

BT403 – AGRICULTURAL BIOTECHNOLOGY

FINAL TERM MOST IMPORTANT QUESTIONS

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1. Why plant virus use gene transgene virus?

Plant viruses use gene transgene viruses to manipulate the genetic makeup of the host plant, aiding in viral replication and spread by altering the plant's gene expression to favor viral propagation.

2. How synthetic seeds are produced?

Synthetic seeds are produced by encapsulating somatic embryos, shoot tips, or other plant tissues in a protective coating composed of gel or other materials, allowing them to be stored and propagated like seeds.

3. Write principles of anther culture.

The principles of anther culture involve isolating anthers from plants, culturing them in a nutrient medium containing growth regulators such as auxins and cytokinins, inducing the formation of haploid cells or embryos, and regenerating plants from these cells through tissue culture techniques.

4. Write principles of meristem culture.

The principles of meristem culture involve isolating meristematic tissue from plants, sterilizing it to remove contaminants, culturing it in a nutrient medium supplemented with growth regulators, inducing the formation of shoots or roots, and regenerating whole plants from these tissues.

5. What is Agrobacterium mediated transformation?

Agrobacterium-mediated transformation is a genetic engineering technique where *Agrobacterium tumefaciens*, a soil bacterium, is used to transfer foreign DNA (transgene) into the genome of a plant cell. This process involves the bacterium's natural ability to transfer a segment of its DNA (T-DNA) into the plant cell, leading to the integration of the transgene into the plant's genome.

6. What is embryo culture?

Embryo culture is a technique where embryos are isolated from seeds or fruits and grown in a nutrient medium under sterile conditions to support their development into mature plants.

7. Write advantages of biofertilizers.

Advantages of biofertilizers:

- Enhance soil fertility and structure
- Increase nutrient availability to plants
- Improve plant growth and yield
- Reduce the need for chemical fertilizers
- Promote sustainable agriculture practices
- Enhance soil microbial activity and diversity
- Facilitate nutrient cycling in the ecosystem
- Decrease environmental pollution and soil degradation
- Can be cost-effective in the long term
- Compatible with organic farming practices

8. What are advantages and disadvantages of herbicides?

Advantages of herbicides:

- Effective in controlling weeds, reducing competition with crops
- Facilitate efficient land preparation and weed management
- Can be applied selectively, targeting specific weed species
- Aid in conserving soil moisture and nutrients by minimizing weed interference
- Help prevent the spread of weed-borne diseases and pests

Disadvantages of herbicides:

- Potential harm to non-target plants and organisms, including beneficial insects and wildlife
- Risk of contaminating water sources through runoff or leaching, leading to environmental pollution
- Development of herbicide-resistant weed species over time
- Concerns about human health risks associated with exposure to herbicide residues
- Disruption of ecological balance and biodiversity in agricultural ecosystems
- Dependency on chemical inputs, which may not be sustainable in the long term

9. Write outcomes of controlled pollination in plants.

Outcomes of controlled pollination in plants:

- Increased genetic diversity: Controlled pollination allows breeders to introduce desired traits into the offspring by selecting specific parent plants with desired characteristics.
- Maintenance of genetic purity: By controlling the pollination process, breeders can prevent unwanted cross-pollination with undesired varieties, ensuring the genetic purity of the resulting seeds or offspring.

- Facilitation of breeding programs: Controlled pollination enables breeders to efficiently produce hybrids or new varieties with desirable traits, such as disease resistance, high yield, or improved quality.
- Preservation of valuable traits: By controlling the pollination process, breeders can preserve and propagate valuable genetic traits present in specific plant varieties, helping to conserve genetic diversity and heritage varieties.
- Acceleration of plant improvement: Controlled pollination allows breeders to expedite the development of improved plant varieties by selectively crossing parent plants with complementary traits, leading to faster progress in breeding programs.

10. Write effect of charcoal in the anther and pollen culture.

The effects of charcoal in anther and pollen culture include:

- Adsorption of toxic compounds: Charcoal can adsorb toxic compounds released during tissue culture, thereby reducing their negative effects on the cultured cells or tissues.
- Enhanced growth and development: Charcoal can provide a source of carbon, which may stimulate growth and development of the cultured cells or tissues, leading to improved regeneration efficiency.
- Reduction of oxidation: Charcoal has antioxidant properties that can help protect the cultured cells or tissues from oxidative damage, thereby enhancing their viability and survival during culture.
- Prevention of browning: Charcoal can help prevent browning of the culture medium or explants by scavenging phenolic compounds and other substances responsible for oxidation reactions.
- Promotion of organogenesis: Charcoal may stimulate organogenesis in anther and pollen culture systems by providing a favorable environment for the initiation and development of shoots or roots from the cultured tissues.
- Overall improvement of culture performance: Charcoal supplementation in the culture medium can contribute to overall improvements in culture performance, including enhanced regeneration efficiency, reduced contamination, and improved quality of regenerated plants.

11. Write applications of micro propagation.

Applications of micropropagation include:

- Mass production of plants: Micropropagation enables rapid and efficient production of large numbers of uniform plants with desirable traits, such as disease resistance, high yield, or ornamental features.
- Clonal propagation: Micropropagation allows for the clonal propagation of elite plant varieties, ensuring the preservation and dissemination of valuable genetic material.
- Germplasm conservation: Micropropagation facilitates the conservation of endangered or rare plant species by providing a method for the propagation and preservation of germplasm in vitro.

- **Rapid multiplication of hybrids:** Micropropagation accelerates the multiplication of hybrid plants, enabling breeders to quickly produce large populations of hybrid plants for evaluation and selection.
- **Disease elimination:** Micropropagation provides a means for the production of disease-free plant material through the use of tissue culture techniques, such as meristem or shoot-tip culture, which can help in the elimination of pathogens from infected plant material.
- **Production of transgenic plants:** Micropropagation serves as a tool for the production of transgenic plants by facilitating the regeneration of whole plants from genetically modified cells or tissues, allowing for the study and application of genetic engineering in plant breeding and biotechnology.
- **Plant breeding:** Micropropagation supports plant breeding efforts by providing a rapid and efficient method for the multiplication, evaluation, and selection of plant variants with desired traits, leading to the development of improved crop varieties.

12. How plants response to diseases?

Plants respond to diseases through various mechanisms, which can include:

- **Physical barriers:** Plants have physical barriers such as cell walls, cuticles, and bark that act as a first line of defense against pathogens. These barriers can prevent pathogens from entering plant tissues.
- **Chemical defenses:** Plants produce a wide range of chemical compounds, such as phytoalexins and secondary metabolites, which can inhibit the growth of pathogens or deter herbivores. These chemicals may be produced constitutively or induced in response to pathogen attack.
- **Immune responses:** Plants possess sophisticated immune systems that recognize and respond to pathogen invasion. This involves the activation of various defense mechanisms, including the production of antimicrobial proteins, reactive oxygen species (ROS), and pathogenesis-related (PR) proteins. The recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) triggers these immune responses.
- **Systemic acquired resistance (SAR):** When a plant is infected by a pathogen, it can induce systemic acquired resistance throughout the plant. This involves the production of signaling molecules such as salicylic acid (SA), which activate defense responses in uninfected parts of the plant, making them more resistant to subsequent infections.
- **Hypersensitive response (HR):** In some cases, plants can mount a localized cell death response called the hypersensitive response (HR) at the site of pathogen infection. This response restricts the spread of the pathogen by killing the infected cells and forming a physical barrier around the site of infection.

- **Induced systemic resistance (ISR):** Beneficial microbes in the rhizosphere can induce systemic resistance in plants against pathogens. This involves the activation of defense responses by signaling molecules produced by the beneficial microbes.

13. Define transformation with examples and tell how plants are transformed.

Transformation, in biological terms, refers to the process of introducing foreign DNA (usually from another organism) into the genome of an organism, resulting in a heritable change in its genetic makeup. This genetic modification allows researchers to introduce new traits or characteristics into an organism for various purposes, such as enhancing crop yield, improving resistance to pests or diseases, or producing valuable compounds.

Examples of transformation include:

1. Bacterial Transformation: In molecular biology, bacterial transformation is a common technique used to introduce foreign DNA into bacterial cells. For example, the insertion of a gene encoding antibiotic resistance into bacterial cells allows for the selection of transformed cells on antibiotic-containing media.

2. Plant Transformation: Plant transformation involves the introduction of foreign DNA into plant cells, leading to the generation of transgenic plants. This can be achieved through various methods, including *Agrobacterium*-mediated transformation and biolistic (gene gun) transformation.

- ***Agrobacterium-mediated transformation:*** *Agrobacterium tumefaciens*, a soil bacterium, naturally transfers a segment of its DNA (T-DNA) into plant cells, leading to the integration of the foreign DNA into the plant genome. This method is widely used for transforming many plant species, including crops like soybean, cotton, and maize.

- ***Biolistic transformation:*** In biolistic transformation, foreign DNA is coated onto microscopic particles (such as gold or tungsten), which are then delivered into plant cells using a device known as a gene gun. The high-speed particles penetrate the plant cell wall and deliver the DNA into the cell nucleus, where it integrates into the genome. This method is particularly useful for transforming plants that are recalcitrant to *Agrobacterium*-mediated transformation, such as cereals and trees.

3. Animal Transformation: Animal transformation involves the introduction of foreign DNA into animal cells, resulting in genetically modified animals. This can be achieved through techniques such as microinjection, where DNA is directly injected into the nucleus of fertilized eggs, or viral vectors, where viruses are used to deliver the foreign DNA into animal cells.

14. Explain Murashige & Skoog medium.

Murashige and Skoog (MS) medium is a widely used nutrient medium in plant tissue culture for the growth and propagation of plant cells, tissues, and organs in vitro. It was developed by Toshio Murashige and Folke K. Skoog in 1962. MS medium provides essential nutrients, vitamins, and plant growth regulators necessary for the growth and development of plant cultures.

Key components of Murashige and Skoog medium include:

- 1. Macro and micronutrients:** MS medium contains inorganic salts that provide essential macro and micronutrients required for plant growth, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo).
- 2. Vitamins:** MS medium is supplemented with vitamins, particularly thiamine (vitamin B1), pyridoxine (vitamin B6), nicotinic acid (niacin), and myo-inositol, which are essential for plant growth and metabolism.
- 3. Plant growth regulators (PGRs):** MS medium can be supplemented with plant growth regulators such as auxins (e.g., indole-3-acetic acid, IAA; indole-3-butyric acid, IBA) and cytokinins (e.g., kinetin, zeatin) to regulate cell differentiation, proliferation, and organogenesis in plant tissue cultures. The concentrations and combinations of PGRs can be adjusted based on the specific requirements of the culture system and the desired outcomes.
- 4. Sucrose:** MS medium typically contains sucrose as a carbon source to provide energy for plant growth and development. The concentration of sucrose can vary depending on the type of plant tissue and the stage of culture.
- 5. Solidifying agent:** To solidify the medium and provide support for plant growth, MS medium is often supplemented with agar or agarose at a concentration of around 0.7-0.8%.

15. Write markers which help in plant breeding.

Markers used in plant breeding are genetic or phenotypic traits that can be easily observed, measured, or detected and are associated with specific genes or genomic regions of interest. These markers help plant breeders in selecting plants with desired traits and in accelerating the breeding process. Some common types of markers used in plant breeding include:

- 1. Molecular markers:** These markers are based on variations in DNA sequences and are detected using molecular techniques such as polymerase chain reaction (PCR) or DNA sequencing. Examples include:
 - Single nucleotide polymorphisms (SNPs)
 - Simple sequence repeats (SSRs) or microsatellites
 - Amplified fragment length polymorphisms (AFLPs)

- Restriction fragment length polymorphisms (RFLPs)
- Insertion-deletion polymorphisms (InDels)

2. Phenotypic markers: These markers are observable physical traits or characteristics that are controlled by one or more genes. Phenotypic markers can include:

- Morphological traits such as plant height, leaf shape, flower color, and fruit size
- Physiological traits such as disease resistance, drought tolerance, and nutrient use efficiency
- Agronomic traits such as yield, seed size, and maturity date

3. Quantitative trait loci (QTLs): QTLs are regions of the genome associated with quantitative traits that are controlled by multiple genes. Marker-assisted selection (MAS) is used to identify and select plants with desirable QTLs linked to traits of interest.

4. Genomic selection markers: Genomic selection involves using genome-wide marker data to predict the breeding value of individuals based on their genomic profiles. This approach utilizes statistical models to estimate the genetic merit of plants for multiple traits simultaneously.

5. Functional markers: These markers are linked to specific genes or gene variants that directly influence traits of interest. Functional markers can be derived from coding sequences, regulatory regions, or other functional elements in the genome.

16. What is post mitotic stage of anther?

The post-mitotic stage of anther development refers to the phase during which mitotic cell divisions have ceased, and the cells within the anther undergo differentiation to form the specialized structures involved in pollen production. This stage follows the mitotic phase of anther development and precedes the maturation and release of pollen grains.

During the post-mitotic stage, several key events occur:

1. Differentiation of cell layers: Cells within the anther differentiate into distinct layers or regions, including the outer epidermis, endothecium, middle layer, and innermost tapetum layer. Each layer plays a specific role in pollen development and maturation.

2. Formation of microsporocytes: Within the anther, specialized cells called microsporocytes undergo meiosis to produce haploid microspores, which are the precursors of pollen grains. This process occurs within the sporogenous tissue located in the center of the anther.

3. Degradation of tapetum: The tapetum, a nutritive tissue layer surrounding the developing microspores, undergoes programmed cell death (PCD) or degeneration. This degradation

provides essential nutrients and materials to support microspore development and pollen wall formation.

4. Wall formation and pollen maturation: The microspores undergo further differentiation and development, forming mature pollen grains with distinct outer walls (exine) and inner contents (cytoplasm and nucleus). The exine of pollen grains often exhibits intricate patterns and structures that aid in pollen dispersal and fertilization.

17. How sterilization of surface of explant is done?

Surface sterilization of plant explants is a critical step in tissue culture to remove any external contaminants, such as fungi, bacteria, and other microbes, that may interfere with the growth of cultured tissues or introduce unwanted infections. The method of surface sterilization typically involves a series of steps, which may vary depending on the type of plant material being used. Here's a general procedure for surface sterilization of plant explants:

1. Preparation of sterilization solution: Prepare a suitable sterilization solution, commonly consisting of a disinfectant such as bleach (sodium hypochlorite) or ethanol, diluted in water. The concentration of the sterilizing agent and the duration of sterilization may vary depending on the plant species and the sensitivity of the explant.

2. Rinsing: Rinse the plant material thoroughly under running tap water to remove any soil, dust, or debris adhering to the surface.

3. Immersing in sterilization solution: Place the plant material in a container filled with the sterilization solution. Ensure that the explants are fully submerged in the solution.

4. Agitation: Agitate the container gently to ensure thorough contact of the sterilization solution with the entire surface of the explants. This helps in dislodging and removing any adhering contaminants.

5. Sterilization: Depending on the protocol and plant material, immerse the explants in the sterilization solution for a specific duration, typically ranging from a few seconds to several minutes. The duration of sterilization may vary based on factors such as plant species, tissue type, and the presence of contaminants.

6. Rinsing in sterile water: After sterilization, transfer the explants to a container filled with sterile water (distilled or autoclaved) to rinse off the sterilization solution and remove any residual disinfectant.

7. Surface drying: Optionally, allow the explants to air-dry briefly on sterile filter paper or tissue paper to remove excess moisture from the surface. However, ensure that the explants do not desiccate excessively during this process.

18. Write name of area where protoplast fusion performed.

Protoplast fusion is typically performed in a laboratory setting, specifically in a controlled environment such as a tissue culture laboratory or a biotechnology research facility. There isn't a specific "area" designated for protoplast fusion, but rather it is conducted in specialized equipment and under sterile conditions to ensure the success of the fusion process.

19. Define parasitoids.

Parasitoids are organisms that are parasitic during a specific stage of their life cycle, typically during their larval stage, and ultimately kill or incapacitate their host organism. Unlike true parasites, which usually do not kill their hosts directly, parasitoids ultimately cause the death of their host as a result of their feeding or development. Once the host is killed, the parasitoid typically completes its development, undergoes metamorphosis, and emerges as an adult. Parasitoids are commonly used in biological control of pests, where they are employed to manage populations of insect pests in agriculture, forestry, and other ecosystems. Examples of parasitoids include certain species of wasps, flies, and some beetles.

20. Define oxidative stress.

Oxidative stress is a physiological condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms in cells. Reactive oxygen species, such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\bullet$), are highly reactive molecules that can damage cellular components, including proteins, lipids, and DNA. Under normal physiological conditions, cells maintain a balance between ROS production and antioxidant defenses. However, when this balance is disrupted, either due to excessive ROS production or impaired antioxidant capacity, oxidative stress occurs. Oxidative stress can lead to cellular damage, dysfunction, and ultimately contribute to the development of various diseases, including neurodegenerative disorders, cardiovascular diseases, cancer, and aging-related conditions. Factors such as environmental pollutants, UV radiation, toxins, inflammation, and metabolic processes can contribute to oxidative stress.

21. Write principles of electroporation.

The principles of electroporation involve the use of brief electrical pulses to create temporary pores or openings in the cell membrane, allowing the entry of exogenous molecules such as DNA, RNA, proteins, or drugs into the cell. Here are the key principles of electroporation:

1. Application of electric field: Electroporation is initiated by applying a short, high-voltage electrical pulse to the cells or tissues of interest. The electric field disrupts the lipid bilayer of the cell membrane, causing the formation of transient aqueous pores or nanopores.

2. Pore formation: The electric field-induced disruption of the cell membrane leads to the formation of temporary aqueous pores, which allow the passage of molecules across the

membrane. These pores can vary in size and duration depending on the amplitude and duration of the electrical pulse.

3. Facilitation of molecular uptake: Once the pores are formed, exogenous molecules present in the surrounding medium can diffuse into the cell through the openings in the membrane. This process enables the delivery of various biomolecules, such as nucleic acids (DNA, RNA), proteins, dyes, or drugs, into the cytoplasm or nucleus of the cell.

4. Reversible membrane permeabilization: Importantly, electroporation-induced membrane permeabilization is reversible, meaning that the pores reseal shortly after the electric field is removed. This allows the cells to recover their normal membrane integrity and physiological function.

5. Optimization of parameters: The efficiency of electroporation can be optimized by adjusting various parameters, including the amplitude and duration of the electrical pulse, the number of pulses, the cell density, and the composition of the electroporation buffer. Optimization of these parameters ensures efficient molecular delivery while minimizing cell damage and maintaining cell viability.

22. How growth regulators are classified on the basis of origin?

Growth regulators, also known as plant hormones or phytohormones, can be classified into different groups based on their origin. The major classes of growth regulators based on origin are:

1. Endogenous or Natural Plant Hormones: These growth regulators are synthesized naturally within plant tissues. They play crucial roles in regulating various physiological processes, including growth, development, and responses to environmental stimuli.

Endogenous plant hormones include:

- Auxins
- Cytokinins
- Gibberellins
- Absciscic acid (ABA)
- Ethylene
- Brassinosteroids
- Jasmonates
- Salicylic acid

2. Exogenous or Synthetic Growth Regulators: These growth regulators are synthesized artificially and applied externally to plants to manipulate their growth and development.

Exogenous growth regulators include:

- Synthetic auxins (e.g., 2,4-D, NAA)
- Synthetic cytokinins (e.g., kinetin, benzyladenine)
- Synthetic gibberellins (e.g., GA3)

- Synthetic abscisic acid analogs
- Synthetic ethylene-releasing compounds (e.g., ethephon)

23. Write advantages of biological markers.

Advantages of biological markers, also known as biomarkers, include:

- 1. Early disease detection:** Biomarkers can serve as early indicators of disease or physiological abnormalities, enabling early detection and intervention before symptoms manifest, which can improve treatment outcomes and prognosis.
- 2. Disease diagnosis and classification:** Biomarkers can aid in the diagnosis and classification of diseases, providing valuable information about disease subtype, severity, and progression.
- 3. Monitoring disease progression:** Biomarkers can be used to monitor the progression of diseases over time, assess treatment efficacy, and predict patient outcomes.
- 4. Personalized medicine:** Biomarkers can help identify individuals who are likely to respond to specific treatments or therapies, allowing for personalized treatment approaches tailored to individual patient needs.
- 5. Drug development and clinical trials:** Biomarkers can be used as surrogate endpoints in clinical trials to evaluate the safety and efficacy of new drugs or therapies, potentially speeding up the drug development process and reducing costs.
- 6. Prognostic prediction:** Biomarkers can provide prognostic information about disease outcomes, recurrence risk, and overall survival, aiding in patient counseling and treatment decision-making.
- 7. Non-invasive monitoring:** Many biomarkers can be measured using minimally invasive or non-invasive techniques, such as blood tests, urine tests, or imaging studies, making them convenient and accessible for patient monitoring.
- 8. Research tools:** Biomarkers serve as valuable tools for biomedical research, facilitating the study of disease mechanisms, biological pathways, and therapeutic targets.
- 9. Environmental monitoring:** Biomarkers can be used to assess environmental exposures, such as toxins or pollutants, and their effects on human health or ecological systems.
- 10. Predictive toxicology:** Biomarkers can help predict and assess the potential toxicity of drugs, chemicals, or environmental agents, enabling early identification of potential hazards and risk mitigation strategies.

24. What is germplasm preservation/conservation?

Germplasm preservation, or conservation, is the storage and management of genetic material (seeds, tissues, etc.) from plants, animals, or microorganisms to maintain biodiversity and ensure future use in agriculture, research, and conservation efforts.

25. Write 3 advantages of somatic embryogenesis.

Three advantages of somatic embryogenesis are:

1. Mass propagation: Somatic embryogenesis allows for the production of a large number of embryos from a small amount of starting plant material. This mass propagation capability facilitates rapid multiplication of elite or genetically modified plant lines for agricultural, horticultural, or forestry purposes.

2. Genetic uniformity: Somatic embryos are derived from single cells or tissues and develop into genetically identical plants. This uniformity ensures consistency in traits such as growth rate, yield, and quality among propagated plants, making somatic embryogenesis useful for producing clonal populations with desirable characteristics.

3. Genetic manipulation: Somatic embryogenesis provides a platform for genetic manipulation and transformation of plants. By introducing specific genes or genetic modifications into somatic cells, researchers can generate transgenic plants with desired traits, such as disease resistance, abiotic stress tolerance, or improved agronomic characteristics, for research, breeding, or commercial applications.

26. How crown gall disease occurs?

Crown gall disease is caused by the bacterium *Agrobacterium tumefaciens*. Here's how it occurs:

1. Infection: The bacterium enters the plant through wounds or natural openings, such as leaf scars or root tips.

2. Transfer of T-DNA: Once inside the plant, *A. tumefaciens* transfers a segment of its DNA, known as the T-DNA (transfer DNA), into the plant genome. This process is facilitated by the bacterium's natural ability to insert T-DNA into the plant's cells.

3. Tumor formation: The transferred T-DNA contains genes that cause the plant cells to proliferate uncontrollably, leading to the formation of a tumor-like growth known as a gall. These galls typically form at the crown of the plant, near the soil line, but can also occur on roots, stems, or other plant parts.

4. Nutrient supply: The bacterium modifies the plant's metabolism to provide nutrients and growth factors necessary for its own survival and the development of the gall.

5. Symptom development: As the gall grows, it disrupts the flow of water and nutrients within the plant, leading to symptoms such as stunted growth, wilting, and reduced yield. In severe cases, the plant may die due to the interference with its normal physiological functions.

27. What is importance of callus in tissue culture?

The importance of callus in tissue culture lies in its ability to regenerate into whole plants, serve as a target tissue for genetic transformation, produce secondary metabolites, and provide a source of pathogen-free plant material.

28. Write steps of tissue culture.

The steps of tissue culture typically involve:

- 1. Selection of explant:** Choose a small piece of plant tissue (explant) from the desired plant species, such as leaves, stems, or embryos.
- 2. Surface sterilization:** Treat the explant with disinfectants to remove surface contaminants and sterilize it to prevent microbial contamination.
- 3. Initiation of culture:** Place the sterilized explant onto a nutrient medium containing sugars, vitamins, minerals, and growth regulators, which provide the necessary nutrients and hormones for tissue growth.
- 4. Incubation:** Incubate the culture under controlled environmental conditions, including temperature, light, and humidity, to promote tissue growth and development.
- 5. Subculture:** Periodically transfer the growing tissue to fresh nutrient medium to maintain its growth and prevent overcrowding.
- 6. Differentiation:** Manipulate the growth conditions or hormone concentrations to induce the formation of specific cell types, organs, or embryos from the cultured tissue.
- 7. Rooting (if needed):** Stimulate the development of roots in the tissue culture to produce whole plants, especially for shoots or embryos that lack roots.
- 8. Acclimatization:** Transfer the rooted plants to soil or a potting mix and gradually acclimate them to ambient environmental conditions to prepare them for transplantation to the field or greenhouse.

29. Why viruses are used in gene transfer?

Viruses are used in gene transfer because they possess natural mechanisms for infecting host cells and delivering their genetic material into the cell's genome. This ability to transfer genetic material makes viruses effective vectors for introducing foreign genes into target cells for various purposes, including:

- 1. Efficiency:** Viruses have evolved sophisticated mechanisms to efficiently infect host cells and deliver their genetic material. This high efficiency of gene delivery increases the likelihood of successful gene transfer and expression in target cells.
- 2. Cell specificity:** Different viruses have tropism for specific cell types or tissues, allowing for targeted gene delivery to particular cell populations. This specificity can be advantageous for gene therapy applications targeting specific tissues or organs.
- 3. Integration into host genome:** Some viruses, such as retroviruses and lentiviruses, have the ability to integrate their genetic material into the host cell's genome. This integration allows for stable, long-term expression of the transferred gene in the host cell and its progeny.
- 4. Large cargo capacity:** Viral vectors can accommodate relatively large DNA inserts, making them suitable for delivering genes of interest, regulatory elements, and other genetic components into target cells.
- 5. Transient or stable expression:** Depending on the type of virus used and its integration characteristics, gene expression can be transient or stable. This flexibility allows researchers to choose the appropriate viral vector based on the desired duration and level of gene expression.
- 6. Versatility:** There is a wide variety of viral vectors available, each with unique characteristics and advantages. Researchers can select the most suitable viral vector for their specific application based on factors such as cell type, payload size, integration preferences, and safety considerations.

30. Describe haploid cells.

Haploid cells are cells that contain a single set of chromosomes, consisting of one copy of each chromosome (n), as opposed to diploid cells, which contain two sets of chromosomes ($2n$). Haploid cells are produced through the process of meiosis, which is a specialized type of cell division that reduces the chromosome number by half.

In sexually reproducing organisms, haploid cells are typically gametes, such as sperm cells in males and egg cells in females, which fuse during fertilization to form a diploid zygote.

Haploid cells contain a unique combination of genetic material due to the random assortment and recombination of chromosomes that occur during meiosis.

In some organisms, such as certain fungi and algae, haploid cells can exist as the predominant or only form of the organism's life cycle. These organisms undergo a type of life cycle called haplontic or haploid-dominant, where the haploid phase is the dominant phase, and the diploid phase is short-lived and transient. Examples include many types of fungi and some types of algae.

31. Write disadvantages of particle bombardment system.

Disadvantages of particle bombardment (biolistic) systems, which are used for genetic transformation in plants, include:

- 1. Low transformation efficiency:** Particle bombardment often results in low transformation efficiencies compared to other methods such as *Agrobacterium*-mediated transformation. This lower efficiency can make it challenging to obtain a sufficient number of transformed cells or plants.
- 2. Random integration:** The integration of exogenous DNA into the plant genome through particle bombardment tends to be random, resulting in unpredictable insertion sites and potential disruption of endogenous genes. This can lead to variable expression levels of the introduced gene and undesirable effects on host plant physiology.
- 3. Tissue damage:** The high-pressure shockwaves generated during particle bombardment can cause physical damage to the target tissues or cells, leading to reduced viability and regeneration capacity. This tissue damage may result in necrosis, cell death, or impaired growth, particularly in sensitive or delicate tissues.
- 4. Requirement for specialized equipment:** Particle bombardment systems require specialized equipment, such as a gene gun or particle accelerator, which can be expensive to purchase, operate, and maintain. Additionally, the procedure may require access to facilities with appropriate safety measures due to the high-pressure conditions involved.
- 5. Genetic instability:** The mechanical forces exerted during particle bombardment can induce genetic instability and chromosomal rearrangements in the target cells or tissues, potentially leading to unintended genomic alterations or instability in subsequent generations.
- 6. Limited host range:** Particle bombardment is not equally effective across all plant species and tissues. Some plants may be more amenable to transformation via particle bombardment, while others may exhibit low transformation efficiencies or poor regeneration capacity, limiting the applicability of the technique.

7. Regulatory considerations: The use of particle bombardment for genetic modification of plants may be subject to regulatory restrictions or public concerns regarding the safety and environmental impact of genetically modified organisms (GMOs), which can pose challenges for obtaining regulatory approval and market acceptance of transgenic crops.

32. Why synthetic seeds are produced?

Synthetic seeds are produced for clonal propagation, germplasm preservation, ease of handling, disease control, and customization.

33. What is inoculation in culturing?

In culturing, inoculation refers to the introduction of microorganisms, such as bacteria, fungi, or viruses, into a growth medium or host organism for the purpose of initiating or promoting their growth and reproduction. Inoculation is a crucial step in microbiological and biotechnological processes, such as microbial fermentation, cell culture, and plant tissue culture. It involves transferring a small quantity of the microbial inoculum (e.g., a culture or suspension of microorganisms) into the appropriate culture medium or substrate under sterile conditions. This allows the microorganisms to proliferate and form colonies or cultures, which can be further studied, manipulated, or utilized for various applications in research, industry, agriculture, and medicine.

34. Why synthetic seeds are better than natural?

Synthetic seeds offer advantages in terms of clonal propagation, germplasm preservation, ease of handling, disease control, and customization, making them superior to natural seeds in certain applications.

35. Write characteristics of cell suspension culture.

Characteristics of cell suspension culture include:

- 1. Homogeneous cell population:** Consists of single cells or small aggregates of cells suspended in a liquid medium.
- 2. High proliferation rate:** Cells exhibit rapid growth and division under optimal culture conditions.
- 3. Versatility:** Can be used for production of secondary metabolites, genetic transformation, and large-scale biomass production.
- 4. Ease of scale-up:** Culture volumes can be easily scaled up for industrial production.
- 5. Facilitates genetic manipulation:** Provides a platform for genetic engineering and selection of desired traits.

36. Write benefits of Agricultural Biotechnology.

Benefits of agricultural biotechnology include:

1. Increased crop yield and productivity
2. Enhanced nutritional quality of crops
3. Improved resistance to pests, diseases, and environmental stresses
4. Reduced reliance on chemical pesticides and fertilizers
5. Conservation of natural resources, such as land and water
6. Expansion of agricultural opportunities for smallholder farmers
7. Contribution to food security and sustainable agriculture.

37. Why legumes are preferred over tobacco?

Legumes are preferred over tobacco for certain biotechnological applications due to several reasons:

- 1. Economic importance:** Legumes, such as soybeans, peas, and beans, are important food crops that contribute significantly to global food security and agricultural economies. Therefore, research and development efforts focused on legumes align with broader agricultural priorities and have direct relevance to food production and human nutrition.
- 2. Wide acceptance:** Legumes are widely accepted as food crops and have a long history of consumption in various cultures worldwide. This acceptance facilitates the adoption of biotechnological advancements in legume crops for agricultural and commercial purposes.
- 3. Nutritional value:** Legumes are rich sources of protein, dietary fiber, vitamins, and minerals, making them valuable components of a healthy diet. Biotechnological improvements in legumes can enhance their nutritional quality, addressing malnutrition and dietary deficiencies in populations dependent on legume-based diets.
- 4. Sustainability:** Legumes have the ability to fix atmospheric nitrogen through symbiotic associations with nitrogen-fixing bacteria (rhizobia), which enhances soil fertility and reduces the need for synthetic nitrogen fertilizers. Biotechnological interventions in legumes can further enhance their nitrogen-fixing capabilities, contributing to sustainable agricultural practices and environmental conservation.
- 5. Diverse applications:** Legumes have diverse agricultural applications beyond food production, including animal feed, biofuel production, soil improvement, and agroforestry systems. Biotechnological advancements in legumes can benefit these various applications, promoting agricultural diversification and sustainability.

38. What are protoplasts? Write two methods of protoplast fusion.

Protoplasts are plant, fungal, or bacterial cells that have had their cell walls removed, leaving behind the plasma membrane and organelles. Protoplasts are used in various biotechnological applications, including genetic engineering, fusion experiments, and cell culture studies.

Two methods of protoplast fusion are:

1. Chemical fusion: Protoplasts from different organisms are treated with chemicals, such as polyethylene glycol (PEG), which promotes the fusion of their plasma membranes. The protoplasts are then mixed together in the presence of PEG, leading to the formation of hybrid cells through fusion of their membranes. This method is relatively simple and widely used for protoplast fusion experiments.

2. Electrofusion: Protoplasts are subjected to brief electrical pulses, which induce temporary pores or openings in their plasma membranes. When protoplasts from different organisms are exposed to these electrical pulses simultaneously, the pores allow for the fusion of their membranes, leading to the formation of hybrid cells. Electrofusion is a precise and controlled method that enables the fusion of protoplasts with high efficiency and specificity.

39. Write names of three plastids.

Three types of plastids are:

1. Chloroplasts: Chloroplasts are responsible for photosynthesis in plant cells, containing chlorophyll pigments that capture light energy to produce carbohydrates.

2. Chromoplasts: Chromoplasts are plastids that contain pigments other than chlorophyll, such as carotenoids and xanthophylls. They are responsible for providing colors to fruits, flowers, and other plant tissues.

3. Leucoplasts: Leucoplasts are colorless plastids that primarily function in the storage of starch, lipids, or proteins. Depending on their content, they can be further categorized into amyloplasts (starch-storing), elaioplasts (lipid-storing), and proteinoplasts (protein-storing).

40. What are pathogen attack strategies?

Pathogen attack strategies include penetration, secretion of toxins, manipulation of host physiology, evasion of host defenses, nutrient acquisition, disruption of communication, formation of specialized structures, and horizontal gene transfer.

41. Write disadvantages of PEG mediated transformation.

Disadvantages of PEG-mediated transformation include:

1. Low transformation efficiency.
2. Non-specific integration of exogenous DNA.
3. Damage to protoplasts during the process.
4. Risk of somaclonal variation.
5. Labor-intensive and time-consuming procedure.

42. Write importance of biotechnology derived vaccines.

Importance of biotechnology-derived vaccines includes:

- 1. Safety:** Biotechnology-derived vaccines are produced using highly controlled and standardized processes, resulting in vaccines that are generally safer and more consistent in quality compared to traditional vaccines.
- 2. Efficacy:** Biotechnology allows for the production of vaccines with improved efficacy, including recombinant vaccines that can stimulate a more robust immune response and provide better protection against infectious diseases.
- 3. Disease prevention:** Biotechnology-derived vaccines have played a crucial role in preventing and controlling infectious diseases, leading to significant reductions in morbidity, mortality, and healthcare costs associated with vaccine-preventable diseases.
- 4. Rapid development:** Biotechnology enables the rapid development and production of vaccines against emerging infectious diseases or newly identified pathogens. This agility is particularly valuable in responding to pandemics or outbreaks where timely vaccine deployment is critical.
- 5. Customization:** Biotechnology allows for the customization of vaccines to target specific strains or variants of pathogens, improving vaccine effectiveness and reducing the risk of vaccine escape mutants.
- 6. Reduced reliance on animal-derived components:** Biotechnology-derived vaccines can be produced using recombinant DNA technology or cell culture systems, reducing the need for animal-derived components and addressing ethical concerns associated with traditional vaccine production methods.
- 7. Global access:** Biotechnology-derived vaccines have the potential to be produced on a large scale and distributed globally, addressing vaccine shortages and improving access to vaccines in underserved regions of the world.

8. Platform for novel vaccine technologies: Biotechnology serves as a platform for the development of novel vaccine technologies, such as mRNA vaccines, viral vector vaccines, and virus-like particle vaccines, which offer new opportunities for vaccine design, delivery, and efficacy.

43. What are multitier viruses?

Multitier viruses, also known as multi-segmented or multi-component viruses, are viruses that have a genome consisting of multiple segments of nucleic acid (RNA or DNA) instead of a single contiguous molecule. Each segment typically encodes one or more proteins necessary for the virus's replication, assembly, or pathogenesis. These segments may be packaged separately into distinct viral particles or encapsidated together into a single virion.

Multitier viruses are found in various virus families across different taxa, including animal, plant, and bacterial viruses. Examples of multitier viruses include influenza viruses, which have segmented genomes consisting of eight RNA segments, and reoviruses, which have genomes composed of 10-12 segments of double-stranded RNA.

The presence of multiple genome segments in multitier viruses offers several advantages, including increased genetic flexibility, enhanced reassortment potential, and potential for rapid evolution. Reassortment, in particular, can lead to the emergence of novel virus strains with altered virulence, host range, or antigenic properties, posing challenges for disease control and vaccine development.

44.