxin secreted by the pathogen itself. These toxin inhibit some of the enzymes that are directly involved in photosynthesis. Tabtoxin is not so toxic but when it hydrolyzed in the host cell, it releases Tabtoxinine which is the active toxin molecule.

iv. Reduction in the activity of Calvin cycle enzyme

After disaese infection the activity of some key enzymes of Calvin cycle is progressively reduced due to changes in the concentration of soluble carbohydrates in infected tissues which in turn effects photosynthetic fixation of carbon dioxide. Arabidopsis, leaves infected by the biotrophic fungus Albugo candida ,the reduction in the rate of photosynthesis is parallel to the decrease in the amount of Rubisco enzyme present in the host tissue.

68. Physiological alteration in host plants due to biotic stress II

Respiration

The rate of respiration generally increases in diseased plants , because the affected tissues consume their reserve carbohydrates faster than the healthy tissue would and it continues rising during the multiplication and sporulation of the pathogen.

The mechanism of respiratory increase in diseased plants is described by four theories.

They are — i. Uncoupling of oxidative phosphorylation.

ii. Augmentation in biosynthesis pathways.

iii. Changes in respiratory pathways.

iv. Increased activity of terminal oxidation systems.

Uncoupling of oxidative phosphorylation

The rate of respiration increase in diseased plants is brought about by uncoupling electron transfer from oxidative phosphorylation of ADP to generate ATP.

Certain chemical agents such as 2,4-dinitrophenol get involved in this case .

As a result no utilizable energy in the form of ATP is produced through normal respiration .

ll. Augmentation of biosynthetic pathways

It is the most satisfactory explanation regarding the increased respiration rate in diseased plants.

According to this theory respiration is increased due to increased metabolism and augmentation of biosynthetic pathways, in other words there is a general enhancement in the biosynthetic activities of the host during disaese infection.

This increase in turn utilizes more ATP rapidly and respiration rate increases.

Changes in respiratory pathways

The major metabolic pathway of respiration, the "glycolytic pathway" is replaced by an alternative route called pentose Phosphate Pathway" during disaese infection or stressed condition.

This alteration of cycles produces less ATP. So to generate adequate ATP, respiration rate get increased. Alteration in the operation of Tricarboxylic Acid cycle [TCA] have also been reported . eg. Rust and Powdery mildew of Wheat.

Increased activity of terminal oxidation system

Apart from the usual cytochrome system terminating with cytochrome oxidase, alternative terminal oxidation systems involving phenol oxidases and ascorbic acid oxidase operate in plants that enhances the activity of the terminal oxidation system subsequently.

eg. Increased activity of phenol oxidases have been shown in disaeses caused by phytophthora and Fusarium species

Nuc1eic Acid metabolism

Marked increase in nucleic acid synthesis has been noted in some fungal, bacterial diseases especially those characterized by outgrowth and gall formation. eg. In club root disease of crucifers, DNA content is 16 folds greater in infected cells in comparison to healthy one.

Protein Metabolism

The alterations of protein metabolism in diseased plant tissue and concluded that the total content of the protein normally increases during early stages of development. Synthesis of some novel proteins have been reported during infection in resistant plant varieties called pathogenesis-related protein [PR proteins], usually associated with host defense.

69. Plant response against biotic stresses

Plants exhibit certain physical and chemical defense response against biotic stresses .

They are as follows

Physical response

1.Cork layer formation:

Formation of cork layer takes place after infection of certain disease [scab of potato, soft root of sweet potato] in the host plant below the point of infection.

This happens due to stimulation of the host cells by certain chemicals secreted by the pathogen.

It prevents further spread of the pathogen and block the inward flow of toxic substances.

2.Tyloses formation

Tyloses are outgrowths of the protoplast of adjacent parenchymatous cells protruded into xylem vessels. Tyloses are formed in response to the pathogen attacks in many plants and prevent them from being infected. eg. Tyloses formation in certain varieties of sweet potatoes protects themselves from being infected by wilt disaese caused by Fusarium oxysporum.

3.Swelling of the cell wall

Swelling of the outer wall of epidermal and sub epidermal cells takes place as defense response in certain plant cells when they come in contact with pathogenic hyphae. Swelling inhibit further pathogen penetration within the host.Swelling device has been reported in pea leaves after attacked by Botrytis cinerea.

4.Hypersensitive response:

This type of defense very commonly occurs in disaeses caused by biotrophic fungal parasites, viruses and nematodes.

Challenged host cells and those in their immediate vicinity die rapidly [react hypersensitivity] and this necrotic behavior is associated with defense of the plant as a whole.

eg. Hypersensitivity is exemplified in Wart of potato [Synchytrium endobioticum] and Blast of rise [Pyricularia oryzae]

5.Nuritional response

The reduction in growth and spore production of pathogen is generally supposed to be due to unfavorable physiological conditions within the host.

Actually a resistant host doesn't fulfill nutritional requirements of the pathogens, thus inhibit their growth and reproduction.

Chemical Response

1.Phynolic Compounds :

These are the main inhibitory compounds synthesized by the plant cells in response to the infection or injury, which retard the growth of pathogens.

Eg. Chlorogenic acid, Caffeic acid, umbelliferon etc. Phenolics are consist of one or more benzene ring with one or more hydroxyl groups.

Some phenolics are pre-exist in healthy plants but their synthesis or accumulation is accelerated when gets infected by a pathogen, eg Chlorogenic acid is present in sweet potato ,white potato and carrot but its synthesis or accumulation accelerated when get infected by the fungus.

2. Cross-linking of Cell Wall Proteins

This defense device developed very rapidly in response to pathogen attacks.

They have been linked to a self sealing car tyre and thought to make the host cell wall more defensive against pathogen penetration.

Extensin has been considered such a cell wall protein which is subjected to cross linking.

3. Phytoalexins

These are Phenolic or non phenolic compounds which don't pre exist in healthy plants but synthesized de novo in response to injury, physiological stimuli, infectious agents or their products.

These are low molecular weight lipophilic compounds.

Examples : Piasins . reported to be produced in the exposed endocarp of

i) detached pea pods in response to inoculation of many fungi. [Monilinia fimbricata, Ascochyta pisi].

Ii) Phaseolin : Detected in bean leaves incubated with the bacterium Pseudomonas phaseolicola

Medicarpin :

Antifungal phytoalexins isolated from the diffusate solutions of leaves when inoculated with pathogenic fungi such as Colletotrichum phomoides, Helminthosporium turcicum.

It is commonly occurs in legumes.

70. Plant Response to Biotic Stress

Pathogen attack strategies

Necrotrophy, in which the plant cells are killed

Biotrophy, in which the plant cells remain alive

Hemibiotrophy, in which the pathogen initially keeps cells alive but kills them at later stages of infection

Failure of a pathogen to cause disease

The plant species attacked is unable to support the life-strategy of the particular pathogen

The plant possesses preformed structural barriers or toxic compounds. Defence mechanisms are activated such that the invasion remains localized. Environmental conditions change and the pathogen perish

Successful pathogen infection & disease occurs:

Only if the environmental conditions are favorable. If the preformed plant disease defenses are inadequate. If the plant fail to detect the pathogen

If activated defense responses are ineffective

Preformed defense: Secondary metabolites

Plants possess different secondary metabolites with antimicrobial properties may be present in their biological active form or may be store as inactive precursors that are converted to their active forms by host enzymes in response to pathogen attack or tissue damage

Secondary metabolites

pre-formed inhibitors are the **saponins** and the **glucosinolates**

Saponins

Glycosylated compounds, classified as either triterpinoids, steroids, or steroidal glycoalkaloids.

A biologically active triterpinoid saponin found in the roots of oat plants, **avenacin A-1**, is highly effective against the root infecting Takeall fungus, a major pathogen of cereal roots

This pathogen affects wheat and barley, but not oat plants

Hypersensitive response

1st line of activated defence, occurs within 24hr

Recognition of a genetically incompatible pathogen

Creates unfavourable conditions for pathogen growth and reproduction

Impair the spread of harmful enzymes & toxins

Leads to localised cell and tissue death

Reactive oxygen species (ROS)

the production of ROS is often the first response detected, occurring within 5 min

superoxide and hydrogen peroxide (H_2O_2).

The mechanism plants have for producing superoxide from molecular oxygen probably involves a plasma membrane-associated NADPH oxidase

Role of ROS in plant defense

H_2O_2 maybe directly toxic to pathogens

may contribute to the structural reinforcement of plant cell walls, either by cross-linking various hydroxyproline and proline rich glycoprotein to the polysaccharide matrix or by increasing the rate of lignin polymer formation by way of peroxidase enzyme activity

make the plant cell wall more resistant to microbial perpetration and enzymatic degradation

Role of ROS in cell signaling

H_2O_2 induces benzoic acid 2 hydrolase (BA 2- H) enzyme activity, which is required for biosynthesis of SA

H_2O_2 is known to induce genes for proteins involved in certain cell protection mechanisms e.g. glutathione *S*-transferase

71. Plant Response to Biotic Stress

Nitric oxide synthesis (NO)

In plants, rapid *de novo* synthesis of NO accompany the recognition of avirulent pathogenic bacteria. NO has the capacity to potentiate induction of plant cell death by ROS

NO is known to bind heme and could inhibit catalase and ascorbate peroxidase, which detoxifies H2O2

Nitric oxide

In the presence of inhibitors of NO production, the HR diminishes, disease symptoms become more severe, and bacterial growth is increased

NO and ROS play an important synergistic role in the rapid activation of a wide repertoire of defence responses after pathogen attack

Benzoic acid and salicylic acid

Both SA and BA are derived from the phenylpropanoid pathway and have many roles in plant defense responses

Accumulate to high concentrations in the vicinity of incompatible infection sites

Jasmonic acid and Ethylene

Jasmonic acid (JA) is an oxylipin-like hormone derived from oxygenated linolenic acid

Increases in JA in response to pathogen/insect attack occur both locally and systematically

Spraying methyl-JA onto plants increases their resistance to some (but not all) necrotrophic fungi, but not to biotrophic fungi or bacteria

Jasmonic acid and ethylene

Ethylene is frequently synthesised during both incompatible and compatible interactions

Ethylene is required to mediate both resistance against necrotrophic fungal pathogens and against soil borne fungal species that are not ordinarily plant pathogens

Ethylene and JA are required for activation of proteinase inhibitor (PI) genes and certain PR and chitinase genes

Pathogenesis-related (PR) proteins

- fungal cell wall-degrading enzymes
- chitinases
- glucanases

- lipoxygenase
- anti-microbial polypeptides
- components of signal transduction cascades
- **PR proteins**

SA-mediated signal transduction cascades regulate the transcriptional activation of many PR genes

Ethylene and SA have been shown to act synergistically, further enhancing the expression of PR genes.

71. Biotic and abiotic stress resistance

Stress

External conditions that adversely affect growth, development, or productivity. When some factors of the environment interfere with the expression of genotypic potential Stresses trigger a wide range of plant responses: altered gene expression, cellular metabolism, changes in growth rates and crop yields

Types of Stress

Biotic - imposed by other organisms

Abiotic - arising from an excess or deficit in the physical or chemical environment

Biotic and abiotic stresses can reduce average plant productivity by 65% to 87%, depending on the crop

ABIOTIC STRESSES

Environmental, non-biological

Temperature (high / low)

Water (high / low)

Salt

Radiation

Chemical

BIOTIC STRESSES

Caused by living organisms

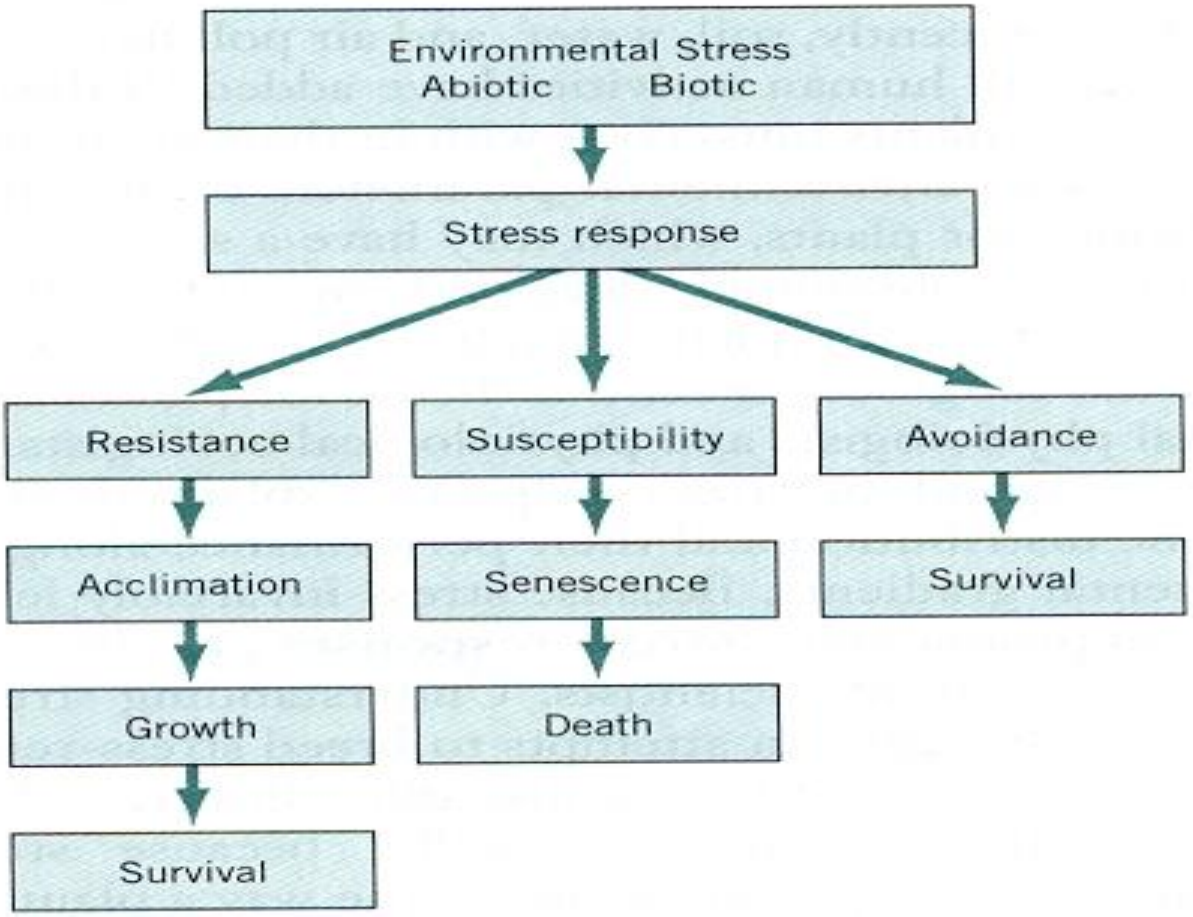
Fungi

Bacteria

Insects

Herbivores

Other plants/competition



Plants are sessile and must deal with stresses in place

Plants cannot avoid stress after germination

How plants deal with stress has implications in

Ecology: Stress responses help explain geographic distribution of species

Crop science: Stress affects productivity

Physiology and biochemistry: Stress affects the metabolism of plants and results in changes in gene expression

Environmental conditions that can cause stress

- water-logging
- drought
- high or low temperatures
- excessive soil salinity
- inadequate mineral in the soil
- too much or too little light

Productivity losses due to stress

- Loss due to diseases range from 20 to 30 %, in case of severe infection, total crop may be lost.
- Estimated global loss due to insect pests in potential yields of all crops is ~14%.
- Losses due to insect pests ranges from 10 to 20 %
- Abiotic stresses reduce average yield of crops by upto50% .
- Most of the agricultural area is rain fed and crops in these areas invariably experience droughts of different magnitudes.

Annually about 42% of the crop productivity is lost owing to various abiotic stress factors.

73. Plants must be stress resistant to survive

stress avoidance

In the whole growth process does not meet with the face of adversity

stress tolerance

Plant has a capacity of environmental stress defense, and a variety of physiological processes remain normal

Resistance or sensitivity of plants to stress depends on:

- the species
- the genotype
- development age

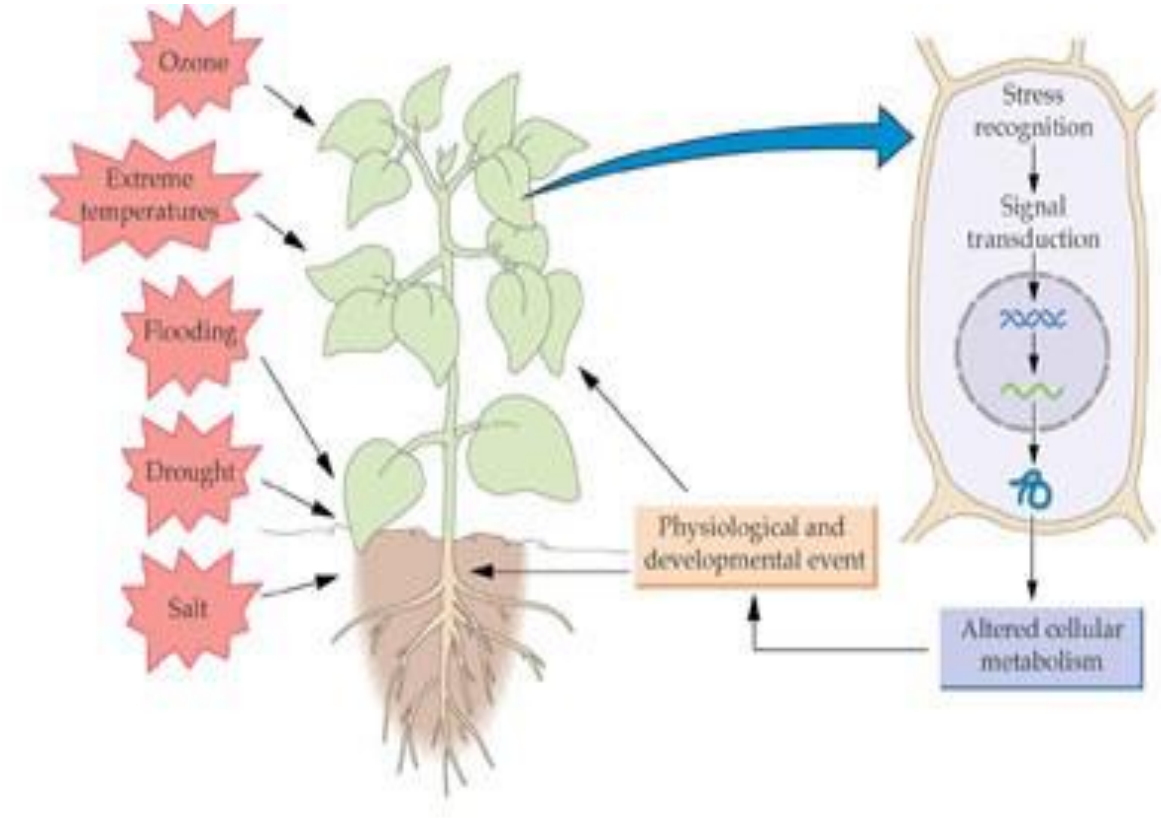
Plants may become stress tolerant through

- Adaptation: heritable modifications to increase fitness
    - CAM plants' morphological and physiological adaptations to low H2O environment
- Acclimation: non heritable physiological and biochemical gene expression
    - Cold hardening induced by gradual exposure to chilling temps, e.g. cold-hardy plants
- Avoidance also possible by morphological adaptations
    - Deep tap roots in alfalfa allow growth in arid conditions
    - Desert CAM plants store H2O in fleshy photosynthetic stems

- Stress resistant plants can tolerate a particular stress
    - Resurrection plants (ferns) can tolerate dessication of protoplasm to <7% H_2O à can rehydrate dried leave

Changes in gene expression to stress

- A stress response is initiated when plants recognizes stress at the cellular level.
- Stress recognition activates signal transduction pathways that transmit information within the individual cell and throughout the plant.
- Changes in gene expression may modify growth and development and even influence reproductive capabilities.



ABIOTIC STRESS: Temperature

- Plants exhibit a wide range of T(opt) (optimum temperature) for growth
- We know this is because their enzymes have evolved for optimum activity at a particular T
- Properly acclimated plants can survive overwintering at extremely low Ts
- Environmental conditions frequently oscillate outside ideal T range
- Deserts and high altitudes: hot days, cold nights

- Three types of temperature stress affect plant growth

Suboptimal growth Temperatures result in suboptimal plant development

Chilling injury

- Common in plants native to warm habitats
    - Peas, beans, maize, Solanaceae
- Affects
    - seedling growth and reproduction
    - multiple metabolic pathways and physiological processes
        - Cytoplasmic streaming
        - Reduced respiration, photosynthesis, protein synthesis
        - Patterns of protein expression
- Initial metabolic change precipitating metabolic shifts thought to be alteration of **physical state** of cellular membranes
- Temperature changes **lipid** and thus **membrane** properties
- Chilling sensitive plants have more saturated FAs in membranes: these congeal at low temperature (like butter!)
- Liquid crystalline → gel transition occurs abruptly at **transition temperature**

74. Regulation of plant stress responses

Stresses

External conditions that adversely affect growth, development, or productivity Stresses trigger a wide range of plant responses:

- altered gene expression
- cellular metabolism

changes in growth rates and crop yields

Types of Stress

- Biotic

- imposed by other organisms

- Abiotic

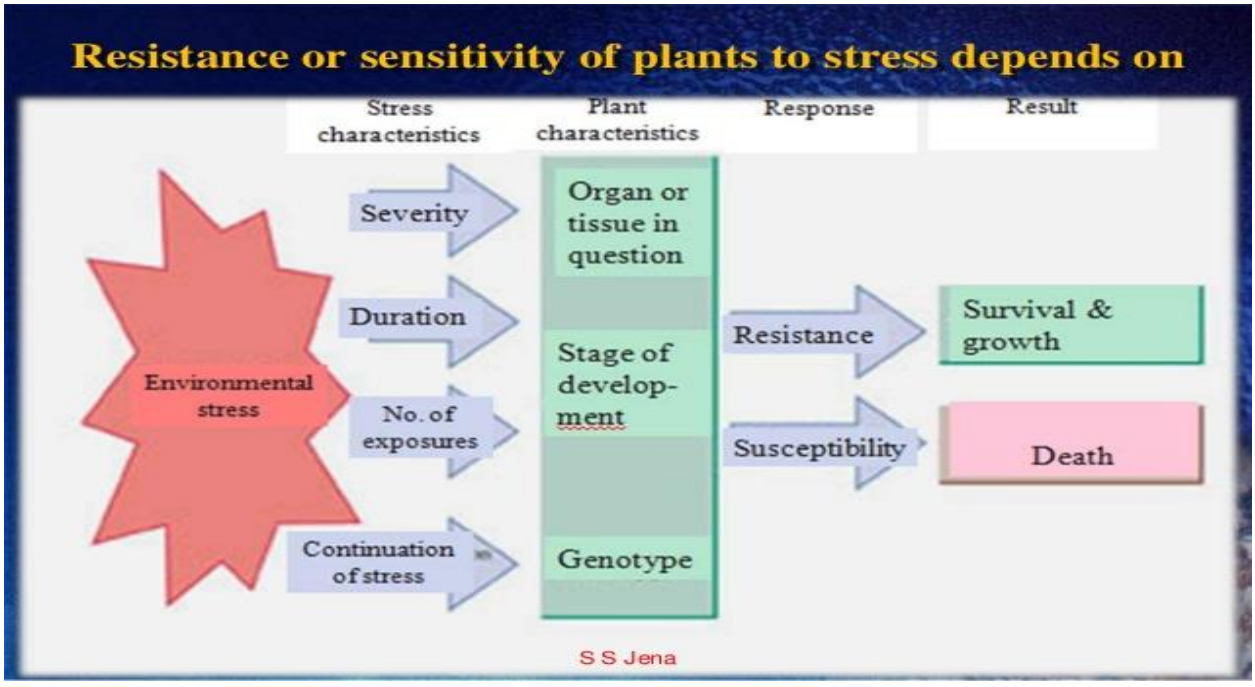
arising from an excess or deficit in the physical or chemical environment

Biotic and abiotic stresses can reduce average plant productivity by 65% to 87%, depending on the crop

Plant Response to Abiotic Stress

- arising from an excess or deficit in the physical or chemical environment
- Environmental conditions that can cause stress
- water-logging
- drought
- lihigh or low temperatures
- excessive soil salinity
- inadequate mineral in the soil
- too much or too little light
- Plant resistance and stress
- Resistance or sensitivity of plants to stress depends on
- the species
- the genotype
- development age

How plants respond to environmental stress



- Stress resistance mechanisms
- Avoidance mechanisms
- prevents exposure to stress
- Tolerance mechanisms

- permit the plant to withstand stress
- Acclimation
- alter their physiology in response stress
- Changes in gene expression to stress
- A stress response is initiated when plants recognizes stress at the cellular level
- Stress recognition activates signal transduction pathways that transmit information within the individual cell and throughout the plant
- Changes in gene expression may modify growth and development and even influence reproductive capabilities

Gene expression results in

- Increase amounts of specific mRNA
- Enhance translation
- Stabilize proteins
- Altered protein activity
- A combination of the above

75. Oxidative Stress in plants

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.

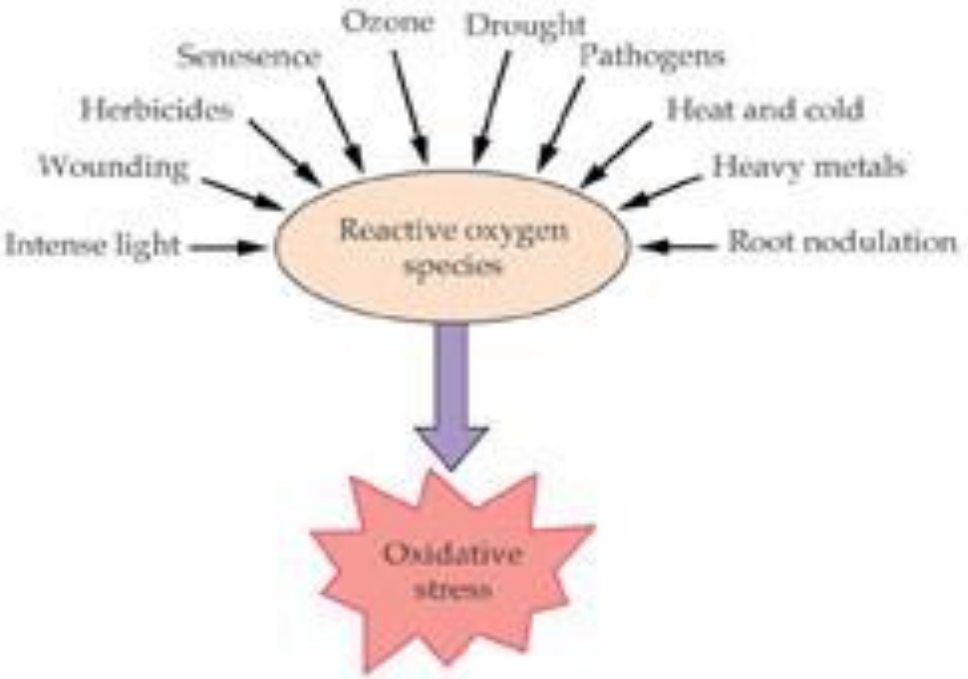
Oxidative stress results from conditions promoting the formation of active oxygen species that damage or kill cells

Environmental factors that causs oxidative stress

- air pollution (increased amounts of ozone or sulfur
- dioxide)
- oxidant forming herbicides e.g. paraquat dichloride
- heavy metals
- drought
- heat and cold stress
- wounding
- UV light

intense light that stimulate **photoinhibition**

Oxidative stress



Reactive oxygen species (ROS)

Formed during certain redox reactions and during incomplete reduction of oxygen or oxidation of water by the mitochondrial or chloroplast electron transfer chain

Singlet oxygen, hydrogen peroxide, superoxide anion, hydroxyl and perhydroxyl radicals

Ozone and oxidative stress

Hydrocarbons and oxides of nitrogen (NO, NO2) and sulfur (SOx) react with solar UV radiation to generate ozone (O3).

Ozone is a highly reactive oxidant.

The negative effects of ozone on plants

- decreased rates of photosynthesis
- leaf injury
- reduced growth of shoots and roots
- accelerated senescence
- reduced crop yield

Ozone Damage

- alters ion transport
- increases membrane permeability

- inhibits H+-pump activity
- collapses membrane potential
- increases Ca2+ uptake from the apoplasm
- Oxidative damage to biomolecules

Resistance to ozone

Utilizes either **avoidance** or **tolerance**

Avoidance involves physically excluding the pollutant by closing the stomata, the principal site at which ozone enters the plant

Tolerance - biochemical responses that induce or activate the antioxidant defence system and possibly also various repair mechanisms

Tolerance to oxidative stress

Stress conditions, antioxidants and antioxidant enzymes

Antioxidant or antioxidant enzyme	**Stress condition**
Anionic peroxidases	Chilling, high CO2
Ascorbate peroxidase	Drought, high CO2, high light Intensity, ozone, paraquat
Catalase	Chilling
Glutathione	Chilling drought, irradiation, heat stress, high CO2, ozone, SO2
Glutatione reductase	Chilling, drought, high CO2, ozone, paraquat
Polyamines	Deficiency of K, P, Ca, Mg, Mn, S, or B; drought, heat, ozone
Superoxide dismutase	Chilling, high CO2, high light, increased O2, ozone, paraquat,

Salicylic acid and ethylene

Ozone exposure results in increased amounts of H2O2, which stimulate the production of SA

Results in a transient increase in the number of transcripts that encode defence-related secondary metabolites e.g. phytoalexins, cellular barrier molecules e.g. lignins, callose, and

extensins, PR proteins e.g. glucanase, chitinase, gluthatione S-transferase and phenylalanine ammonia lyase

Increases ethylene production by inducing increases in ACC synthase and ACC oxidase gene transcription

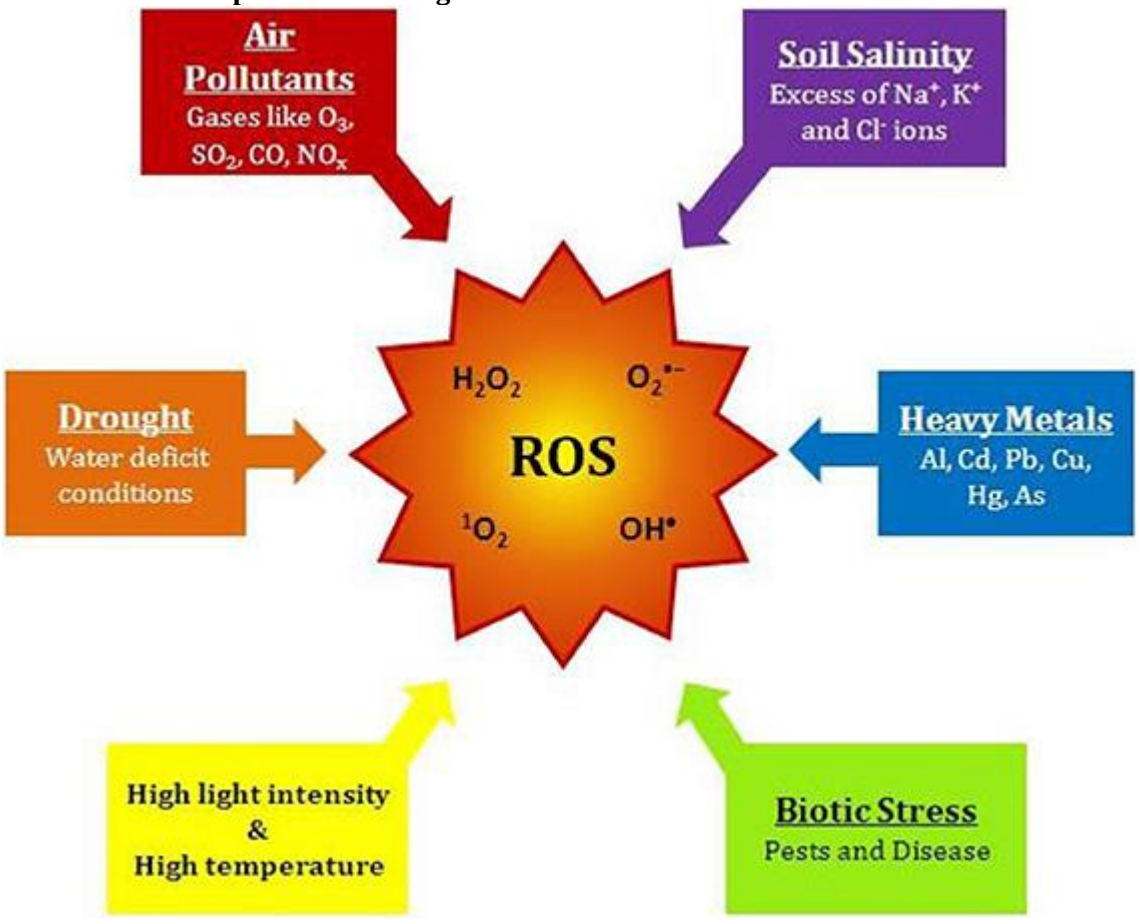
76. Production and damage by ROS

Reactive oxygen species (ROS) were initially recognized as toxic by-products of aerobic metabolism. In recent years, it has become apparent that ROS plays an important signaling role in plants, controlling processes such as growth, development and especially response to biotic and abiotic environmental stimuli. The major members of the ROS family include free radicals like $O^{\bullet-}_2$, $OH^{\bullet}$ and non-radicals like H_2O_2 and $^1O_2$.

The ROS production in plants is mainly localized in the chloroplast, mitochondria and peroxisomes. There are secondary sites as well like the endoplasmic reticulum, cell membrane, cell wall and the apoplast.

The role of the ROS family is that of a double edged sword; while they act as secondary messengers in various key physiological phenomena, they also induce oxidative damages under several environmental stress conditions like salinity, drought, cold, heavy metals, UV irradiation etc.

Various causes responsible for the generation of ROS



Types of ROS

Different types of ROS, namely, $^1O_2$(singlet oxygen), H_2O_2(hydrogen peroxide), $O^{\bullet-}_2$ (superoxide radical), and $OH^{\bullet}$ (hydroxyl radical), generated as unwanted byproducts

Sites of ROS Production in Plant Cells

The ROS is being produced under both normal and stressful conditions at various locations in the chloroplasts, mitochondria, peroxisomes, plasma membranes, ER and the cell wall. In presence of light, chloroplasts and peroxisomes are the major sources of ROS production, while the mitochondrion is the leading producer of ROS under dark conditions

Chloroplast

The chloroplast comprises of an extremely ordered system of thylakoid membranes which houses the light capturing photosynthetic machinery as well as anatomical requirements for efficient light harvesting.

The photosystems, PSI and PSII which form the core of the light harvesting system in the thylakoids are the major sources of ROS production.

Abiotic stress factors like drought, salinity, temperature extremes, all of which cause water stress and limit CO2 concentrations, coupled with excess light, leads to the formation of O•−2 at the PS

Mitochondria

Mitochondria are also the site of generation of harmful ROS, like H2O2 and O•−2 though in a smaller scale.

Plant mitochondria differ from animal counterparts in having O2 and carbohydrate-rich environment and also being involved in photorespiration

Peroxisomes

Peroxisomes are single-membrane-bound spherical microbodies and are the major sites of intracellular H2O2 production due to their integral oxidative metabolism

They also produce O•−2, like chloroplasts and mitochondria during the course of various metabolic process.

Apoplast

Apoplast, the diffusible space around the plant cell membrane is responsible for converting the incoming CO2 into a soluble, diffusible form which enters the cytosol to undergo photosynthesis.

At times of adverse environmental conditions, stress signals combined with abscisic acid (ABA) make the apoplast a prominent site for H2O2 production

Plasma Membranes

Plasma membrane which surrounds the entire plant cell plays an important role in interacting with the ever changing environmental conditions and provides information necessary for the continual survival of the cell.

The NADPH-dependent-oxidases which are localized in the plasma membrane are in the spotlight due to their gene expression and presence of different homologs during different stress conditions

The NADPH oxidase produces O•−2 by transferring electrons from cytosolic NADPH to O2

Cell Walls

During stress, the cell wall-localized lipoxygenase (LOX) causes hydroperoxidation of polyunsaturated fatty acids (PUFA) making it active source of ROS like $OH^{\bullet}$, $O^{\bullet-}_2$, H_2O_2, and $^1O_2$.

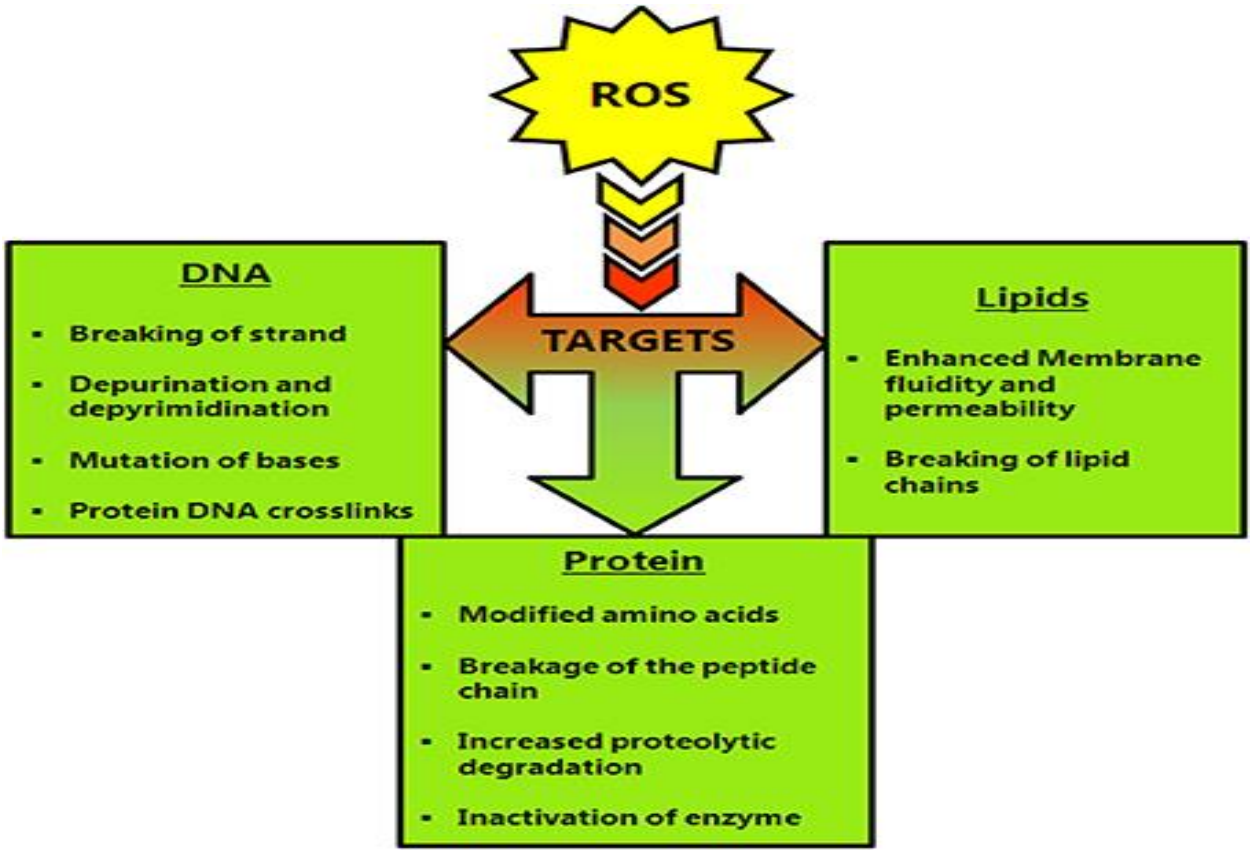
The cell wall-localized diamine oxidases utilize diamines or polyamines to generate ROS in the cell wall.

Endoplasmic Reticulum (ER)

Cytochrome P45 reacts with triplet oxygen (3O2) to form an oxygenated complex (Cyt P450-ROO−). The complex may occasionally decompose to Cyt P450-Rh by generating O•−2 as byproduct.

77. Targets of ROS

ROS is known to cause damages to biomolecules such as lipids, proteins and DNA



Lipids

Lipids form a major portion of the plasma membrane which envelopes the cell and helps it to adapt to the changing environment.

However, under stressful conditions, when the level of ROS rise above the threshold value, LPO becomes so damaging that it is often considered as the single parameter to gauge lipid destruction.

The two main targets of the ROS in membrane phospholipids are the double bond between C-atoms and the ester linkage between glycerol and fatty acids.

The PUFA which are important components of the plasma membrane are the hotspots for ROS damage.

PUFAs like linoleic and linolenic acid are specifically prone to attack by ROS like $^1O_2$ and $OH^{\bullet}$.

The hydroxyl radical ($OH^{\bullet}$) is the most damaging member as it has the ability to trigger a cyclic chain reaction and cause further peroxidation of other PUFAs.

Proteins

The ROS produced during stress conditions causes the oxidation of proteins. The protein undergoes different types of modifications which may either be direct or indirect.

During direct modifications, the activity of the protein becomes varied as a result of different chemical modifications such as nitrosylation, carboxylation, disulfide bond formation, and glutathionylation.

Protein carbonylation is often used as a marker for evaluating protein oxidation

The ROS concentration, on crossing its threshold value, leads to the site-specific modification of amino acids like Arg, Lys, Pro, Thr, and Trp, and increased susceptibility to proteolytic degradation

The amino acids differ in their susceptibility to ROS attack. Amino acids containing thiol groups and sulfur are the most vulnerable.
The Cys and Met are both prone to damage by the reactive $^1O_2$ and $OH^{\bullet}$.
The enzymes containing iron-sulfur centers are irreversibly inactivated on getting oxidized by $O^{\bullet-}_2$.

DNA

Since the plant nuclear DNA is well protected by histones and associated proteins, both mitochondrial and chloroplastic DNA bears the brunt of the ROS attack due to lack of protective histones as well as the close proximity to ROS production machinery.
Oxidative damage of DNA as a result of ROS occurs at multiple levels which include oxidation of the deoxyribose sugar residue, modification of the nucleotide base, abstraction of a nucleotide, breaks in either DNA strand, and cross-linking of the DNA and protein.
The $OH^{\bullet}$ is also notorious for creating DNA-protein cross-links when it reacts with either DNA or associated proteins.
These cross-links are not easily reparable and may be lethal to the plant cell, if not repaired in time before commencement of critical cellular processes like replication or transcription.

78. ROS Defense Machinery

The ROS defense mechanism consists of the antioxidant machinery which helps to mitigate the oxidative stress-induced damages.
The antioxidant machinery has two arms with the enzymatic components and non-enzymatic antioxidants

Enzymatic antioxidants	Enzyme code	Reaction catalyzed	Subcellular location
Superoxide dismutase (SOD)	1.15.1.1	$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow 2H_2O_2 + O_2$	Peroxisomes, Mitochondria, Cytosol, and Chloroplast
Catalase (CAT)	1.11.1.6	$2H_2O_2 \rightarrow O_2 + 2H_2O$	Peroxisome and Mitochondria
Ascorbate peroxidase (APX)	1.11.1.11	$H_2O_2 + AA \rightarrow 2H_2O + DHA$	Peroxisomes, Mitochondria, Cytosol, and Chloroplast
Monodehydroascorbate reductase (MDHAR)	1.6.5.4	$2MDHA + NADH \rightarrow 2AA + NAD$	Mitochondria, Cytoplasm, and Chloroplast
Dehydroascorbate reductase (DHAR)	1.8.5.1	$DHA + 2GSH \rightarrow AA + GSSG$	Mitochondria, Cytoplasm, and Chloroplast
Glutathione reductase (GR)	1.6.4.2	$GSSG + NADPH \rightarrow 2GSH + NADP^+$	Mitochondria, Cytoplasm, and Chloroplast
Guaiacol peroxidase (GPX)	1.11.1.7	$H_2O_2 + DHA \rightarrow 2H_2O + GSSG$	Mitochondria, Cytoplasm, Chloroplast, and ER
Non-enzymatic Antioxidants		**Function**	**Subcellular location**
Ascorbic Acid (AA)		Detoxifies H_2O_2 via action of APX	Cytosol, Chloroplast, Mitochondria, Peroxisome, Vacuole, and Apoplast
Reduced Glutathione (GSH)		Acts as a detoxifying co-substrate for enzymes like peroxidases, GR and GST	Cytosol, Chloroplast, Mitochondria, Peroxisome, Vacuole, and Apoplast
α -Tocopherol		Guards against and detoxifies products of membrane LPO	Mostly in membranes
Carotenoids		Quenches excess energy from the photosystems, LHCs	Chloroplasts and other non-green plastids
Flavonoids		Direct scavengers of H_2O_2 and $^1O_2$ and $OH^{\bullet}$	Vacuole
Proline		Efficient scavenger of $OH^{\bullet}$ and $^1O_2$ and prevent damages due to LPO	Mitochondria, Cytosol, and Chloroplast

Enzymatic Antioxidants

The enzymes localized in the different subcellular compartments and comprising the antioxidant machinery include Superoxide Dismutase (SOD), Catalase (CAT Glutathione Reductase (GR) and APX.

Superoxide Dismutase (SOD)

SOD belongs to the family of metalloenzymes present in all aerobic organisms.
Under environmental stresses, SOD forms the first line of defense against ROS-induced damages.

The SOD catalyzes the removal of O•−2 by dismutating it into O2 and H2O2. This removes the possibility of OH• formation by the Haber-Weiss reaction

SODs are classified into three isozymes based on the metal ion it binds, Mn-SOD (localized in mitochondria), Fe-SOD (localized in chloroplasts), and Cu/Zn-SOD (localized in cytosol, peroxisomes, and chloroplasts)

SOD has been found to be up regulated by abiotic stress conditions

Catalase (CAT)

CAT is a tetrameric heme-containing enzyme responsible for catalyzing the dismutation of H_2O_2 into H_2O and O_2. It has high affinity for H_2O_2, but lesser specificity for organic peroxides (R-O-O-R).

It has a very high turnover rate (6×10^6 molecules of H_2O_2 to H_2O and O_2 min^{-1}) and is unique amongst antioxidant enzymes in not requiring a reducing equivalent.

Peroxisomes are the hotspots of H_2O_2production due to β-oxidation of fatty acids, photorespiration, purine catabolism and oxidative stress

However, recent reports suggest that CAT is also found in other subcellular compartments such as the cytosol, chloroplast and the mitochondria, though significant CAT activity is yet to be seen

Angiosperms have been reported to have three CAT genes. CAT1 is expressed in pollens and seeds (localized in peroxisomes and cytosol), CAT2 predominantly expressed in photosynthetic tissues but also in roots and seeds (localized in peroxisomes and cytosol) and finally CAT3 is found to be expressed in leaves and vascular tissues

Ascorbate peroxidase (APX)

APX is an integral component of the Ascorbate-Glutathione (ASC-GSH) cycle. While CAT predominantly scavenges H2O2 in the peroxisomes, APX performs the same function in the cytosol and the chloroplast.

The APX reduces H2O2 to H2O and DHA, using Ascorbic acid (AA) as a reducing agent.

The APX family comprises of five isoforms based on different amino acids and locations, viz., cytosolic, mitochondrial, peroxisomal, and chloroplastid (stromal and thylakoidal).

Since APX is widely distributed and has a better affinity for H2O2 than CAT, it is a more efficient scavenger of H2O2 at times of stress.

Glutathione Reductase (GR)

GR is a flavoprotein oxidoreductase which uses NADPH as a reductant to reduce GSSG to GSH.

Reduced glutathione (GSH) is used up to regenerate AA from MDHA and DHA, and as a result is converted to its oxidized form (GSSG).

GR, a crucial enzyme of ASC-GSH cycle catalyzes the formation of a disulfide bond in glutathione disulfide to maintain a high cellular GSH/GSSG ratio.

It is predominantly found in chloroplasts with small amounts occurring in the mitochondria and cytosol.

GSH is a low molecular weight compound which plays the role of a reductant to prevent thiol groups from getting oxidized, and react with detrimental ROS members like 1O2 and OH.

79. ROS Defense Machinery II

Non-Enzymatic Antioxidants

The non-enzymatic antioxidants form the other half of the antioxidant machinery, comprising of AA, GSH, α-tocopherol, carotenoids, flavonoids.

They not only protect different components of the cell from damage, but also play a vital role in plant growth and development by tweaking cellular process like mitosis, cell elongation, senescence and cell death

Ascorbic Acid (AA)

AA is the most abundant and the most extensively studied antioxidant compound. It is considered powerful as it can donate electrons to a wide range of enzymatic and non-enzymatic reactions.

It reacts with H_2O_2, $OH^{\bullet}$, $O^{\bullet-}_2$, and regenerates α-tocopherol from tocopheroxyl radical, thereby protecting the membranes from oxidative damage

Reduced glutathione (GSH)

Glutathione is a low molecular weight thiol tripeptide (γ-glutamyl-cysteinyl-glycine) abundantly found in almost all cellular compartments like cytosol, ER, mitochondria, chloroplasts, vacuoles, peroxisomes, and even the apoplast

Reduced glutathione (GSH)

Glutathione is a low molecular weight thiol tripeptide (γ-glutamyl-cysteinyl-glycine) abundantly found in almost all cellular compartments like cytosol, ER, mitochondria, chloroplasts, vacuoles, peroxisomes, and even the apoplast

It is involved in a wide range of processes like cell differentiation, cell growth/division, cell death and senescence, regulation of sulfate transport, detoxification of xenobiotics, conjugation of metabolites, regulation of enzymatic activity, synthesis of proteins and nucleotides, synthesis of phytochelatins and finally expression of stress responsive genes

This versatility of GSH is all due to its high reductive potential.

A central cysteine residue with nucleophilic character is the source of its reducing power. GSH scavenges H_2O_2, $^1O_2$, $OH^{\bullet}$, and $O^{\bullet-}_2$ and protects the different biomolecules by forming adducts (glutathiolated) or by reducing them in presence of ROS or organic free radicals and generating GSSG as a by-product.

α-tocophero

The α-tocopherol belongs to a family of lipophilic antioxidants which are efficient scavengers of ROS and lipid radicals, making them indispensable protectors and essential components of biological membranes

Tocopherols are known for their ability to protect lipids and other membrane constituents of the chloroplasts by reacting with O_2 and quenching its excess energy, thus protecting the PSII, both structurally and functionally.

It reacts with the lipid radicals $RO^{\bullet}$, $ROO^{\bullet}$)perhydrooxyl), and RO* at the membrane-water interface, where α-tocopherol reduces them and itself gets converted into $TOH^{\bullet}$.

The $TOH^{\bullet}$ radical undergoes recycling to its reduced form by interacting with GSH and AA

Carotenoids

Carotenoids belong to family of lipophilic antioxidants which are localized in the plastids of both photosynthetic and non-photosynthetic plant tissues.

They are found not only in plants, but also in micro-organisms.

Carotenoids exhibit their antioxidative activity by protecting the photosynthetic machinery in four ways,

(a) reacting with LPO products to end the chain reactions,

(b) scavenging $^1O_2$ and generating heat as a by-product,

(c) preventing the formation of $^1O_2$ by reacting with ^{3}Chl* and excited chlorophyll (Chl*), and

(d) dissipating the excess excitation energy, via the xanthophyll cycle.

Flavonoids

Flavonoids are widely found in the plant kingdom occurring commonly in the leaves, floral organs and pollen grains.

Flavonoids can be classified into four classes on the basis of their structure, flavonols, flavones, isoflavones, and anthocyanins.

They have diverse roles in providing pigmentation in flowers, fruits and seeds involved in plant fertility and germination of pollen and defense against plant pathogens.

Flavonoids have been considered as a secondary ROS scavenging system in plants experiencing damage to the photosynthetic apparatus, due to the excess excitation energy.

They also have a role in scavenging 1O2 and alleviate the damages caused to the outer envelope of the chloroplastic membrane.

80. Heat Stress in plants

Introduction

Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. Heat stress affects plant growth throughout its ontogeny, though heat-threshold level varies considerably at different developmental stages. Heat stress due to high ambient temperature is a serious threat to crop production worldwide Different greenhouse gases will gradually increase world's average ambient temperature

At very high or moderately high temperatures

Due to high temperature severe cellular injury and even cell death can occur, direct injuries include protein degradation can happen and increased fluidity of membrane lipids can also happens.

Direct injury

Direct injury can cause protein denaturation and aggregation and increased fluidity of membrane lipids.

Indirect injuries

Indirect injuries can cause enzymes inactivation, inhibition of protein synthesis and degradation and loss of membrane integrity.

Due to high temperatures changes occur

At the molecular level altering gene expression, there is accumulation of transcripts and there is formation of stress related proteins (HSP). There exists tremendous variation within and between species, providing opportunities to improve crop heat stress tolerance through genetic means

Heat-stress threshold

It is a value of daily mean temperature at which a detectable reduction in growth begins / the temperature at which growth and development of plant cease. Upper threshold: is the temperature above which growth and development cease. Lower threshold (base temperature): is the temperature below which plant growth and development stop

Threshold high temperatures for some crops plants

Crop plants	Threshold tem.(°C)	Growth stage	References
Wheat	26	Post -anthesis	Stone and Nicolas (1994)
Corn	38	Grain filling	Thompson (1986)
Cotton	45	Reproductive	Rehman et al., (2004)
Pearl millet	35	Seedling	Ashraf and Hafeez (2004)
Tomato	30	Emergence	Camejo et al., (2005)
Brassica	29	Flowering	Morrison Stewart (2002)
Cool season pulses	25	Flowering	Siddique et al., (1999)
Ground nut	34	Pollen production	Vara Prasad et al., (2000)
Cow Pea	41	Flowering	Patel and Hall (1990)
Rice	34	Grain yield	Morita et al., (2004)

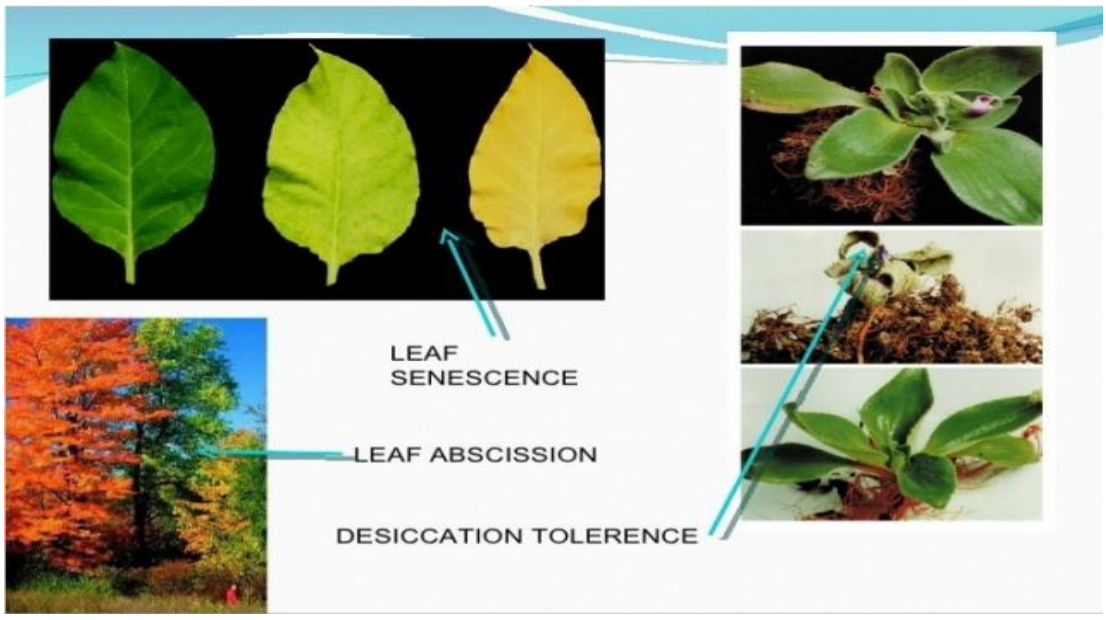
PLANT RESPONSES TO HEAT STRESS

Morphological Symptoms

PLANT RESPONSES TO HEAT STRESS

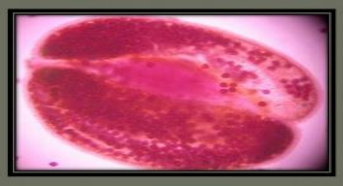
✓**Morphological symptoms :**

- Scorching of leaves and twigs
- Sunburns on leaves branches and stems
- Leaf senescence and abscission
- Shoot and root growth inhibition
- Fruit discoloration and damage and reduced yield
- Reduction in the internodes length
- Reproductive phases most sensitive to high temperature are gametogenesis (8–9 days before anthesis) and fertilization (1–3 days after anthesis) in various crop plants (Foolad, 2005).

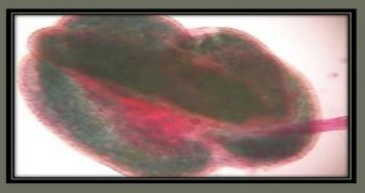


Fruit discoloration and damage and reduced yield

Reproductive phases most sensitive to high temperature



Chickpea Pollen Tolerant to high temperature



Chickpea Pollen sensitive to high temperature

Scorching of leaves

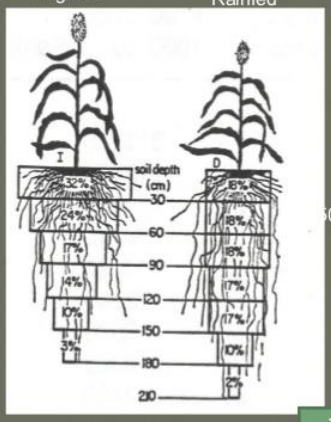
Sunburns on leaves

ROOT SYSTEM GROWTH

Irrigated

Rainfed

>50% water from 60cm



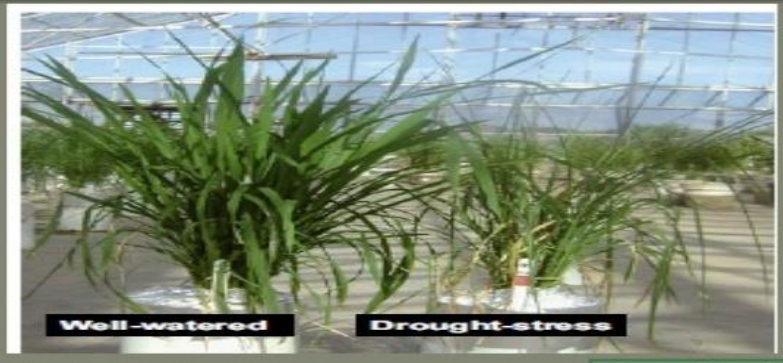
0% water from 90cm

Farooq et al. 2009

Effect on Growth:

- Reduction in Turgor Pressure,
- Reduction in Cell size

Reduce growth



Farooq M et al. 2009

81. Heat Stress in plants II

Plant responses to heat stress

Effect on Growth: the effects of heat on growth of plants are: reduction in turgor pressure, reduction in cell size and reduced growth.

Effect on Photosynthesis: the effects of heat on photosynthesis of plants are: disruption of PS II (Photo System II), stomatal closure and decrease in electron transport, so reduced photosynthesis.

Effect on proteins: the effects of heat on proteins are: Protease activity increases, Protein content falls down and Irrigated Drought Stress.

Reduction in N metabolism: Nitrate reductase activity decrease, Nitrate to nitrite to ammonia to take part in amino acid synthesis and the importance of regulating nitrate reductase activity is to limit the amount of nitric oxide being produced.

Phenological changes:

Heat stress is a major factor affecting the rate of plant development, which may increase to a certain limit and decrease afterwards. Heat and high temperature can cause damage; there is opened flowers abortion during reproduction and impairment of pollen and anther development. Furthermore, there is decrease in days to ear emergence and grain filing duration is also decreased.

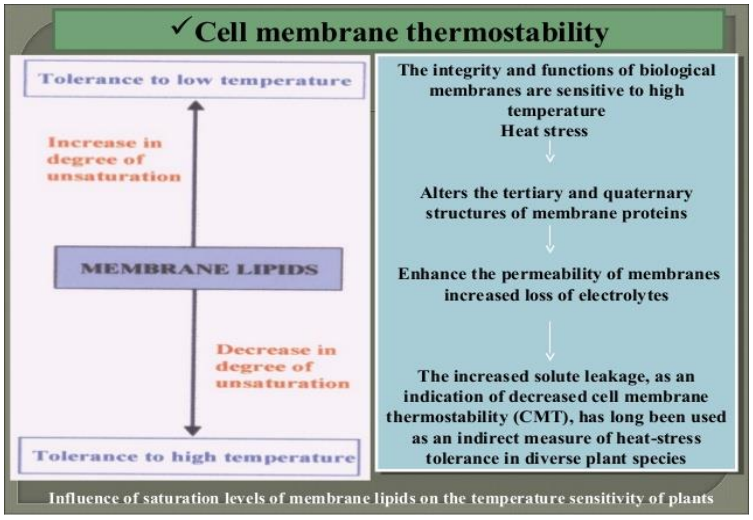
Physiological responses Waters relations

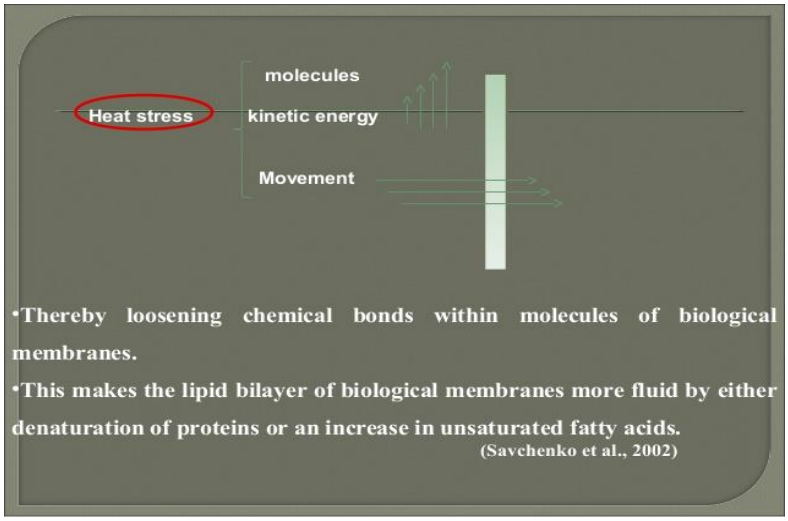
Heat stress perturbed the leaf water relations and root hydraulic conductivity. Enhanced transpiration induces water deficiency in plants, causing a decrease in water potential and leading to perturbation of many physiological processes. High temperature seems to cause water loss in plant more during day time than night time.

Accumulation of compatible Osmolytes

Plant species may accumulate osmolytes such as: Sugars and sugar alcohols, Proline, Ammonium compounds, Sulphonium compounds. Glycinebetaine (GB) is an amphoteric quaternary amine, plays an important role as a compatible solute in plants. High level of GB accumulation was reported in sugarcane

Primary sites of injury at high temperatures are: Photochemical reactions in thylakoid lamellae and Carbon metabolism in the stroma of chloroplast. In tomato genotypes differing in their capacity for thermotolerance as well as in sugarcane, an increased chlorophyll a:b ratio and a decreased chlorophyll:carotenoids ratio were observed in the tolerant genotypes under high temperatures. High temperature influences the photosynthetic capacity of C3 plants more than in C4 plants





82. Hormonal changes due to heat stress

Two hormones named as Abscisic acid (ABA) and ethylene (C2H4), are used as stress hormones, they are involved in the regulation of many physiological processes, they act as signal molecules.

Action of ABA: it involves modification of gene expression, helps in modulating the up- or down-regulation of numerous genes

Action of C2H4: It is involved in induced abscission of reproductive organs.

Heat-stress tolerance mechanisms in plants: it is the ability of plant to grow and produce economic yield under high temperature.

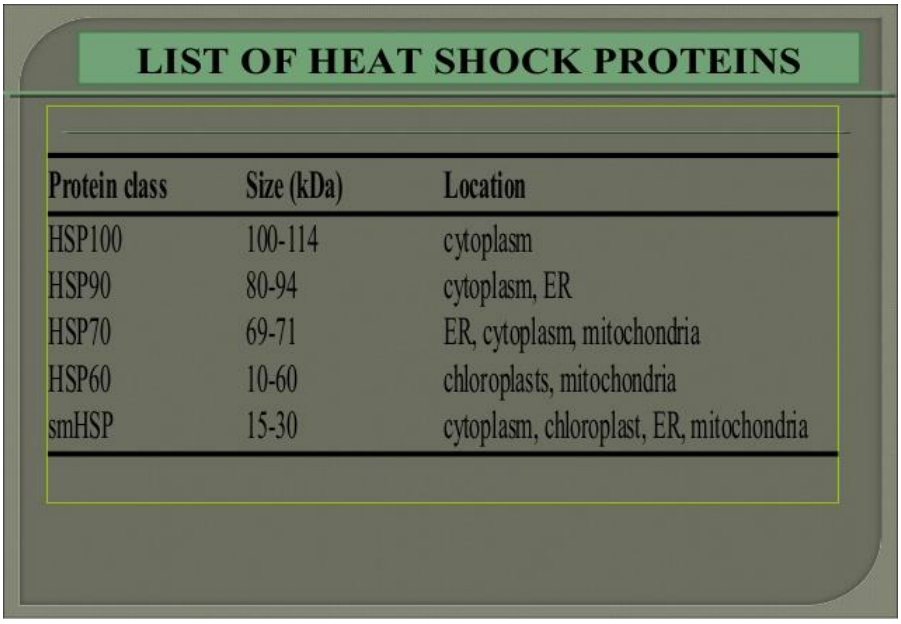
Expression of stress proteins:

Expression of stress proteins is an important adaption to cope with environmental stresses. Most of the stress proteins are soluble in water and therefore contribute to stress tolerance presumably via hydration of cellular structures. In higher plants, HSPs is usually induced under heat shock at any stage of development.

Heat shock proteins: A specific set of proteins that are induced by a rapid rise in temperature.

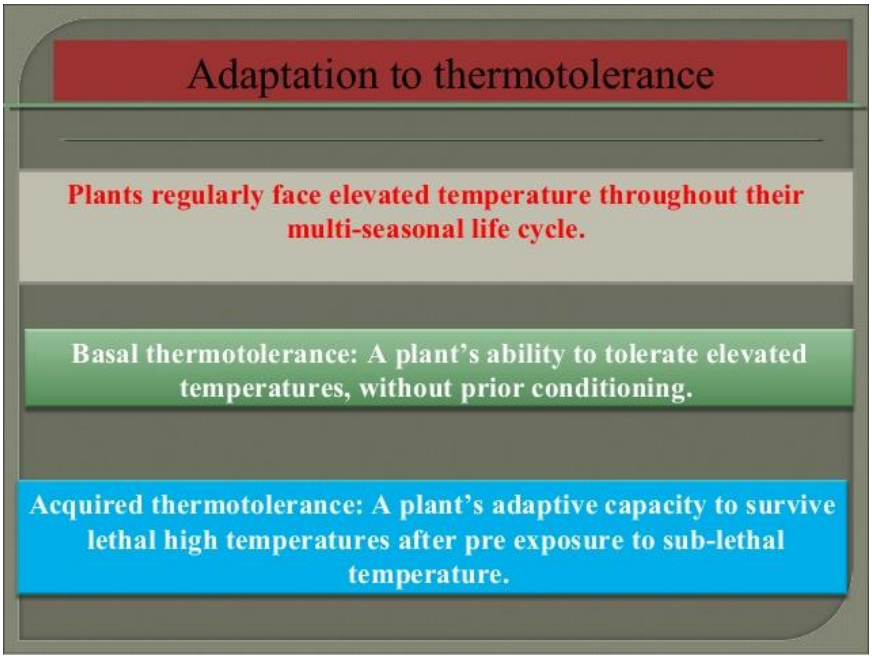
List of heat shock proteins:

Some heat shock proteins with their respective properties are listed below:



Protein class	Size (kDa)	Location
HSP100	100-114	cytoplasm
HSP90	80-94	cytoplasm, ER
HSP70	69-71	ER, cytoplasm, mitochondria
HSP60	10-60	chloroplasts, mitochondria
smHSP	15-30	cytoplasm, chloroplast, ER, mitochondria

Adaptation to thermo tolerance:



Plants exhibit a variety of responses to high temperatures. High temperatures affect plant growth at all developmental stages. Stress proteins are helping in folding and unfolding of essential proteins under stress, and ensuring three-dimensional structure of membrane proteins for sustained cellular functions and survival under heat stress. The induction of signaling cascades leading to profound changes in specific gene expression is considered an important heat- stress adaptation

83. Genetically modified organism

What is GMOs?

GMO is an organism whose genetic material has been altered using genetic engineering techniques. Organisms that have been genetically modified include micro-organisms such as bacteria and yeast, plants, fish, and mammals. Source of genetically modified foods, and are also widely used in scientific research and to produce useful goods other than food.

PRODUCTION OF GENETICALLY MODIFIED ORGANISMS

Genetic modification involves the insertion or deletion of genes. When genes are inserted, they usually come from a different species, which is a form of horizontal gene transfer. In nature this can occur when exogenous DNA penetrates the cell membrane for any reason. To do this artificially may require attaching the genes to a virus or just physically inserting the extra DNA into the nucleus of the intended host with a very small syringe, or with very small particles fired from a gene gun. Agrobacterium's ability to transfer genetic material to plants or the ability of lentiviruses to transfer genes to animal cells are natural examples of gene transfer.

PRINCIPLE OBJECTIVE OF PRODUCTION

The basic principle for producing a GMO was to add new genetic material into an organism's genome. This is called genetic engineering and was made possible through the discovery of DNA and the creation of the first recombinant DNA molecules by Paul Berg in 1972.

TRANSGENIC PLANTS

Transgenic plants have been engineered for scientific research, to create new colors in flowers, and to create improved crops. In research, plants are engineered to help discover the functions of certain genes. One way to do this is to knock out the gene of interest and see what phenotype develops. Another strategy is to attach the gene to a strong promoter and see what happens when it is over expressed.

GM CROPS

In agriculture, genetically engineered crops are created to possess several desirable traits, such as resistance to pests, herbicides, or harsh environmental conditions, improved product shelf life, increased nutritional value, or production of valuable goods such as drugs (pharming). Plants, including algae, jatropha, maize, and other plants have been genetically modified for use in producing fuel, known as biofuel.

MICROBES

Bacteria were the first organisms to be modified in the laboratory, due to their simple genetics. These organisms are now used for several purposes, and are particularly important in producing large amounts of pure human proteins for use in medicine. Genetically modified bacteria are used to produce the protein insulin to treat diabetes. Similar bacteria have been used to produce clotting factors to treat haemophilia, and human growth hormone to treat various forms of dwarfis.

MAMMALS

Ralph L. Brinster and Richard Palmiter developed the techniques responsible for transgenic mice, rats, rabbits, sheep, and pigs in the early 1980s. They established many of the first transgenic models of human disease, including the first carcinoma caused by a transgene. The process of genetically engineering animals is a slow, tedious, and expensive process. However, new technologies are making genetic modifications easier and more precise.

84. What Plants and Foods Are GMOs?

There are only a few types of transgenic, or "genetically modified," plants that have been approved for commercial production in the United States. The table below shows those different plants and what genetic traits in the plants have been added or changed by scientists. These plants have one or more of the following traits modified by genetic engineering.

Herbicide Resistance

Herbicides are chemicals used to kill weeds. On large farms that use herbicides, these chemicals can leak into the environment or they can stick to the crops, ending up in your food in small amounts. If farmers could use less toxic chemicals to kill weeds, it would be safer for people eating the crops and for the environment. Many transgenic plants have a gene added making them resistant to a specific, low-toxicity herbicide. This allows farmers to use herbicides that do less harm to the environment and people.

Pest Resistance

Some plants have been modified to have a bacterial gene. This bacterial gene makes a protein which kills only certain types of insects that harm plants. The protein is not toxic to people or to other insects or animals, but it protects the plants from that specific pest. Because of this, farmers do not have to use toxic pesticides on these plants.

Virus Resistance

These viruses do not make people sick, but they can damage crops. Genes have been added to some plants so they won't catch these specific viruses.

Changed Metabolism

Genes have also been added to plants to change the types of sugars or fats that a plant makes. This can be used to make the plant safer to eat. Some of these genes make the plant less likely to get bruised or damaged during shipping which means less food gets thrown in the trash.

85. 6 Different Processes Used to Genetically Modify Crops

There are six most common crop modification techniques, which are as follows:

1. **Cross Breeding:**

This technique has been used since the 1700s, it's when you take two sexually compatible crops and cross pollinate them to produce a hybrid. Some examples are the plumcot (plum and apricot), tangelos (tangerine and grapefruit), the limequat (lime and kumquat) and most famously the rabbage (cabbage and radish).

2. **Mutagenesis**

Mutations (*muta*) are genetic changes that can switch, add, or delete nucleotides (those A,T,G and C bases), these genetic changes can sometimes lead to new/enhanced traits which is why plant breeders sometimes induce (*genesis*) these genetic changes using radiation or chemicals. Hermann Muller, Charlotte Auerbach and J. M. Robson founded this technique in the first half of the 20th century. For example, radiation was used to produce a deeper color in the red grapefruit.

3. **Protoplast Fusion**

Sounds scary right? It's actually when you take two plant cells which have their hard cell walls removed (*Protoplasts*) and you add a chemical called polyethylene which allow the two cells to stick together. Once they are stuck together basic chemicals are added to help the two cells combine and exchange genetic information to create a hybridized plant cell (*fusion*). It's much like cross breeding, except it's done in a lab.

Polyploidy

We, humans, are diploid animals, which mean we have two sets of homologous chromosomes. Polyploidy have more than one, and the induction of polyploidy is used by plant breeders to control reproduction. Introducing polyploidy by soaking seeds in colchcine can either make sterile crosses fertile, like the Triticale (hybrid of wheat and rye), or sterilizes crops, like watermelon, to make seedless strains.

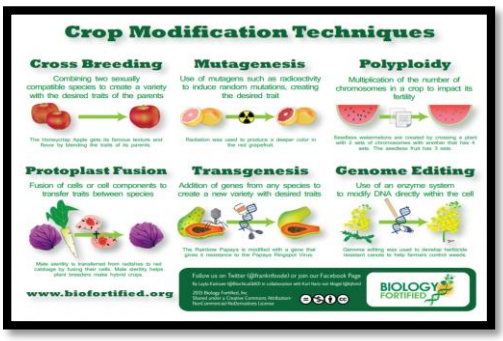
Genome Editing

This process has the ability to cut, replace or insert genes within the seed cells using "molecular scissors" called nucleases—enzymes which have the ability to loosen, remove and add nucleotides. These nucleases are artificially engineered to accurately place in desired genes, or traits, into the

genome of the crop. Herbicide tolerant canola was created using this technique to help famers control weeds.

Transgenisis:

It occurs when genes from one crop are incorporated into another crop. Since the genetic code is readable by all living organisms, this means that the genes introduced will code for the same proteins as it did before. There are many ways to introduce these new genes, like using agrobacterium to carry it into the genome, or using electricity. I was even learning how to transform yeast in my biology classes at school.



86. Types of Techniques Used to Genetically Modify Food

While genetically modified (GM) foods are a controversial subject given the questions about their effect on the environment and human health, little attention is given to the techniques and experiments used to modify these foods. These techniques are important to understand because they affect the final organism that results. . In fact, the development of these techniques and experiments is quite fascinating given how rapidly the biotechnology field has advanced over the last two decades. Ensuring that the techniques are safe and that food is safe as well are important considerations.

Bacterial Carriers

Bacterial carriers can be very effective for delivering DNA. Typically, a bacterium would be prepped in a solution that makes the cell walls extremely porous. The chosen gene would then be inserted into a plasmid and put in the solution. After heating the solution, the plasmid is able to 'merge' with the bacterium and show the new gene. Once the genetically modified bacterium recovers and grows, it can make additional replicates of the new gene. After infecting the targeted plant, it can deliver the plasmid and the new gene.

Calcium Phosphate Precipitation

In this biotechnology technique, the chosen DNA would be exposed to calcium phosphate, which results in the creation of miniscule granules. The targeted cells react to the granules by essentially 'swarming' them and ingesting them, thereby facilitating the granule release of DNA and the subsequent delivery to the host's nuclei and chromosomes.

Using Electroporation to Create GM Organisms

In electroporation, the prepped target cells are saturated in a solution with the chosen DNA. A brief but strong electric shock is transmitted through the solution, causing little tears in the walls of the cells. This allows for the new genetic material to penetrate the nuclei. Afterwards, the cells are put in a different solution that coaxes the repair of their walls, which works to 'trap' the DNA of the donor in the cell. The chosen DNA becomes joined with the host chromosomes to give the host this new gene.

Biolistics for GM Technology

This technique uses the chosen DNA to attach it to tiny gold particles. The particles – now 'carrying' DNA – are forced into the target cells using an intense burst of gas.

Gene Silencing Technique

With GM techniques, they are sometimes used to remove a gene that is responsible for an undesirable trait. When gene silencing is used, the gene that is responsible for this trait will first be identified in the organism. Then, another copy of the gene is attached but in the other direction, which prevents the expression of that trait. For instance, an allergen that triggers an allergic reaction in humans could be 'silenced' in this manner.

Gene Splicing

With this GM technique, biotechnologists can modify DNA, and then insert it into target host cells to allow for genes and resulting traits to be modified. An enzyme is then used to fuse the newly added gene into the chromosome.

Using a Viral Carrier

A virus can make an effective carrier for modifying an organism. The virus chosen will be one that does not cause any kind of disease or death. Through the addition of the chosen DNA to the virus genome, the virus can infect the target. Once the virus invades the cell and makes copies of itself, the chosen DNA can be added to the targeted cell.

87. Most common types of GMO

The two most common types of GMO’s

1. FOODS

Crops are modified to develop resistance to herbicides and increase their nutrient content, for example corn and soybeans. Fruits are modified to make them ripen later. This help them available fresh in marketplace during a longer time or for fruits that ripen after being picked, make it easier to transport them.

2. MEDICINES

These can be produced cheaper and easier some are: insulin, thyroid hormones and the Hepatitis B vaccine. GM bacteria's have been particularly important in producing large amounts of pure human proteins for use in medicine like clotting factors for hemophilia and human growth hormones to treat dwarfism.

Other types of GMO's are

MAMMALS

Mammals are used to do research on human diseases, to develop animal models for many diseases, to produce industrial or consumer products (pharmaceutical products or tissue implantation), to enrich the animal's interactions with humans (Hypo-allergic pets), to enhance production or food quality traits (faster growth fish, pigs that digest food more efficiently) and improve animal health (disease resistance).

INSECTS

Insects are used to find effects of genetic changes on development (malaria resistant mosquitoes).

AQUATIC LIFE

Aquatic life is used in evolution of immunity and developmental processes, rapid growth, (MADAKA -fish to detect pollutions in waterways).

Transgenic monkey

It is so similar to humans, hence it used in clinical trials and used for studying: HIV, Huntington's disease.

DISADVANTAGES: Disadvantages of using transgenic monkey are: Expensiveness, Difficulty in experiment and there are also breeding problems.

Topical microbicides for blocking HIV-1 transmission

Lactobacilli or *E. Coli* are altered to secrete or express proteins with anti–hiv-1 activity, in colonization of the vagina or rectum with recombinant bacteria, to secret fusion inhibitory peptides or proteins, in lactobacilli that maintains a low vaginal ph, and it also lowers the risk of HIV-1 infection.

88. Use of GMO

Genetically modified pigs

In MEDICINE: In medicine these are the following uses of genetically modified pigs. Production of pharmaceuticals (human hemoglobin in blood of pigs for treating Trauma patients)**,** Organs for Xenotransplantation into humans**,** development of models for human diseases

In Agriculture: In agriculture these are being used in: resistance to disease, altering the carcass composition, improving pig's resistance to heat stress and protecting environment.

Pigs given spinach genes

It is world's first genetically engineered mammal to contain DNA from plants to produce pork that is healthier normal pigs. It produces less fat than normal, less fat intake. It is confirmed for the first time in the world that a plant gene is functioning properly in a living mammal, not in a cultured cell," said by professor akira.

Pigs given spinach genes experiment

In experiment these steps were followed: Inserting the spinach gene into a fertilized pig egg, Implanted in a female pig's womb and FAD2 gene converted about a fifth saturated fatty acids into linoleic acids.

Advantages of pig

Selection of pig was due to these characters: physiology and size, they can be raised in pathogen free condition, have less chance to transmit infectious disease to humans; they have fewer ethical issues as donor, they have short generation interval(114 days) and their genome is quite similar to humans (3x times than mouse).

Goat that produce spider silk

There are two key genes that allow a spider to weave their silk inserted into their genetic code, which produce milk that contain spider silk proteins, proteins are then harvested through the goat's milk, goats are separated into two groups, each contains one of the two proteins, and proteins must be extracted and combined. They are stronger than steel and more flexible, they are used to replace damaged tendons and ligaments, suture damaged eyes, or even nerve sake stronger and safer parachutes for soldiers, bulletproof vests etc.

Silk from milk

Goats are milked, then milk is frozen and the cream is separated, thawed milk is pushed into a micro filter that blocks the larger fat molecules and lets the smaller proteins through, a smaller filter then further isolates the silk proteins and when it is dried, it looks like a white powder.

The challenge: how they take a powder and spin it into a fiber, like a spider does?

The two proteins are combined into a solution, and then these are transformed into microfibers using wet-spinning fiber production methodologies. "Biosteel biopolymer" had been transformed into nanofibres and nanomeshes using electro spinning technique.

Ways GMO toxicity affects animals, plants and soil

The ways by which GMO toxicity can affect animals, plants and soil are: Cancer, damage to native species, they can pollute the environment, these can deplete soil minerals, destroy beneficial bacteria, there are also 'Super weeds,' 'superbugs.' they can also cause infertility, stillbirths, miscarriages.

Lab animals tested with GM foods

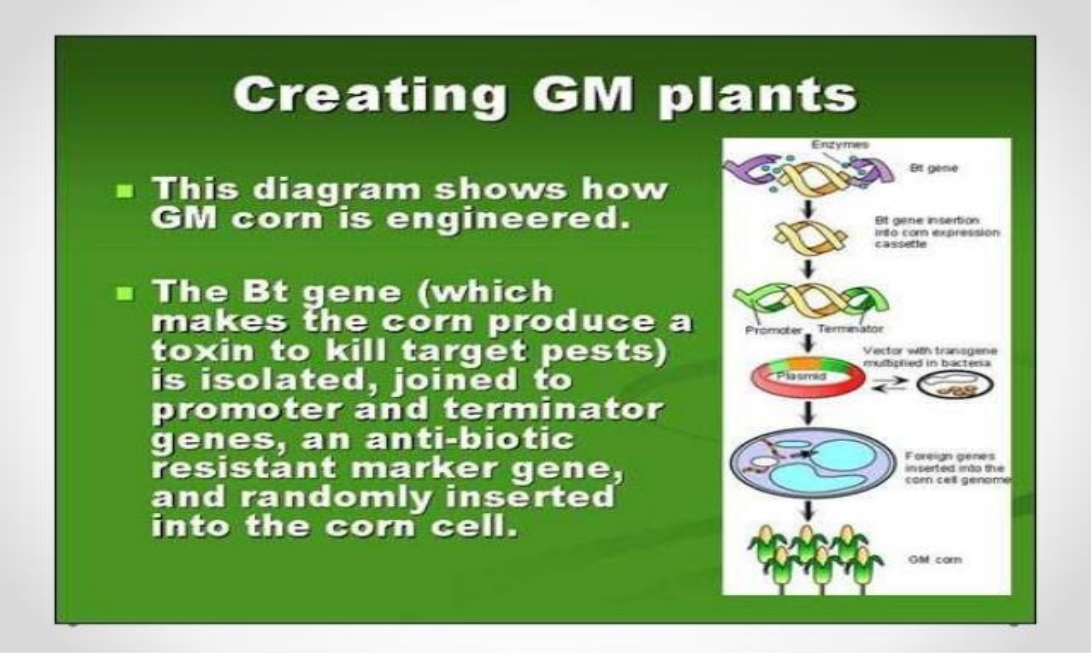
The features which were observed after the lab animals were tested with GM foods are: Stunted growth, Impaired immune systems, Bleeding stomachs, Impaired blood cell development, Misshapen cell structures in the liver, pancreas, and testicles, Altered gene expression and cell metabolism, Their offspring have a lower chance of survival, they have higher blood sugar, have enlarged livers, pancreases, have inflamed kidneys, have less developed brains and testicles, have intestines reduced digestive enzymes, the animal's life spans are shortened and they have inflamed lung tissue.

Adverse effects of GMO's

Already 37 people have died from side effects, 1,500 are partially paralyzed, 5,000 were temporarily handicapped by chemicals used in harvesting, Glyophosate "caused malformations in frog and chicken embryos, malformations of the head and there is increased death rates and higher offspring mortality.

89. Genetically modified crops 1

Genetically modified crops (GMCs, GM crops, or biotech crops) are plants used in agriculture, the DNA of which has been modified using genetic engineering methods. In most cases, the aim is to introduce a new trait to the plant which does not occur naturally in the species. Examples in food crops include resistance to certain pests, diseases, or environmental conditions, reduction of spoilage, or resistance to chemical treatments (e.g. resistance to a herbicide), or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation.



4 examples of genetically modified crops

Pesticide resistant rape plants

Scientists have transferred a gene to the rape plant which enables the plant to resist a certain pesticide. When the farmer sprays his genetically modified rape crop with pesticides, he or she can destroy most of the pests without killing the rape plants.

Advantages:

The farmer can grow a larger crop because it is easier to fight pests. In some cases the farmer can use a more environmentally friendly crop spray. The farmer can also protect the environment by using less crop spray.

Disadvantages:

Genes from the genetically modified rape crop could be transferred to the pests. The pests then become resistant to the crop spray and the crop spraying becomes useless. Rape plants can pollinate weeds - for example navew which is found in rape fields. When rape plants pollinate the navew their genes are transferred. Corn, soya beans and sugar cane have also been genetically modified by scientists so they are able to tolerate crop spray.

Insecticide sweet corn

Scientists have genetically modified sweet corn so that it produces a poison which kills harmful insects. This means the farmer no longer needs to fight insects with insecticides. The genetically modified corn is called Bt- corn, because the insect-killing gene in the plant comes from the bacteria Bacillus thuringiensis.

Advantages:

The farmer no longer has to use insecticide to kill insects, so the surrounding environment is no longer exposed to large amounts of harmful insecticide. The farmer no longer needs to walk around with a drum of toxic spray wearing a mask and protective clothing.

Disadvantages:

This type of genetically modified corn will poison the insects over a longer period than the farmer who would spray the crops once or twice. In this way the insects can become accustomed (or resistant) to the poison. If that happens both crop spraying and the use of genetically modified Bt-corn become ineffective. A variety of insects are at risk of being killed. It might be predatory insects that eat the harmful ones or, perhaps attractive insects such as butterflies. Cotton and potatoes are other examples of plants that scientists have , genetically modified to produce insecticide.

Golden rice

Golden rice is genetically modified rice that now contains a large amount of A-vitamins. Or more correctly, the rice contains the element beta- carotene which is converted in the body into Vitamin-A. So when you eat golden rice, you get more vitamin A. Beta-carotene gives carrots their orange colour and is the reason why genetically modified rice is golden. For the golden rice to make beta-carotene three new genes are implanted: two from daffodils and the third from a bacterium.

Advantages:

The rice can be considered a particular advantage to poor people in underdeveloped countries. They eat only an extremely limited diet lacking in the essential bodily vitamins. The consequences of this restricted diet causes many people to die or become blind.This is particularly true in areas of Asia, where most of the population live on rice from morning to evening.

Disadvantages:

Critics fear that poor people in underdeveloped countries are becoming too dependent on the rich western world. Usually, it is the large private companies in the West that have the means to develop genetically modified plants. By making the plants sterile these large companies can prevent farmers from growing plant- seed for the following year - forcing them to buy new rice from the companies. Some opposers of genetic modification see the "golden rice" as a method of making genetic engineering more widely accepted. Opponents fear that companies will go on to develop other genetically modified plants from which they can make a profit. A situation could develop where the large companies own the rights to all the good crops.

Long-lasting tomatoes

Long-lasting, genetically modified tomatoes came on to the market in 1994 and were the first genetically modified food available to consumers. The genetically modified tomato produces less of the substance that causes tomatoes to rot, so remains firm and fresh for a long time.

Advantages:

Because the GM tomatoes can remain fresh longer they can be allowed to ripen in the sun before picking - resulting in a better tasting tomato. GM tomatoes can tolerate a lengthier transport time. This means that market gardens can avoid picking tomatoes while they are green in order that they will tolerate the transport. The producers also have the advantage that all the tomatoes can be harvested simultaneously.

Disadvantages: Scientists today can genetically modify tomatoes without inserting genes for antibiotic resistance; however the first genetically modified tomatoes contained genes that made them resistant to antibiotics. Doctors and vets use antibiotics to fight infections. These genes spread to animals and people, doctors would have difficulties fighting infectious diseases.

90. Genetically Modified Plants 2

Herbicide-Tolerant Plants

The development of herbicide-tolerant varieties of agronomically important plants such as corn, soybeans and the cereals promises to have a major impact on agriculture, both economically and on production practices. Weeds compete with crops for soil nutrients and routinely lead to significant losses in yield. Modern agriculture makes use of herbcides to control weeds and minimize the losses. Unfortunately, the available herbicides seldom provide the degree of

specifications that is desired, and most herbicides will control only certain classes and not others. The most promising of the alternate approaches is the development of herbicide-tolerant plant varieties for use with broad-spectrum or totally nonspecific herbicides. Obviously, the potential economic value of herbicide-tolerant plant varieties is significant. Herbicides are simple chemical compounds that kill or inhibit the growth of plants without deleterious effects on animals. Herbicides usually inhibit the processes that are unique to plants, for example, photosynthesis. Most frequently, herbicides act as inhibitors of essential enzyme reactions. Thus, anything that diminishes the level of inhibition will provide increased herbicide tolerance.

The two most common sources of herbicide tolerance are:

(1) Over-production of the target enzyme and (2) Mutations resulting in enzymes that are less sensitive to the inhibitor (usually due to a lower affinity of the enzyme for the inhibitor). It seems likely that the most successful strategy for developing herbicide- tolerant plants will be to combine both sources of tolerance, that is, to engineer plants that overproduce herbicide-tolerant mutant enzymes. Herbicide resistant crops are -soybean, corn, canola, Transgene = EPSP synthase.

Disease-Insect-Resistant Varieties

Several microorganisms and certain native plants produce proteins that are toxic to specific plant pathogens, both microbial pathogens and insects that feed on plants. One goal of plant genetic engineering is to transfer the genes encoding these protein toxins to agronomically important plants with the hope that expressing the toxin genes in these plants will provide biological control Disease-insect of at least some plant diseases and insect pests. Currently, plant diseases resistant plants and insect pests are controlled almost exclusively by the use of broad- spectrum chemical bacteriocides, fungicides and insecticides. However, there is reason for concern about the potential damage to ecosystems and pollution of groundwater that might result from the widespread use of these chemicals on agricultural crops. Thus, scientists are searching for alternate methods for controlling these pathogens. The best-known example of the use of natural gene products to control plant pests are the insect toxins of Bacillus thuringiensis. Each of the toxin genes of B. thuringiensis encodes a large protein that aggregate, to form protein crystals in spores and these protein crystals are highly toxic to certain insects. Some of the insects that are killed by these protein toxins are plant pests of major economic importance. Different subspecies of B. thuringiensis produce toxins that kill different insects. For example, the toxin produced by B. thuringiensis subspecies kurstaki kills lepidopteran larvae such as the tobacco hornworm. The gene that encodes this toxin has been isolated and shown to synthesize a functional toxin in E. coli. A chimeric gene with the structure CaMV35S promoter/B. thuringiensis subspecies kurstaki toxin coding sequence/Ti nos 3′ termination sequence was constructed. This chimeric gene was placed in a Ti vector, and tomato leaf disc cells were transformed by co-cultivation with A .tumefaciens harboring the engineered Ti vector-chimeric gene construct.

BT Cotton:

The transgenic technology provides alternative and innovative method to improve pest control management which is ecofriendly, effective, sustainable and beneficial in terms of yield. The first genes available for genetic engineering of crop plants for pest resistance were cry genes (popularly known as Bt genes) from bacterium Bacillus thuringiensis. These genes are specific to particular group of insect pests and are not harmful to other useful insects such as butterfly, silk worms and honeybee. Transgenic crops (e.g., cotton, rice, maize, potato, tomato, brinjal, cauliflowers, cabbage, etc.) with Bt genes have been developed and such transgenetic variety proved effective in controlling insect pests and it has been claimed worldwide that it has led to significant increase in yield along with dramatic reduction in pesticide use.

Insect resistant cotton – Bt toxin kills the cotton boll worm transgene = Bt gene from Bacillus thuringensis

91. Genetically Modified Plants 3

Male Sterility:

Male sterile plants are very important to prevent unnecessary pollination and to eliminate the process of emasculation during the production of hybrid plants. Such sterile male plants are created by introducing a gene coding for an enzyme (barnase), which is an RNA hydrolyzing enzyme) that inhibits pollen formation. This gene is expressed specifically in the tapetal cells of anther using tapetal specific promoter TA29 to restrict its activity only to the cells involved in pollen production.

Transgenic Plants as Bioreactors (Molecular Farming)

Plants are amazing and cheap chemical factories that need only water, minerals, sunlight and carbon dioxide to produce thousand types of chemical molecules. Given the right genes, plants can serve as bioreactors to new compounds such as amino acids, proteins, vitamins, plastics, pharmaceuticals (peptides and proteins), drugs, and enzymes for food industries and so on.

Transgenic plants can be used for the following purposes:

Under the heading of ‘High-lysine com’, we have described how cereals rich in certain essential amino acids such as lysine, methionine and tryptophan can be developed by genetic engineering. Likewise, rice is being modified into Golden rice by Prof. Inge Potrykus and Dr. Peter Beyer. This is done so that vitamin A potential is maintained even after the husks are removed, a procedure adopted to allow for storage since the husks become rancid. This change may improve health of millions of people throughout the world.

(ii) Diagnostic and therapeutic proteins:

Transgenic plants can also produce a variety of proteins used in diagnosis for detecting human diseases and therapeutics for curing human and animal diseases in large-scale with low-cost. The monoclonal antibodies, blood plasma proteins, peptide hormones and cytokinins are being produced in trangenic plants and their parts such as tobacco (in leaves), potato (in tubers), sugarcane (in stems) and maize (in seed endosperm).

(iii) Edible vaccines:

Crop plants offer cost-effective bioreactors to express antigens which can be used as edible vaccines. The genes encoding antigenic proteins can be isolated from the pathogens and expressed in plants and such transgenic plants or their tissues producing antigens can be eaten for immunization (edible vaccines).The expression of such antigenic proteins in crops such as banana and tomato are useful for immunization of humans since both of these fruits can be eaten raw. Such edible vaccines of transgenic plants have the following advantages: lessening of their storage problems, their easy delivery system by feeding and low cost as compared to the recombinant vaccines produced by bacteria.

(iv) Biodegradable plastics:

Transgenic plants can be used as factories to produce polyhydroxy butyrate (PHB, biodegradable plastics). Genetically engineered Arabidopsis plants produced PHB globules exclusively in their chloroplasts without effecting plant growth and development. The large-scale production of PHB may be easily achieved in tree plants such as populus, where PHB can be extracted from leaves.

92. What Are the Advantages of GMOs

It allows for more profit

GMOs are an effective way to provide farmers a larger profit, while making them spend less time on resources.

It introduces the knowledge of genetic alterations.

This is done through mapping genetic material for GMO crops. This way, we would get the ability to enhance crop genes and make them more beneficial for human production and consumption.

Plants can be engineered to resist temperature or produce higher yields, which is good for regions where climate limits productivity.

It is economically efficient.

Because GMOs are designed to resist pests, there will be no need for pesticides to be used, which means more savings.

It is known to decrease food prices.

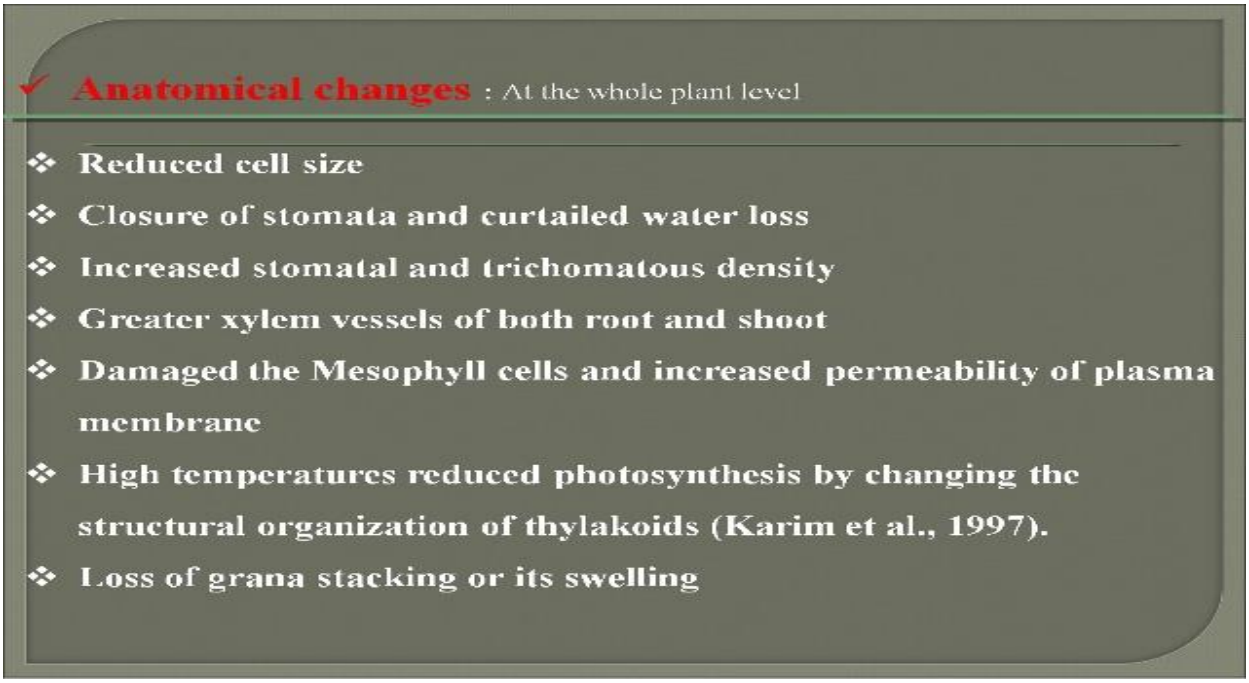
Advanced crops and lower costs can lead to cheaper food. This will certainly help families who cannot afford to buy their needed supply for everyday consumption, so starvation will be prevented.

It adds more nutritional value to crops.

The GMO method can put in added nutritional value to crops that lack necessary vitamins and minerals. Considering that there are places in the world relying on rice or corn as their daily staple, plant genes may be added to these crops to increase their nutritional value. This would help malnourished populations receive more nutrients from their diet.

Its products are found to be safe.

The precise evaluation and testing of GMOs crops and other products means they are safe for human consumption. In fact, research shows that they are safer compared with traditional crops.



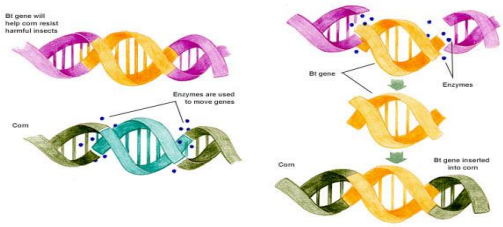
93.Hidden Threats from Genetically Modified Crops

GM crops and Food Safety

Since the 1980s several companies are aiming to develop genetically modified crops and to market them. Chemical company **Monsanto** dominates the market for seeds of GM crops - holding 70%–100% market share for various crops. Other companies: Pioneer, Syngenta and Bayer

Artificial organisms

GM crops are produced using laboratory techniques where **genetic material** from the cells of one species is transferred into another species and to artificially create **new** living organisms that would never naturally occur through breeding.



Risks for consumers

Because genetic engineering is a very imprecise technology, the insertion of foreign genes can stimulate the production of unexpected proteins. These may prove toxic or allergenic to those who consume them.

Types of GM Crops

There are two types of crops that are economically important: 1- Herbicide resistant crops → that can stand high doses of a specific herbicide 2- and in case of Monsanto's seeds: → Roundup Ready crops.

Types of GMO

There are 2 types of Bt crops, such as Bt corn and Bt cotton, these crops have an inserted gene from the bacteria *Bacillus thuringiensis* (*Bt*) which enables them to produce a poison which shall kill the dominant pests e.g. → corn borer, cotton bollworm.

Importance of GM crops

Worldwide, there is 9 percent global primary crop production from genetically modified crops, like in 2006: over 100 million hectares in 22 countries were of GM crops in which 64% of total were soya crop and 24% are of the global maize crop. Many processed foods contain Soybean oil and/or glucose syrup (from corn). It is difficult to avoid food containing genetically modified material, especially in the USA.

Animal feeds

Likewise animal feeds commonly contain corn and soybean meal from GM crops also in Europe GM material was found in milk and meat.

94. What are genetically modified foods?

What are genetically modified foods?

When scientists alter the genetic structure of a plant or animal in order to manufacture advantageous traits in the organism, they are engaging in what is called genetic modification (GM). The resulting product is a genetically modified organism (GMO). GM is a form of food biotechnology.

7 Most Common Genetically Modified Foods

Corn

Almost 85 percent of corn grown in the U.S. is genetically modified. Even Whole Foods's brand of corn flakes was found to contain genetically modified corn. Many producers modify corn and soy so they are resistant to the herbicide glyphosate, which is used to kill weeds.

Soy

Soy is the most heavily genetically modified food in the country. The largest U.S. producer of hybrid seeds for agriculture, Pioneer Hi-Bred International, created a genetically engineered soybean, which was approved in 2010. It is modified to have a high level of oleic acid, which is naturally found in olive oil. Oleic acid is a monounsaturated omega-9 fatty acid that may lower LDL cholesterol (traditionally thought of as "bad" cholesterol) when used to replace other fats.

Yellow Crookneck Squash and Zucchini

Numbers of this GMO veggie are relatively small, but genetically modified yellow squash and zucchini can be found in two different species. The species contain protein genes that protect against viruses. Just like their other GMO counterparts, you won't be able to tell the difference between non-GMO and GMO zucchini or squash.

Alfalfa

Cultivation of genetically engineered alfalfa was approved in 2011, and consists of a gene that makes it resistant to the herbicide Roundup, allowing farmers to spray the chemical without damaging the alfalfa.

Canola

Canola is genetically engineered form was approved in 1996, and as of 2006, around 90 percent of U.S. canola crops are genetically modified.

Sugar Beets

A very controversial vegetable, sugar beets were approved in 2005, banned in 2010, then officially deregulated in 2012. Genetically modified sugar beets make up half of the U.S. sugar production, and 95 percent of the country's sugar beet market.

Milk

To increase the quantity of milk produced, cows are often given rBGH (recombinant bovine growth hormone), which is also banned in the European Union, as well as in Japan, Canada, New Zealand and Australia.

95. Food Safety 1

Food Safety: Concerns

GM food is not labeled as such, the industry argues GM crops are "substantially equivalent" to their conventional counterparts and consequently no need for special considerations concerning safety.

Producers ensure

All food developers and manufacturers are required to ensure the safety and quality of their products. Producers of new foods have an obligation to ensure that the foods they offer consumers are safe and in compliance with applicable legal requirements (US Food, Drug and Cosmetic Act).

Are GM crops safe to eat?

According to the National Academy of Sciences, "genetic transfers between unrelated organisms do not pose hazards or risks different from those encountered by natural selection or traditional cross-breeding between similar species." "The process itself by which genes are transferred does not make living organisms harmful."

The risks: effects due to the insertion of genes into chromosomes of crops, roundup residuesand residues from Bt.

Risks due to the GM transformation process:

Advocates of GM crops: no concerns about safety because GM crop material is degraded during processing into feed and during digestion. (→ secretions of nucleases, enzymes which break down DNA, along the gut.)

Risks due to the GM transformation process

Since late 2005, three published studies by three different scientific teams and one unpublished study detected transgenic plant DNA in animal tissues and milk. It is likely that people are being frequently exposed to GM DNA by eating milk and meat from GM-fed animals, albeit at very low levels. Harmless protein in one organism can be harmful in another organism. One factor: post – translation modification. Rat feeding with GM potatoes showed that lesions in the gut wall of the rats were due to substances resulting from the process.

96. Food Safety 2

Roundup Ready crops are:

Most widely grown GM crop variety is 'Roundup Ready' soya: it tolerates applications of Monsanto's 'broad spectrum' glyphosate herbicide. Roundup destroys all other plants. Italian mouse trial: Roundup Ready soya affects key body organs. FSA human feeding trial: entire transgenic gene in GM soy survives the passage through the stomach and small intestine, though not through the colon. Roundup residues cause cell damage. Portions of transgenic DNA had 'horizontally' transferred from GM food into the intestinal bacteria of some of the volunteers another rat feeding: unexplained changes in testicle cells → indicator of toxins. Russian study: rats fed with **GM soy** showed a five-fold increase in mortality, lower birth weights, and the inability to reproduce. This Photo is showing stunted growth - the larger rat, 19days old, is from the control group; the smaller rat, 20 days old, and is from the GM soy" group.

Residues from Bt

Bt toxin is present in all the cells in Bt maize, it is the main GM maize used in animal feed and it can cause toxic reactions in rats (several studies). Twelve cows died mysteriously in Germany

when fed with Bt corn. In India, sheep died after grazing in cotton fields. More than 20 farmers observed: pigs and cows became sterile from GM corn.

Conclusion

Numerous feeding trails with rats and several observations by farmers indicate that the novel substances of GM crops are not as harmless as Monsanto and others state. Assessment of the list of risks indicates that GM crops are currently far too risky to be used for food or animal feed. To some extent: Humanity has been turned into a pack of guinea pigs*somebody or something experimented on: somebody or something used as the subject of an experiment or test. Producers and consumers need to be aware of the looming dangers. Information concerning products containing GM material is essential. In food preparations soy oil, cornstarch and glucose syrup should not be used unless origin is clear.

97. What Are the Disadvantages of GMOs?

It can be dangerous to other insects that are important to our ecosystem.

GMOs are believed to be dangerous to some insects because new crop genes can be deadly to them. This is worth noting when it comes to certain insects, such as butterflies, that are not actually dangerous to crops.

It sparks concerns on changing the field of agriculture.

The process of making GMOs includes adding new genetic material into an organism's genome. In agricultural ecology, this means introducing new genes in the genome of crops like corn. Research on the effects of cultivation of GM crops in a large scale has sparked various concerns, specifically those ideas on ecosystems with GMO strains. As proven by certain studies, GMO strains have the potential to change agriculture.

It can damage the environment.

Genetically modified crops can cause a threat to the environment due to the fact that they are not a natural way to plant and cultivate plants.

It causes unwanted residual effects.

A genetically modified plant can leave unwanted residual substances that can remain in the soil for extended periods of time. Agricultural regulators were alerted by research that strains from GM crops would remain in the soil for years after the crops were removed. Its data even reported that despite the absence of these plants, the strain persisted for up to more than 5 years.

It can create more weeds.

Take note that engineered crops can act as mediators in transferring genes to wild plants, which can create more weeds. To keep these new weeds under control, scientists then invented new herbicides that were not necessary for non-GMO weeds. These chemicals are also toxic to various mammals and amphibians, who are feeding on GMO crops. Tests even show that the uptake of these herbicides also has toxic consequences on aquatic ecosystems.

It threatens crop diversity.

There is opposition to introducing GM genes on genetic diversity because these genes can spread to other organic farm crops and threaten crop diversity in agriculture and if crop diversity decreases, it will have a direct impact on our entire ecosystem and would affect the population dynamics of other organisms. The chance that a single genetically modified crop strain could pollinate an already existing non-GM crop is unlikely and unpredictable, and there are many conditions that must be met for cross pollination to occur. However, when a large scale plantation releases a GM strain during pollination, this risk increases, where the cross pollination to non-GM plants could create a hybrid strain. This means there is a greater possibility of ecological novelty or new artificial strains that are being introduced into the environment that could potentially reduce biodiversity through competition.

It has trade issues.

In other countries and regions in the world, there may be problems regarding trade matters, such as tariff and quota.

98. Ethical Aspects of Agricultural Biotechnology 1

Ethics

Ethics can usefully be defined as the branch of philosophy concerned with how we should decide what is morally wrong and what is morally right. Ethical conclusions need to be based on reason, take into account historically well-established ethical principles, be based on consensus, take

account of minority interests and be open to the possibility of change. A useful tradition of ethical reasoning in the European Union and elsewhere is beginning to accumulate about moral questions concerning biotechnology. The simplest approach to deciding whether an action would be right or wrong is to look at what its consequences would be. Controversy exists as to whether that is all which is needed. Traditionally, ethics has concentrated mainly upon actions that take place between people at one point in time. In recent decades, however, moral philosophy has widened its scope by taking into account interspecific and intergenerational issues. Ethical decisions can be taken at a number of levels from the individual to the international. Some general ethical questions which relate to all applications of modern biotechnology include: 1 How to weigh the potential benefits against the possible costs? 2 Do the processes themselves constitute an "unnatural" interference with Nature, particularly in breaching natural species boundaries and violating the integrity of species? 3 What is ethically wrong with interfering with Nature? 4 Do the processes involve the taking of ethically unjustifiable risks? 5 From a religious viewpoint, is modern biotechnology to be interpreted as "playing God" or as collaborating in the on-going work of creation? 6 Do these questions suggest any significant ethical differences between modern biotechnology and more traditional techniques?

Food

Agriculture has always depended on plant and animal breeding and modern biotechnology provides new possibilities. Public perceptions of agro-food biotechnology are more critical than of its applications in healthcare. This probably results from the cultural and symbolic functions of food together with most people's relative ignorance about modern agriculture and food production. The most important areas where biotechnology can provide benefits for European consumers can be in improved price, quality and nutritional value of foods. Regulation has been mostly directed to the safety of foods. Labelling is still a controversial issue at the international level. The EC regulation on novel foods and novel food ingredients (258/97) includes a labelling requirement for a material not present in conventional equivalent foods which "gives rise to ethical concerns" to inform population groups with "well established" food practices. In the USA the Food and Drug Administration considers that no special labelling is required. Ethical considerations of agrofood biotechnology relate to the environment, biodiversity, sustainability, animal welfare and its socio-economic impacts. Consumers rights concerning biotechnological food products relate to the rights to health from safe foods, to be informed and to choose genetically engineered products or not. It is crucial that balanced information is provided to the public. Communication strategies should bring together the scientific, industrial and general communities to promote openness, dialogue and mutual understanding.

Environment

The current environmental problems that arise from agriculture stem from modern, intensive agricultural practices and not from the use of genetically modified crops, as the latter are only currently being introduced into European agriculture. The use of biotechnology may either

exacerbate or ameliorate these effects depending on how it is applied. It is therefore important, before applying new biotechnology, to consider the precautionary principle, the need for sustainable development and the need to maintain and possibly enhance agriculturally-important biodiversity. The latter is emphasised in the two case studies, one on the introduction of GMO crops into their centres of origin (frost tolerant potato) and the other on the importance of mediterranean biodiversity, taking the case of Greece.

99. Ethical Aspects of Agricultural Biotechnology 2

Medicine

Biotechnology offers many opportunities for the production of medicines, vaccines and other medical products using agricultural sources for further improvement of human and animal health. New products can be developed using this technology, or the production of already existing products made more cost-effective. The disadvantages of the use of biotechnology must not be overlooked: although safety regulations do exist unforeseen and unwanted consequences may still occur. The continued development of biotechnology in relation to the use of agriculture for medicine will undoubtedly raise new ethical questions and controversies. However, there is good reason to expect that the very considerable body of expertise that exists in relation to medical and other areas of ethics will help to give rise to some degree of consensus in many of these novel areas, though it must be recognized that ethical debate is characterized by conflicting arguments and viewpoints.

Industry

Industrial development of biotechnology in Europe varies both between different sectors of biotechnology and different areas within Europe. Environmental and food-related issues are more important in northern European countries, whereas production and employment tend to prevail in southern and eastern countries. The conclusions from public opinion surveys concerning applications of biotechnology are that:1 Usefulness is a precondition of support, in no case is a "not useful" application given support.2 People will accept some risk if the application is (a) useful and (b) morally acceptable.3 Moral concerns act as a veto regardless of views on risk and use.4 If risk is less significant than moral acceptability in shaping public perceptions, then public concerns are unlikely to be alleviated by technically based reassurances and other policy initiatives dealing solely with risks. Employment is in itself an ethical issue where biotechnology in Europe is concerned.

Developing Countries

The issues that are identified need to be addressed regardless of the technology used to manufacture or market a product; these need to be within the context of (agricultural) need and/or food resources. The view in countries where there is enough to eat, and where choice of what to eat is assumed may be significantly different from that pertaining in other countries. Choices need to be made by those who have to live with their consequences. Many of the issues identified which are of ethical concern are not specific to developing countries, but occur within parts of countries considered to be developed. The developing world is much too diverse to be treated as a whole. The issues which result from moving from "traditional" agriculture to industrialized agriculture are those which need consideration. Biotechnology *does* not lead to a loss of biodiversity; all modern agricultural techniques contribute both positively and negatively. Technology has the capacity to contribute to the empowerment of rural communities. 6 The increase in the world population will mainly occur in the developing countries and therefore food increase needs to occur in those countries. The developed world is supplying food to the developing world. Furthermore, governmental agencies control access to the staple crops that form the major starch, oil and protein sources. It is important to provide a mechanism for sustainable food production where and when needed. Developing countries contribute significantly to the added value made in agriculture. In this respect it could therefore be beneficial for these to set up a balanced system of intellectual property rights. There are five major players that contribute to agricultural research: governmental and public institutions, international institutions, non-governmental organizations and industry. Developing countries should be free to use their land according to their own view. The prejudices and views on industrialized agriculture which color the "Northern" approach to agricultural produce should not be imposed on those not getting enough to eat**.** Developing countries should be helped to have access to biotechnology based on their genetic resources (Convention on Biological Diversity). All parties to the Convention on Biological Diversity have an obligation "to provide for the effective participation in biotechnological research activities by those Contracting Parties, especially developing countries which provide the genetic resources for such research, and where feasible in such Contracting Parties". There will be costs associated with maintaining biodiversity within a center of origin, which should be borne by the international community.

100. Bio fertilizer

A bio fertilizer (also bio-fertilizer) is a substance which contains living microorganisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing

phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio fertilizers can be expected to reduce the use of synthetic fertilizers and pesticides. The microorganisms in bio fertilizers restore the soil's natural nutrient cycle and build soil organic matter. They can be grouped in different ways based on their nature and function.

Different types of bio fertilizers:

1. *Rhizobium* - This belongs to bacterial group and the classical example is symbiotic nitrogen fixation. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is reality utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40-250 kg N / ha / year is fixed by different legume crops by the microbial activities of Rhizobium.

***2. Azotobacter* -**

It is the important and well known free living nitrogen fixing aerobic bacterium. It is used as a Bio-Fertilizer for all non-leguminous plants especially rice, cotton, vegetables etc. Azotobacter cells are not present on the rhizosplane but are abundant in the rhizosphere region. The lack of organic matter in the soil is a limiting factor for the proliferation of Azotobaceter in the soil.

3. Azospirillum- It belongs to bacteria and is known to fix the considerable quantity of nitrogen in the range of 20- 40 kg N/ha in the rhizosphere in non- non-leguminous plants such as cereals, millets, Oilseeds, cotton etc.

4. Cyanobacteria- A group of one-celled to many-celled aquatic organisms. Also known as blue green algae

5. Azolla - Azolla is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga Anabaenaazollae. Azolla fronds consist of sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. Azolla is considered to be a potential biofertilizer in terms of nitrogen contribution to rice. Long before its cultivation as a green manure, Azolla has been used as a fodder for domesticated animals such as

pigs and ducks. In recent days, Azolla is very much used as a sustainable feed substitute for livestock especially dairy cattle, poultry, piggery and fish.

6. Phosphate solubilizing microorganisms(PSM)

7. AM fungi- An arbuscular mycorrhiza (AM Fungi) is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant.

8. Silicate solubilizing bacteria (SSB)- Microorganisms are capable of degrading silicates and aluminum silicates. During the metabolism of microbes several organic acids are produced and these have a dual role in silicate weathering.

9. Plant Growth Promoting Rhizobacteria (PGPR)-The group of bacteria that colonize roots or rhizosphere soil and beneficial to crops are referred to as plant growth promoting rhizobacteria (PGPR).

Importance of Bio-fertilizers: (i) They increase the yield of plants by 15-35%. (ii) Bio-fertilizers are effective even under semi-arid conditions,(iii) Farmers can prepare the inoculum themselves,(iv) They improve soil texture,(v) Bio-fertilizers do not allow pathogens to flourish, (vi) They produce vitamins and growth promoting bio-chemical's(vii) They are non-polluting