Amaan Khan Contact: 0305-4716616	BT 403 Current Final Terms

Current Subjective Final Terms

2 marks

Qno1: What is Bio Compost?

It is one of the Eco-friendly products composed of waste material released from sugar industries which are decomposed. It is magnified with human-friendly bacteria, fungi, and various plants.

Qno2: What is biopesticides?

Biopesticide is a formulation made from naturally occurring substances that controls pests by non toxic mechanisms and in ecofriendly manner. Biopesticides are typically microbial biological pest control that is applied in a manner similar to chemical pesticides.

Qno3: What is need germplasm preservation?

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

Qno4: What is callus?

The explant gives rise to a callus (a mass of loosely arranged cells) which is manipulated by varying sugar concentrations and the auxin (low): cytokinin (high) ratios to form multiple shoots.

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3 marks

Qno1: Role of auxins in plant?

- Auxins stimulate cell elongation
- Influence a host of other developmental responses,
- Root initiation, vascular differentiation, tropic responses, apical dominance
- Development of auxiliary buds, flowers and fruits

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

Qno2: What are molecular marker and its types?

A sequence of DNA or protein that can be screened to reveal key attributes of its state or composition and thus used to reveal genetic variation it is Also known as "Genetic Marker".

Morphological markers:

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

Biochemical molecular markers

• A molecular marker is a molecule contained within a sample taken from an organism (Biochemical molecules)
• The first biochemical molecular markers used were the protein based markers.

• One of the earliest protein based markers to be used was Isozyme.

• These are different forms of an enzyme exhibiting the same catalytic activity but differing in charge and electrophoretic mobility.

DNA based markers

• The sequence of nucleotides in DNA of an individual is unique and thus determines its identity.

• The ultimate difference between individuals lies in the nucleotide sequence of their DNA.

• These can be used to diagnose the presence of the gene without having to wait for gene effect to be seen.

Qno3: Write down the ozone negative effects on plants.

The negative effects of ozone on plants

• Decreased rates of photosynthesis

• Leaf injury

• Reduced growth of shoots and roots

• Accelerated senescence

• Reduced crop yield

Qno4: Write down the steps for the initiation and maintenance of callus culture.

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Initiation and maintenance of callus cultures

- (1) Selection of suitable parent material
- (2) Choice of explant and method of isolation
- (3) Culture medium and conditions required
- (4) Optimization of culture conditions

Selection of suitable parent material

- Parent plant must be healthy and free from decay or disease
- Mother plant be actively growing and not be about to enter a period of dormancy

Choice of explant and method of isolation

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

Culture medium and conditions required

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

Optimization of culture conditions

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

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5 marks

Qno1: Environmental conditions that cause stress in plants.

Environmental conditions that can cause stress:

• Water-logging

• Drought

• High or low temperatures

• Excessive soil salinity

• Inadequate mineral in the soil

• Too much or too little light

Qno2: Explain protoplast optimum conditions.

• Optimal culture conditions:

1. Optimal density to the culture.

2. Optimal auxin to cytokinin ratio, glucose and sucrose.

3. Maintain osmoprotectant in the medium

4. Temperature: 20-28°C pH: 5.5-5.9 0.25% Casein hydrolysate BAP and NAA

Qno3: Write down the problems lab animals tested with GM food.

Lab animals tested with gm foods

• Stunted growth • Impaired immune systems

• Bleeding stomachs • Impaired blood cell development

• Misshapen cell structures in the liver, pancreas, and testicles

• Altered gene expression and cell metabolism

• Their offspring have a lower

• chance of survival

• Higher blood sugar

• Enlarged livers, pancreases

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• Inflamed kidneys

• Less developed brains and testicles

• Intestines reduced digestive enzymes

• The animal‟s life spans are shortened

• Inflamed lung tissue

Qno4: What is treatment of skin burning pesticides?

If the skin is burned from pesticides:

• Rinse well with cool water.

• Do not remove anything stuck to the burn.

• Do not apply lotions, fats, or butter.

• Do not break blisters.

• Do not remove loose skin.

• Cover the area with a sterile dressing, if available.

10 marks

Qno1: what are the applications of cell culture technique in crop improvement explain only 5 of them??

Applications

• The applications of various tissue culture approaches to crop improvement are

1. Breeding & biotechnology

2. Wide hybridization

3. Haploidy

4. Somaclonal variation

5. Synthetic Seed

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Plant Breeding and Biotechnology

• Plant breeding can be conveniently separated into two activities: manipulating genetic variability and plant evaluation. Historically, selection of plants was made by simply harvesting the seeds from those plants that performed best in the field.

• In spite of the general lack of integration of most plant biotechnology and plant breeding programmes, field trials of transgenic plants have recently become much more common.

• More than 50 different plant species have already been genetically modified, either by vector dependent (e.g. Agrobacterium) or vector independent (e.g. biolistic, micro-injection and liposome) methods.

• In almost all cases, some type of tissue culture technology has been used to recover the modified cells or tissues. In fact, tissue culture techniques have played a major role in the development of plant genetic engineering.

• Tissue culture will continue to play a key role in the genetic engineering process for the prictable future, especially in efficient gene transfer and transgenic plant recovery.

Wide Hybridization

• A critical requirement for crop improvement is the introduction of new genetic material into the cultivated lines of interest, whether via single genes, through genetic engineering, or multiple genes, through conventional hybridization or tissue culture techniques.

Haploids

• Haploid plants have the gametophytic (one-half of the normal) number of chromosomes. They are of interest to plant breeders because they allow the expression of simple recessive genetic traits or mutated recessive genes and because doubled haploids can be used immediately as homozygous breeding lines.

• The efficiency in producing homozygous breeding lines via doubled in vitro produced haploids represents significant savings in both time and cost compared with other methods.

Somaclonal Variation

• In addition to the variants/mutants (cell lines and plants) obtained as a result of the application of a selective agent in the presence or absence of a mutagen, many variants have been obtained through the tissue culture cycle itself.

• These somaclonal variants, which are dependent on the natural variation in a population of cells, may be genetic or epigenetic, and are usually observed in the regenerated plantlets.

Synthetic Seed

• Synthetic seed can be defined as the artificial encapsulation of somatic embryo, shoot, bud, or aggregates of cells or any tissue which has the ability to form a plant in vitro.

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• The use of synthetic seeds as an improvement on more traditional micropropagation protocols in facultatively propagated crops may, in the long term, have a cost saving, as the labour intensive step of transferring plants from in vitro to soil/field conditions may be overcome.

Qno2: Explain Electroparation and particle bombardment.

Electroparation

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

Principle:

• The phospholipid molecules of the plasma membrane are not static.

• When we apply electric field to them their kinetic energy increases resulting in the increase in the membrane permeability at certain points.

• This is exactly where we see the formation of electro-pores.

• The recombinant DNA can pass through these transient pores before they close.

Procedure

• In this process cells are mixed with the recombinant DNA and the mixture is placed in a small chamber with electrodes connected to a specialized power supply.

• Then a brief electric impulse is discharged across the electrodes, which makes pores (holes) in the plasma membrane.

• These pores remain for some time and are again resealed themselves. Recombinant DNA enters the cell which are removed and plated in fresh selective medium.

• The process of selection is then applied to isolate cells carrying recombinant DNA.

Advantages of electroporation:

1. This technique is simple, convenient and rapid, besides being cost-effective.

2. The transformed cells are at the same physiological state after electroporation.

3. Efficiency of transformation can be improved by optimising the electrical field strength, and addition of spermidine.

Limitations of electroporation:

1. Under normal conditions, the amount of DNA delivered into plant cells is very low.

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2. Efficiency of electroporation is highly variable depending on the plant material and the treatment conditions.

3. Regeneration of plants is not very easy, particularly when protoplasts are used.

Particle bombardment

• Biolistics is a method where cells are physically impregnated with nucleic acids or other biological molecules. • A biolistic particle delivery system is a device for plant transformation where cells are bombarded with heavy metal particles coated with DNA/RNA.

• This technique was invented by John Stanford in 1984 for introduction of DNA into cells by physical means to avoid the host-range restrictions of Agrobacterium.Agrobacterium-mediated genetic transformation system works well for dicotyledonous plants but has low efficiency for monocots.

• Biolistic particle delivery system provides an effective and versatile way to transform almost all type of cells.

• It has been proven to be a successful alternative for creating transgenic organisms inprokaryotes, mammalian and plant species.

• In this process, construct having gene of interest is coated on the surface oftiny particles of gold or tungsten (0.6 – 1 mm in size).

• Prior to coating, DNA is precipitated with calcium chloride, spermidine and polyethylene glycol.

• These coated microparticles are loaded on to the macrocarrier and accelerated to high speed by using pressurized helium gas.

• Plant cell suspensions, callus cultures, or tissues could be used as the target of these microprojectiles.

• As the microprojectiles penetrate the plant cell walls and membranes to enter the cells, coated DNA is released from its surface and incorporated into the plant's genome.

• In biolistics, use of binary vectors with T-DNA border sequences is not required.

• This method is especially important for monocots, for which efficiency of other transformation methods is not satisfactory.

• A wide range of tissues such as apical and floral meristems, embryos, seedlings, leaves, cultured cells and floral tissues could be used as target in this method.

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