- (a) Which method is used for sterilization of thermolabile components of plant tissue culture medium?
- (a) The method used for sterilization of thermolabile components of plant tissue culture medium is **filter sterilization**. Stock solutions of the heat labile components are prepared and filter sterilized through a 0.22 μ m filter into a sterile container. The filtered solution is aseptically added to the culture medium, which has been autoclaved and allowed to cool to approximately 35-45 °C¹².

(b) What is reporter gene?

(b) A **reporter gene** is a gene that researchers attach to a regulatory sequence of another gene of interest in bacteria, cell culture, animals, or plants. <u>Such genes are called reporters because the characteristics they confer on organisms expressing them are easily identified and measured, or because they are selectable markers³.</u>

©Define fusogen with an example?

© A **fusogen** is a protein that promotes plasma membrane fusion among different cells. To be considered a fusogen, it must be required for fusion, fuse unfamiliar membranes, and be present on the fusing membrane when need be. <u>These cells include but are not limited too: gametes, trophoblasts, epithelial, and other developmental cells⁴.</u>

(d)State Two advantages of artificial seeds?

(d) Two advantages of artificial seeds are: 1 Easy and rapid seed production: Artificial seeds allow for the fast and simple creation of new plants, speeding up the growth cycle⁵. 2 Suitable for rare plant propagation: They are especially useful for growing rare plants that might not naturally produce enough seeds for propagation⁵.

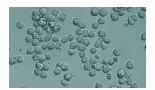
(e)Define Toti potency?

(e) **Totipotency** is defined as the ability of a single cell to produce a completely fertile, species-specific adult. <u>In biology, the word "totipotent" describes a cell with the capacity to divide and produce all the differentiated body cells, embryonic as well as extraembryonic⁶.</u>

(f)What is flavr savr tomato?

(f) The **Flavr Savr tomato** (also known as CGN-89564-2) is a genetically modified tomato and was the first commercially grown genetically engineered food to be granted a license for human consumption. <u>It was developed by the Californian company Calgene in the 1980s</u>⁷.

- (g) A **cybrid**, or cytoplasmic hybrid, is a eukaryotic cell line produced by the fusion of a whole cell with a cytoplast. Cybrids are cells or plants containing the nucleus of one species but cytoplasm from both the parental species⁸.
- (h)Name to direct gene transfer method?
- (h) Two direct gene transfer methods are: 1Physical gene transfer methods: These include electroporation, microinjection, and particle bombardment⁹. 2Chemical gene transfer methods: These include Polyethylene glycol (PEG)-mediated, diethyl amino ethyl (DEAE) dextran-mediated, and calcium phosphate precipitation⁹.
- 2.(a) What is suspension culture? mension its importantance?



Explore

A suspension culture is a type of cell culture in which single cells or small aggregates of cells multiply while suspended in an agitated liquid medium¹². It is also referred to as cell culture or cell suspension culture¹. The cultivation of free cells as well as small cell aggregates in a chemically defined liquid medium as a suspension was initiated to study the morphological and biochemical changes during their growth and developmental phases¹.

The importance of suspension culture includes:

- 1. Synthesis of secondary plant products: Suspension cultures may be used in the future for the whole or partial synthesis of secondary plant products, such as alkaloids, glycosides, etc³.
- 2. Solving problems in applied botany: Many problems in applied botany can be solved by cell culture³.
- 3. Obtaining secondary metabolites: Plant cell suspension cultures are used to obtain secondary metabolites in pharmaceuticals, cosmetics, or used in the food industry⁴.
- 4. <u>Analyzing metabolic pathways</u>: Researchers use plant cell suspension cultures to analyze metabolic pathways for basic science and even knockdown experiments⁴.
 - (b) write a shot note on the components of a plant tissue culture medium?



A plant tissue culture medium is composed of several components that are essential for the growth and development of plant cells, tissues, and organs in vitro¹²³⁴⁵. Here are the key components:

- 1. **Macronutrients**: These include elements like Nitrogen, Phosphorous, Calcium, Magnesium, Potassium, Sulfur, and Carbon. <u>These elements are required in large quantities for the growth and development of the plant²</u>.
- 2. **Micronutrients**: These are required in trace amounts but play a crucial role in cell and tissue growth. <u>It includes elements like iron, manganese, zinc, boron, copper, and molybdenum²</u>.
- 3. **Vitamins**: Vitamins are synthesized by plants in inadequate quantities. For this reason, media cultures are supplemented with vitamins. They are required for the growth and differentiation of the plants. <u>It includes thiamine</u>, riboflavin, niacin, pyridoxine, folic acid, pantothenic acid, biotin, ascorbic acid, myoinositol, Para amino benzoic acid, and vitamin E².
- 4. **Amino Acids**: They are the source of nitrogen for the cells, as it can be easily assimilated by the plants compared to the inorganic nitrogen. <u>Amino acids play a role in the enhancement of cell growth in culture including establishing the culture cells and protoplasts².</u>
- 5. Carbon and Energy Sources: Plant cells and tissues in the culture medium are heterotrophic and therefore, are dependent on the external carbon for energy¹.
- 6. **Growth Regulators**: These are plant hormones that regulate the growth and development of plants¹.
- 7. Solidifying Agents: These are used to provide a solid surface for the growth of plant tissues¹.

The composition of a culture medium is primarily dependent on the particular species of the plant and the type of material used for culture i.e. <u>cells, tissues, organs, protoplasts¹</u>. The media used may be solid (solid medium) or liquid (liquid medium) in nature¹.

Write a short note on Superbug?

A **Superbug** refers to strains of bacteria, viruses, parasites, and fungi that have developed resistance to most of the antibiotics and other medications commonly used to treat the infections they cause¹. The term "superbug" was developed by the media².

Superbugs are a significant concern because they can cause serious and even fatal infections, especially in people with weakened immune systems or those exposed to healthcare settings³. According to the 2019 Antibiotic Resistance Threat Report, published by the Centers for Disease Control and Prevention (CDC), more than 2.8 million drug-resistant infections happen every year in the United States, and more than 35,000 of them are fatal³.

The CDC's report lists 18 bacteria and fungi that endanger human health, classifying them as either urgent, serious, or concerning threats³. They include Carbapenem-resistant Acinetobacter, Candida auris, Clostridioides difficile, Carbapenem-resistant Enterobacteriaceae, Drug-resistant Neisseria gonorrhoeae, and others³.

To protect yourself from harmful germs and lower the risk of illnesses, it's recommended to wash your hands often with soap and water, handle food properly, avoid close contact with people who are ill, make sure your vaccinations are up to date, and use antibiotics as directed and only when needed¹.

what is de-differentiation? state its importance in plant tissue culture.

De-differentiation is a transient process by which cells become less specialized and return to an earlier cell state within the same lineage¹. It involves the reversal of the differentiated state of a cell, making it less specialized and enabling it to regain its ability to divide².

The importance of de-differentiation in plant tissue culture is significant:

- 1. **Generation of New Cells**: De-differentiation allows differentiated cells in a specific area of the plant body to restore their ability to divide. <u>This makes it possible for a part of the plant to produce new cells</u>³.
- 2. **Production of Callus**: The concept of de-differentiation is utilized to create a callus in plant tissue culture. A callus is a mass of unorganized parenchyma cells derived from plant tissue (explants) for use in biological research and biotechnology³.
- 3. Meristem Function: A de-differentiated tissue has the potential to function as a meristem, giving rise to a new set of cells³.
- 4. Plant Regeneration: De-differentiation plays a crucial role in plant regeneration, as it allows cells to revert to a less specialized state and then differentiate into a variety of cell types⁴.
- 3.(a) Discuss the process of T-DNA transfer from Agrobacterium tumefaciens to plant cells with the help of a flow chart.

Agrobacterium tumefaciens is a bacterium commonly used in plant genetic engineering to transfer genes of interest into plants. The process involves the transfer of T-DNA (transfer DNA) from the bacterium to the plant cells. Here's a simplified flow chart to illustrate the main steps of this process:

- 1. **Attachment and Recognition:**
 - Agrobacterium attaches to the wounded plant tissue.
 - Virulence (Vir) proteins aid in the recognition of the plant cells.
- 2. **Virulence (Vir) Proteins Activation:**
 - Activation of Vir proteins occurs in response to specific signals.
- 3. **Tumor-Inducing (Ti) Plasmid Formation:**
- Vir proteins facilitate the transfer of T-DNA from the Ti plasmid to the plant cell.
- 4. **VirD1 and VirD2 Processing:**
 - VirD1 and VirD2 endonucleases process the T-DNA region.
 - VirD2 protein remains attached to the 5' end of the T-DNA.
- 5. **T-DNA Transfer and Transport:**
- T-DNA, along with VirD2, is transported into the plant cell through a type IV secretion system.
- 6. **Integration into Plant Genome: **
 - T-DNA integrates into the plant genome.
- This integration is mediated by host factors and may involve recombination events.

- 7. **Expression of Genes of Interest:**
- Genes of interest carried by T-DNA are now part of the plant genome and can be expressed.
- 8. **Formation of Transgenic Plant:**
- The plant cells containing the integrated T-DNA develop into transgenic tissues.
- 9. **Regeneration of Transgenic Plant:**
 - Transgenic tissues are induced to regenerate into whole transgenic plants.
- 10. **Expression of Transgenes:**
 - Transgenes are expressed in the mature transgenic plant.
- This may result in the production of desired traits, such as resistance to pests or improved nutritional content.

It's important to note that the success of this process depends on various factors, including the type of plant, the specific genes being introduced, and the efficiency of the Agrobacterium strain used. This method is widely used in biotechnology to create genetically modified crops with desirable traits.

(b)

Direct and Indirect Organogenesis:

Organogenesis refers to the process of forming new organs from plant cells. Both direct and indirect organogenesis are methods used in plant tissue culture for the regeneration of whole plants from explants (small pieces of plant tissue).

1. **Direct Organogenesis:**

- In direct organogenesis, plant organs, such as shoots or roots, are directly induced from explants without the need for an intermediate callus phase.
- This process often involves the manipulation of plant growth regulators, particularly cytokinins and auxins, to stimulate the direct formation of shoots or roots.

2. **Indirect Organogenesis:**

- In indirect organogenesis, an intermediate callus phase is formed before the development of new organs.
- The callus is a mass of undifferentiated cells that can be induced to differentiate into shoots or roots by adjusting the concentrations of growth regulators.

Caulogenesis and Rhizogenesis:

Caulogenesis and rhizogenesis are specific types of organogenesis related to the formation of shoot (caulogenetic) and root (rhizogenetic) structures.

1. **Caulogenesis:**

- Caulogenesis is the process of forming new shoots or stems.
- It is an essential aspect of plant regeneration, especially in the production of transgenic plants.

2. **Rhizogenesis:**

- Rhizogenesis is the process of forming new roots.
- Inducing rhizogenesis is crucial for the development of a well-established root system in regenerated plants.

Importance of Organogenesis:

1. **Clonal Propagation:**

- Organogenesis is a key method for clonal propagation, enabling the rapid multiplication of plants with desirable traits. This is particularly useful in agriculture for the mass production of genetically identical plants.

2. **Genetic Transformation:**

- Organogenesis is often employed in genetic transformation techniques to introduce new genes into plants. This is vital for developing genetically modified organisms (GMOs) with improved traits such as resistance to pests, diseases, or environmental stress.

3. **Plant Breeding:**

- Organogenesis plays a role in plant breeding programs by allowing the efficient propagation of elite plant varieties with desirable characteristics.

4. **Conservation of Endangered Species:**

- Organogenesis is used in conservation efforts for endangered plant species. Tissue culture techniques can help preserve and propagate rare and endangered plants, contributing to biodiversity conservation.

5. **Research and Biotechnology:**

- Organogenesis is a valuable tool for studying plant development and physiology. It also supports research in biotechnology for the production of bioactive compounds, secondary metabolites, and pharmaceuticals.

Overall, organogenesis is a versatile and essential process with wide-ranging applications in plant biology, agriculture, and biotechnology.

Bt Cotton¹²³:

Bt cotton is a genetically modified pest-resistant plant cotton variety. It was created by inserting one or more genes from the bacterium Bacillus thuringiensis into the cotton genome¹. This modification allows the cotton plant to produce a natural insecticide in its tissues, which is harmful to many pests, including bollworms¹.

The introduction of Bt cotton has led to a reduction in the use of broad-spectrum insecticides to kill lepidopteran pests (some of which have developed pyrethroid resistance). This spares natural insect predators in the farm ecology and further contributes to noninsecticide pest management¹.

However, Bt cotton is ineffective against many cotton pests such as plant bugs, stink bugs, and aphids; depending on circumstances it may be desirable to use insecticides in prevention¹. It's also worth noting that there have been instances where pests have developed resistance to Bt cotton².

Despite these challenges, Bt cotton has become prevalent and makes up for more than 90% of the cotton fields at present². The high yield is due to the effective control of bollworms². There has been a drastic reduction in the application of chemical insecticides for bollworm control. It has led to higher profits for farmers².

OR

Bt cotton is a genetically modified (GM) variety of cotton that has been engineered to express a toxin called Bacillus thuringiensis (Bt). Here are some key points about Bt cotton:

1. **Bt Toxin Production:**

- Bt cotton is genetically modified to produce a protein toxin derived from the bacterium Bacillus thuringiensis.
- The Bt toxin specifically targets certain insect pests, particularly lepidopteran larvae (caterpillars).

2. **Insect Resistance:**

- The Bt toxin acts as an insecticidal protein. When pests feed on Bt cotton, the toxin binds to receptors in their digestive system, causing the gut lining to rupture and leading to the death of the insect.

3. **Reduced Pesticide Use:**

- Bt cotton has been widely adopted in agriculture due to its ability to resist specific insect pests. This has led to a reduction in the need for chemical insecticides, resulting in lower environmental impact and decreased health risks for farmers.

4. **Increased Yield and Productivity:**

- By reducing losses from insect damage, Bt cotton can contribute to increased crop yields and overall productivity. Farmers benefit from a more reliable and consistent harvest.

5. **Economic Impact:**

- The adoption of Bt cotton has had significant economic implications for farmers. The reduction in pesticide costs, coupled with increased yields, has contributed to improved profitability for many cotton farmers.

6. **Controversies and Concerns:**

- Despite its widespread adoption and benefits, Bt cotton has also faced controversies and concerns. Critics have raised issues related to the potential development of resistance in target pests, unintended ecological effects, and socioeconomic impacts on farmers.

7. **Global Adoption:**

- Bt cotton has been commercially grown in numerous countries around the world, including the United States, China, India, Brazil, and several African nations. Its adoption varies, and it has been a subject of ongoing debate in agriculture and biotechnology discussions.

8. **Continuous Improvement and Varietal Development:**

- Researchers continue to work on improving Bt cotton varieties, addressing challenges such as resistance management and expanding the range of pests targeted by the Bt toxin. New generations of genetically modified cotton varieties are being developed to enhance traits like drought tolerance and fiber quality.

Bt cotton is an example of how genetic engineering can be applied to improve crop traits, increase agricultural sustainability, and address challenges associated with pest management in cotton cultivation.

(c) Write short notes on herbicide resistant transgenic plant.

Herbicide Resistant Transgenic Plants 12345:

Herbicide-resistant crops (HRCs) are crops that have been made resistant to herbicides either through transgene technology or by selection in cell or tissue culture for mutations that confer herbicide resistance³. These crops have been genetically modified to withstand the application of specific herbicides that would normally kill the crop along with the targeted weeds³. This allows farmers to spray fields with herbicides, killing only the weeds and leaving the crop undamaged³.

The first commercial transgenic herbicide-resistant crops were introduced in the 1990s and have since gained widespread adoption in several major crops, including soybeans, corn, cotton, and canola⁴. Glyphosate-resistant (GR) crops now represent well over 80% of all transgenic crops grown worldwide².

The introduction of herbicide-resistant crops has revolutionized weed management in agriculture, leading to higher crop yields and lower costs of production¹. However, the widespread use of these crops has also led to the evolution of herbicide-resistant weeds, posing a significant challenge for sustainable agriculture¹.

Despite these challenges, herbicide-resistant transgenic crops continue to play a crucial role in modern agriculture, providing farmers with an effective tool for weed management¹.

OR/

Herbicide-resistant transgenic plants are genetically modified organisms that have been engineered to withstand the application of specific herbicides, allowing farmers to selectively control weeds while minimizing damage to the crops. Here are some key points about herbicide-resistant transgenic plants:

- 1. **Genetic Modification:** Herbicide resistance is typically achieved through the introduction of specific genes into the plant's genome. These genes often encode for enzymes that can detoxify or modify the herbicide, rendering it ineffective against the transgenic plant.
- 2. **Selective Weed Control:** Herbicide-resistant transgenic plants enable farmers to use potent herbicides to control weeds without harming the crop. This selective approach contributes to more efficient weed management, leading to increased crop yields and reduced reliance on other weed control methods.
- 3. **Types of Herbicide Resistance:** There are two main types of herbicide resistance in transgenic plants:
- **Single Herbicide Resistance: ** Plants engineered to resist a specific herbicide, allowing farmers to use that herbicide while sparing the crop.
- **Multiple Herbicide Resistance:** Some transgenic plants are designed to resist multiple herbicides, providing flexibility in weed management and reducing the risk of developing herbicide-resistant weeds.
- 4. **Common Herbicide-Resistant Crops:** Several crops have been genetically modified for herbicide resistance. Examples include soybeans, corn, cotton, and canola. Each of these crops may have specific herbicide-resistant traits tailored to the needs of the crop and the farming system.

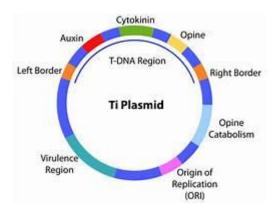
5. **Benefits:**

- **Increased Crop Yield:** Herbicide-resistant transgenic plants often lead to higher crop yields by minimizing competition with weeds.
- **Reduced Environmental Impact:** Targeted herbicide application can reduce the overall use of herbicides and their environmental impact.
- **Improved Efficiency:** Farmers can save time and resources by using herbicides more effectively, leading to increased efficiency in weed control.

- 6. **Controversies and Concerns:** The use of genetically modified organisms, including herbicide-resistant crops, has sparked debates over issues such as environmental impact, potential development of herbicide-resistant weeds, and the long-term effects on ecosystems.
- 7. **Regulation:** The cultivation and commercialization of herbicide-resistant transgenic plants are subject to regulatory approval in many countries. Governments and international organizations assess the safety and environmental impact of these crops before allowing their widespread use.

In summary, herbicide-resistant transgenic plants offer a targeted and efficient approach to weed management, providing benefits such as increased crop yields and reduced environmental impact. However, their adoption also raises concerns that necessitate careful regulatory oversight and ongoing research.

(c) Write short notes on Ti plasmid.



Ti Plasmid¹²³⁴:

The Ti plasmid, short for tumour-inducing plasmid, is an extrachromosomal DNA molecule found in the plant pathogen Agrobacterium tumefaciens and other species of Agrobacterium such as A.rubi, A.vitis, and A.rhizogenes¹⁴. It is part of a family of plasmids carried by many species of Alphaproteobacteria¹.

The Ti plasmid is essential for the bacteria to cause crown gall disease in plants¹. This is facilitated via certain crucial regions in the Ti plasmid, including the vir region, which encodes for virulence genes, and the transfer DNA (T-DNA) region, which is a section of the Ti plasmid that is transferred via conjugation into host plant cells after an injury site is sensed by the bacteria¹.

These regions have features that allow the delivery of T-DNA into host plant cells, and can modify the host plant cell to cause the synthesis of molecules like plant hormones (e.g. <u>auxins</u>, <u>cytokinins</u>) and <u>opines</u> and the formation of crown gall tumours¹.

Because the T-DNA region of the Ti plasmid can be transferred from bacteria to plant cells, it represented an exciting avenue for the transfer of DNA between kingdoms and spurred large amounts of research on the Ti plasmid and its possible uses in bioengineering¹.

The Ti plasmid is relatively large in size, ranging from 100kbp to 2Mbp². It has three important regions²:

- **T-DNA region**: Carries genes responsible for inducing tumors in plants. Other foreign genes of interest can be inserted in this region².
- **Vir region**: Consists of virulence genes. <u>It is responsible for the excision</u>, integration, and transfer of T-DNA into the plant chromosome².
- Opine catabolism region: Catabolizes opines, which are specialized amino acids. It is responsible for catabolizing opines produced by the T-DNA region².

Based on the differences in the T-DNA region, there are two types of Ti plasmids²:

- **Nopaline**: It has a continuous region of T-DNA, which is approximately 25 kb in length. It produces an opine known as Nopaline².
- Octopine: It is subdivided into two regions which are 13 kb and 8 kb long. <u>It produces an opine known as Octopine</u>².

<u>Ti plasmid can be used as a vector for transformation involving Agrobacterium tumefaciens and is used for producing transgenic plants². With the use of restriction enzymes, a particular gene of interest can be inserted into the plasmid and transformed². It is used for the development of stress-tolerant plant varieties².</u>

d)With suitable flow chart enumerate methods of protoplast isolation, purification and culture.

https://www.plantcelltechnology.com/blogprotoplast-culture-isolation-and-culture-methods/

or,

The process of protoplast isolation, purification, and culture involves several steps ¹²:

- 1. Selection of Plant Material: The protoplasts can be isolated from a variety of tissues including leaves, roots, in vitro shoot cultures, callus, cell suspension, and pollen¹². Among these, the mesophyll tissue of fully expanded leaves of young plants or new shoots is used most frequently¹².
- 2. <u>Sterilization of Leaves</u>: Healthy leaves are obtained from greenhousegrown plants and leaves are sterilized².
- 3. Peeling off the Lower Epidermis: After sterilization, the lower epidermis is removed with the help of fine forceps and the stripped leaves are cut into small pieces².
- 4. <u>Incubation in Enzyme Solution</u>: The leaf or callus tissues are placed in an enzyme solution (pH 5.5) prepared in 10-15% sorbitol or mannitol containing a small amount of CaCl2 (7 mM) for membrane stability². This solution is sterilized through a membrane filter (cold sterilization) and incubated for 4-12 hours (sometimes 0.5 to 20 hrs.) on a rocking shaker at 24-26 °C².
- 5. <u>Isolation and Cleaning of the Protoplasts</u>: After incubation, the solution is filtered through a wire or nylon mesh (50-100 µm) to remove debris (undigested cells, tissues, broken cells, etc.), transferred into screw-capped small centrifuge tubes (sterilized), and centrifuged at 100 g². The protoplasts form a pellet while the debris in the supernatant is carefully removed².
- 6. Protoplast Culture: The protoplasts are suspended in a culture medium¹. They are isolated and induced for wall formation¹. After the wall formation, the cells enter into the division phase, forming a clump of a few tissues followed by callus formation¹.
- 7. Plant Regeneration: Shoots are differentiated in callus and then plantlets are regenerated leading to the forming of a whole plant¹.

Or.

Importance of Haploid Culture:

- 1. **Accelerated Breeding:** Haploid culture allows for the rapid production of homozygous lines, speeding up the breeding process. This is particularly valuable in crop improvement programs.
- 2. **Genetic Studies:** Haploid plants provide a simplified genetic system, making it easier to study and understand gene function, inheritance, and the expression of traits.
- 3. **Creation of Genetic Variability:** Through haploid induction and subsequent chromosome doubling (doubling the chromosome number to restore diploidy), novel genetic variability can be generated for crop improvement.
- 4. **Disease Resistance:** Haploid culture can be employed to introduce and study genes associated with disease resistance, contributing to the development of disease-resistant crop varieties.

5. **Simplifying Genetic Modification:** Haploid cultures simplify genetic modification procedures, as the modification is done in a single set of chromosomes before doubling.

In summary, haploid culture is a valuable tool in plant biotechnology, providing a controlled environment for the generation of haploid plants and facilitating various applications in plant breeding and genetic studies.

(e) what is haploid culture? With suitable flow chart enumerate one method for anther culture. State the importance of haploid culture.

Haploid Culture¹²³:

Haploid culture is an in vitro technique used to produce haploid plants¹². Haploid plants are characterized by possessing only a single set of chromosomes (gametophytic number of chromosomes i.e. <u>n</u>) in the sporophyte¹. This is in contrast to diploids which contain two sets (2n) of chromosomes¹. Haploid plants are of great significance for the production of homozygous lines (homozygous plants) and for the improvement of plants in plant breeding programmes¹.

Method for Anther Culture⁴⁵⁶:

Anther culture is a type of tissue culture technique, where a part of a plant (anther) gives rise to an androgenic haploid plant when cultured on a nutrient medium under optimal conditions⁴⁵⁶. The process involves the following steps⁴⁵:

- 1. <u>Selection of Plant Material</u>: The anthers are taken from plants grown under controlled temperature, light, and humidity⁵.
- 2. Sterilization of Anthers: The anthers are sterilized⁵.
- 3. <u>Incubation in Enzyme Solution</u>: The anthers are placed in a suitable nutrient medium⁵.
- 4. <u>Isolation and Cleaning of the Protoplasts</u>: After incubation, the solution is filtered to remove debris⁵.
- 5. Protoplast Culture: The protoplasts are suspended in a culture medium⁵.
- 6. Plant Regeneration: Shoots are differentiated in callus and then plantlets are regenerated leading to the forming of a whole plant⁵.

Importance of Haploid Culture⁷⁸⁹¹⁰¹¹:

Haploid culture is an important component of biotechnology programmes in different countries⁷. They are used in plant breeding to obtain a pure homozygous line by using colchicine to double the chromosome number, or by depending on spontaneous mutations⁸. The major importance of haploid plant production is the improvement of plant or crop production⁸. Haploid production reduces the time

required to produce the improved cultivar of a specific plant². Haploids offer geneticists the opportunity to examine genes in the hemizygous condition and facilitate identification of new mutations¹¹. Plant breeders value haploids as a source of homozygosity following chromosome doubling from which efficient selection of both quantitative and qualitative traits is accomplished¹¹.

"chart dekhe flowchart baniye nibi../."