BT403 –

AGRICULTURAL

BIOTECHNOLOGY

MID TERM MOST IMPORTANT QUESTIONS

PREPARED BY: SIR MUGHAL (ADMIN, BS ZOOLOGY ANNOUNCEMENT)

GROUP LINK: https://chat.whatsapp.com/BjC6W0jZx8o9yaC2ISh1Gu

1. How herbicide resistant crops can be reproduced?

Herbicide-resistant crops can be reproduced through selective breeding or genetic engineering.

1. Selective breeding involves identifying plants that naturally survive herbicide application, then crossbreeding them over generations to enhance resistance.
2. Genetic engineering is more precise and common. Scientists insert specific genes (often from bacteria) that provide resistance to certain herbicides, such as glyphosate. These transgenic plants are then propagated through traditional reproduction or tissue culture.

2. What are multipartite viruses?

Multipartite viruses are viruses that have their genome divided into two or more separate nucleic acid segments, with each segment packaged into a different virus particle. To successfully infect a host and replicate, all the necessary segments must enter the same host cell or at least the same organism. These viruses are common in plants, where multiple viral particles can infect a single cell more easily due to the structure of plant tissues. Each particle carries part of the genetic information, and only together can they complete the infection cycle and produce new virus particles.

3. Explain the process of development in plants.

The process of development in plants involves several coordinated stages:

1. ***Embryogenesis:*** Begins after fertilization, where the zygote divides and forms an embryo. The embryo develops into a basic structure with a root, shoot, and cotyledons (seed leaves).
2. ***Germination:*** When conditions are favorable, the seed absorbs water, swells, and the embryo resumes growth. The radicle (first root) emerges, followed by the shoot.
3. ***Seedling Growth:*** The young plant develops true leaves, and roots expand. Cell division in meristems (growth regions) allows for elongation and differentiation of tissues.
4. ***Vegetative Phase:*** The plant grows in size, developing stems, leaves, and roots. Photosynthesis supports further development.
5. ***Reproductive Phase:*** The plant forms flowers, leading to pollination and fertilization. Floral meristems replace vegetative ones.
6. ***Fruit and Seed Formation:*** Fertilized ovules develop into seeds, and the ovary becomes the fruit, enabling dispersal.
7. ***Senescence:*** The plant or its parts age and die, often after seed production is complete.

4. Write down the principle of Meristem Culture.

The principle of meristem culture is based on the use of the apical meristem, a region of actively dividing, and disease-free cells at the tip of shoots or roots, to regenerate whole plants in vitro. Since viruses usually do not invade the meristematic tissue, culturing this part allows for the production of virus-free, genetically identical plants under sterile, controlled conditions using nutrient media and plant growth regulators.

5. What is the use of CaMV gene transfer?

The CaMV gene transfer refers to the use of the Cauliflower Mosaic Virus (CaMV) 35S promoter in genetic engineering. Its main use is to drive strong, constitutive expression of inserted genes in plant cells. This promoter is widely used in genetically modified (GM) crops because it works efficiently in most plant species, ensuring that the desired gene (e.g., for pest resistance, herbicide tolerance, or improved nutrition) is continuously active in almost all parts of the plant.

6. Explain the mechanism of resistance in plants.

The mechanism of resistance in plants involves a series of defense responses that help the plant detect and defend against pathogens, pests, or environmental stress. It occurs in two main forms:

1. ***Constitutive Resistance:*** Always present in the plant. It includes physical barriers like thick cell walls, waxy cuticles, and chemical compounds (e.g., tannins, alkaloids) that deter invaders.
2. ***Induced Resistance:*** Activated upon detection of a threat. It includes:
   - **Recognition**: Plant receptors detect pathogen-associated molecular patterns (PAMPs) or specific effector molecules from pathogens.
   - **Signal Transduction**: This triggers signaling pathways involving molecules like salicylic acid, jasmonic acid, and ethylene.

- **Defense Activation**: Plants respond with localized defenses like the hypersensitive response (cell death around infection site) and systemic acquired resistance (SAR), producing pathogenesis-related (PR) proteins and antimicrobial compounds.

7. What are environmental effects of pesticides?

Pesticides can have several harmful environmental effects:

1. ***Soil Contamination:*** Pesticides can alter soil microbial communities, reduce fertility, and persist in the soil, affecting future crop growth.
2. ***Water Pollution:*** Runoff from fields can carry pesticides into rivers, lakes, and groundwater, harming aquatic life and contaminating drinking water sources.
3. ***Air Pollution:*** Sprayed pesticides can drift into the air, affecting nearby ecosystems and human health through inhalation.
4. ***Harm to Non-target Species:*** Pesticides can kill beneficial insects (like bees and butterflies), birds, and other wildlife, disrupting ecosystems.
5. ***Bioaccumulation and Biomagnification:*** Some pesticides accumulate in animal tissues and increase in concentration up the food chain, affecting predators, including humans.
6. ***Pest Resistance:*** Overuse can lead to pests developing resistance, making control harder and requiring stronger chemicals.

8. Describe the Crown Gall disease.

Crown Gall disease is a plant disease caused by the bacterium *Agrobacterium tumefaciens*. It primarily affects dicotyledonous plants like grapes, roses, and apples.

Key Features:

- ***Symptoms:*** The disease is characterized by tumor-like swellings (galls) at the crown (junction of root and stem), roots, or stems.
- ***Mechanism:*** The bacterium transfers a portion of its DNA (T-DNA) from a plasmid (Ti plasmid) into the plant genome. This foreign DNA causes uncontrolled cell division, forming the gall.
- ***Spread:*** Enters plants through wounds caused by tools, insects, or other injuries.
- ***Effects:*** Galls can disrupt water and nutrient flow, weaken plants, reduce yield, and sometimes lead to plant death.

9. Who activates charcoal effect on anther and pollen grains?

The activated charcoal effect on anther and pollen grains in plant tissue culture is primarily due to its ability to adsorb inhibitory substances. Activated charcoal is not "activated" by a specific person or agent in the culture; instead, it functions chemically by:

- Adsorbing phenolic compounds released from plant tissues, which can be toxic or inhibit growth.
- Improving nutrient availability and hormone balance in the medium by removing excess or harmful substances.
- Promoting embryogenesis and callus formation in anther and pollen cultures by creating a more favorable environment.

10. Write principle of electroporation.

Electroporation is based on the principle that exposing cells to a brief, high-voltage electric field creates temporary pores in the cell membrane. These pores allow foreign DNA or molecules to enter the cell. Once the electric pulse ends, the membrane reseals, trapping the introduced DNA inside. This method is commonly used in genetic engineering for gene transfer in plant, animal, and bacterial cells.

11. What are genetic markers and its types?

Genetic markers are specific DNA sequences or genes with known locations on chromosomes that can be used to identify individuals, track inheritance, or associate with particular traits.

Types of Genetic Markers:

1. ***Morphological Markers:*** Visible traits like flower color, seed shape, or leaf type used in classical genetics.
2. ***Biochemical Markers:*** Variations in proteins or enzymes detectable by electrophoresis (e.g., isozymes).
3. ***Molecular Markers:*** DNA-based markers that are highly specific and widely used in modern genetics, including:
    - **RFLP (Restriction Fragment Length Polymorphism)**
    - **SSR or Microsatellites (Simple Sequence Repeats)**
    - **AFLP (Amplified Fragment Length Polymorphism)**
    - **SNP (Single Nucleotide Polymorphism)**

12. What are disadvantages of PEG?

Disadvantages of PEG (Polyethylene Glycol), especially in plant genetic engineering and cell fusion, include:

1. ***Toxicity:*** PEG can be toxic to cells at high concentrations, causing reduced viability or cell death.
2. ***Low Efficiency:*** PEG-mediated gene transfer or fusion sometimes shows lower efficiency compared to other methods like electroporation.
3. ***Non-specific Action:*** It can cause unwanted fusion or uptake of molecules in non-target cells.
4. ***Difficult to Control:*** Precise control over dosage and exposure time is needed to avoid damaging cells.
5. ***Cell Damage:*** PEG can cause osmotic stress, damaging cell membranes and organelles.

13. What is direct organogenesis?

Direct organogenesis is a process in plant tissue culture where new organs (like shoots or roots) develop directly from explant tissues without an intermediate callus (undifferentiated cell mass) stage. In this method, cells in the explant differentiate and form organs under the influence of specific plant growth regulators, allowing rapid and genetically stable plant regeneration.

14. Explain charcoal effect on anther and pollen grains.

The charcoal effect on anther and pollen grains in plant tissue culture refers to how activated charcoal improves their growth and development by:

1. ***Adsorbing toxic substances:*** It removes phenolic compounds and other inhibitory metabolites released by anthers and pollen grains, which otherwise can damage cells or inhibit development.
2. ***Enhancing nutrient availability:*** By adsorbing excess hormones or impurities, it creates a more balanced culture environment.
3. ***Promoting embryogenesis and callus formation:*** This improved environment encourages the pollen grains or anthers to develop into embryos or callus, facilitating haploid plant production.

15. What is Plastid transformation?

Plastid transformation is a genetic engineering technique where foreign genes are introduced specifically into the plastid genome (usually chloroplast DNA) of plant cells instead of the nuclear genome.

Key points:

- Plastids have their own DNA and machinery for gene expression.
- Transgenes inserted into plastids are highly expressed and often maternal inherited, reducing gene flow via pollen.
- This method allows stable and high-level expression of desired traits like herbicide resistance or production of pharmaceuticals.
- It is done using biolistic (gene gun) methods or polyethylene glycol (PEG) mediated transformation on isolated plastids.

16. Describe haploid cell.

A haploid cell contains a single set of chromosomes (n), which is half the number of chromosomes found in diploid cells (2n). In plants and animals, haploid cells are usually gametes (e.g., pollen and egg cells in plants, sperm and egg in animals) formed through meiosis. Haploid cells are important for sexual reproduction because when two haploid gametes fuse during fertilization, they restore the diploid chromosome number in the offspring. In plant breeding and genetics, haploid cells can be cultured to produce haploid plants, which help in developing homozygous lines quickly.

17. What are benefits of synthetic seed over natural seed?

Benefits of synthetic seeds over natural seeds include:

1. ***Clonal propagation:*** Synthetic seeds are produced from somatic embryos or shoot buds, allowing exact genetic copies of the parent plant, preserving desirable traits.
2. ***Year-round production:*** Unlike natural seeds that depend on flowering and seed season, synthetic seeds can be produced anytime in vitro.
3. ***Disease-free planting material:*** They are free from pathogens that might infect natural seeds.
4. ***Easy storage and handling:*** Synthetic seeds can be encapsulated and stored under controlled conditions, improving shelf life.

5. ***Facilitates propagation of seedless or sterile plants:*** Useful for plants that do not produce viable seeds naturally.
6. ***Cost-effective mass multiplication:*** Enables large-scale multiplication of elite genotypes in less time.

18. What are symptoms of severe poison of pesticides?

Symptoms of severe pesticide poisoning in humans include:

- Nausea and vomiting
- Headache and dizziness
- Excessive sweating and salivation
- Difficulty breathing and chest tightness
- Muscle twitching or weakness
- Blurred vision or pupil constriction
- Confusion, seizures, or loss of consciousness
- Abdominal pain and diarrhea
- In extreme cases, respiratory failure or death can occur without prompt treatment.

19. Why plastid is used for transformation?

Plastids, especially chloroplasts, are used for transformation because:

1. ***High gene copy number:*** Plastids contain many copies of their genome per cell, leading to high-level expression of introduced genes.
2. ***Maternal inheritance:*** Plastid DNA is usually inherited maternally in most plants, reducing the risk of transgene spread through pollen and gene flow to wild relatives.
3. ***Ability to insert multiple genes:*** Plastids allow insertion of large DNA fragments or multiple genes organized in operons, enabling complex trait engineering.
4. ***Stable gene expression:*** Genes integrated into plastid genomes show stable and consistent expression without gene silencing common in nuclear transformation.
5. ***Reduced position effect:*** Plastid transformation avoids random insertion sites, reducing variation in gene expression.

20. Write steps of tissue culturing.

Steps of Tissue Culturing:

1. ***Selection and Preparation of Explant:*** Choose a healthy plant part (leaf, stem, root, and meristem) and sterilize it to remove microbes.
2. ***Initiation of Culture:*** Place the sterilized explant on a nutrient medium containing minerals, vitamins, sugars, and plant growth regulators under sterile conditions.
3. ***Multiplication (Callus or Shoot Induction):*** Explant cells divide and form callus (undifferentiated mass) or directly produce shoots, depending on the medium and hormones.
4. ***Shoot and Root Formation:*** Transfer the callus or shoots to a medium with hormones that promote shoot elongation and root development.

5. ***Hardening and Acclimatization:*** Gradually adapt the regenerated plantlets to external conditions by transferring them from culture to soil or greenhouse.
6. ***Transfer to Field:*** Once strong and acclimated, plants are transplanted to the field for normal growth.

21. What are steps of micro propagation?

Steps of Micropropagation:

1. ***Selection and Preparation of Explant:*** Choose healthy plant tissue (like shoot tips, nodal segments) and sterilize it to remove contaminants.
2. ***Initiation of Culture:*** Place the explant on a nutrient medium with suitable plant growth regulators to start cell division and growth.
3. ***Multiplication:*** Explants produce multiple shoots or callus through repeated subculturing on media that promote shoot proliferation.
4. ***Rooting:*** Transfer shoots to a rooting medium containing hormones like auxins to induce root formation.
5. ***Hardening (Acclimatization):*** Gradually adapt the plantlets to external conditions by reducing humidity and transferring them to soil or pots in a controlled environment.
6. ***Transfer to Field:*** After successful acclimatization, plantlets are moved to the field for normal growth.

22. What are symptoms of peripheral neuropathy?

Symptoms of **peripheral neuropathy** include:

- Numbness or tingling, often starting in the hands or feet
- Burning, stabbing, or sharp pain
- Muscle weakness or cramps
- Sensitivity to touch or temperature changes
- Loss of coordination or balance problems
- Reduced reflexes
- Feeling of "pins and needles" or electric shocks
- In severe cases, muscle wasting or paralysis

23. Write note on biomolecular markers.

Biomolecular Markers are specific sequences of DNA or proteins used to identify genetic differences, track inheritance, or associate with particular traits at the molecular level. They provide precise and reliable tools in genetics, breeding, and molecular biology.

Key points:

- They are DNA-based markers (molecular markers) like RFLP, SSR, AFLP, and SNP.
- Biomolecular markers are co-dominant, highly polymorphic, and not influenced by the environment.
- Used in genetic mapping, marker-assisted selection, genetic diversity studies, and identifying disease-resistant genes.
- Enable early and accurate detection of traits without waiting for physical characteristics to appear.

24. Explain that pesticides effects kids more than adults.

Pesticides affect kids more than adults because:

1. ***Developing Bodies:*** Children's organs and nervous systems are still growing, making them more vulnerable to toxic effects.
2. ***Higher Exposure Relative to Body Size:*** Kids breathe more air, drink more water, and eat more food per kilogram of body weight, so they absorb a higher pesticide dose proportionally.
3. ***Behavioral Factors:*** Children often play on the ground, put hands or objects in their mouths, increasing pesticide exposure.
4. ***Immature Detoxification Systems:*** Their liver and kidneys, which help detoxify harmful chemicals, are less developed, reducing their ability to eliminate pesticides.
5. ***Long-term Effects:*** Early exposure can disrupt development, causing lasting effects on brain function, growth, and immune health.

25. What is protoplast? Write two methods to isolate protoplast.

Protoplast is a plant cell that has had its cell wall removed, leaving only the plasma membrane enclosing the cytoplasm and organelles.

Two methods to isolate protoplasts:

1. ***Enzymatic Method***
   Uses enzymes like cellulase and pectinase to digest the cell wall gently, releasing protoplasts from plant tissues.
2. ***Mechanical Method***
   Involves physically breaking or crushing cells (less common and often damages cells) to release protoplasts, usually combined with enzymatic treatment.

26. What is Germplasm preservation?

Germplasm preservation is the process of conserving the genetic material (seeds, tissues, pollen, or whole plants) of plants or animals to maintain biodiversity and ensure availability for future breeding, research, or restoration. It helps protect valuable traits like disease resistance, yield, or climate adaptability by storing germplasm long-term under controlled conditions—such as seed banks, cryopreservation, or in vitro culture.

27. Write steps of callus culture.

Steps of Callus Culture:

1. ***Selection and Preparation of Explant***
   Choose a suitable plant tissue (leaf, stem, root) and sterilize it to remove contaminants.
2. ***Inoculation on Culture Medium***
   Place the explant on a nutrient medium (like MS medium) supplemented with appropriate concentrations of auxins and cytokinins to induce callus formation.
3. ***Incubation***
   Keep the culture under controlled conditions (temperature, light, humidity) to promote cell division and callus growth.

4. ***Callus Formation***
   Cells divide and form an unorganized, undifferentiated mass called callus at the explant site.
5. ***Subculturing***
   Transfer growing callus to fresh medium periodically to maintain growth and avoid nutrient depletion.
6. ***Further Differentiation (optional)***
   Callus can be induced to regenerate shoots or roots by changing hormone ratios in the medium.

28. What are characteristics of cell suspension?

Characteristics of Cell Suspension Culture:

1. ***Free-floating cells:*** Cells grow as single cells or small aggregates suspended in a liquid nutrient medium.
2. ***Rapid growth:*** Cells multiply quickly due to constant access to nutrients and oxygen.
3. ***Homogeneous culture:*** Uniform distribution of cells allows easy sampling and uniform treatment.
4. ***Nutrient and gas exchange:*** Better diffusion of nutrients and oxygen compared to solid media.
5. ***Used for secondary metabolite production:*** Ideal for extracting valuable compounds like pharmaceuticals.
6. ***Requires agitation:*** Constant shaking or stirring prevents cell clumping and sedimentation.
7. ***Short life span:*** Cells may lose totipotency over time and require periodic subculturing.

29. Write applications of DNA based biomarker.

Applications of DNA-based Biomarkers:

1. ***Genetic Mapping:*** Locating genes associated with important traits on chromosomes.
2. ***Marker-Assisted Selection (MAS):*** Accelerating plant and animal breeding by selecting individuals carrying desirable genes.
3. ***Genetic Diversity Analysis:*** Assessing variation within and between populations for conservation and breeding.
4. ***Disease Diagnosis:*** Detecting genetic disorders or susceptibility to diseases.
5. ***Forensic Science:*** Identifying individuals through DNA fingerprinting.
6. ***Gene Identification and Cloning:*** Locating and isolating specific genes of interest.
7. ***Population Genetics:*** Studying evolutionary relationships and gene flow.
8. ***Transgenic Confirmation:*** Verifying insertion of foreign genes in genetically modified organisms.

30. When culture of mature embryo is necessary?

Culture of mature embryo is necessary when:

1. Rapid propagation of plants that are difficult to propagate by seeds or cuttings is needed.
2. Overcoming seed dormancy or poor germination in certain species.
3. Producing haploid or doubled haploid plants for breeding programs via embryo culture techniques.
4. Rescuing embryos from wide crosses or interspecific hybridization where seeds may abort naturally.
5. Conserving endangered species by growing embryos under controlled conditions.
6. Studying embryo development and genetic traits in vitro.

31. What is role of chloroplast in gene transfer?

Role of Chloroplast in Gene Transfer:

Chloroplasts have their own genome, making them a target for plastid (chloroplast) transformation in genetic engineering. Their role includes:

1. ***Site for stable gene integration:*** Foreign genes can be inserted into the chloroplast DNA, ensuring stable inheritance and high expression.
2. ***High copy number:*** Multiple copies of chloroplast DNA per cell lead to high-level production of the introduced protein.
3. ***Reduced gene flow risk:*** Since chloroplast DNA is mostly maternally inherited, transgenes are less likely to spread through pollen.
4. ***Expression of multiple genes:*** Chloroplasts can express gene clusters as operons, useful for complex trait engineering.

32. What are advantages of herbicides?

Advantages of Herbicides:

1. ***Effective weed control:*** Quickly and efficiently kills or suppresses unwanted plants that compete with crops.
2. ***Increased crop yield:*** Reduces competition for nutrients, water, and light, boosting crop growth and productivity.
3. ***Labor-saving:*** Reduces the need for manual weeding, saving time and labor costs.
4. ***Cost-effective:*** Over large areas, herbicides are often cheaper than mechanical weed control.
5. ***Selective action:*** Many herbicides target specific weed species without harming crops.
6. ***Improved crop quality:*** Less weed interference means better quality produce.
7. ***Supports conservation tillage:*** Enables minimal soil disturbance farming, reducing erosion.

Disclaimer:

For best results, it is advised NOT to rely on this file only. Go through video lectures, handouts and other sources as well.

Regards,
Sir Mughal,
BS Zoology, Virtual University of Pakistan
B.Sc (Honors), University of Karachi.