VU MEDICAL ZONE

BT 403 Final terms Current Fall 2020

Qno1: Define Inoculation and their function. 2 marks

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

Qno2: What is Bio compost? 2 marks

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.

Qno3: Targets of ROS? 2 marks

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

Qno4: What is Biopesticides? 2 marks

Biopesticide is a formulation made from naturally occurring substances that controls pests by non toxic mechanisms and in ecofriendly manner. Biopesticides may be derived from animals (e.g. nematodes), plants (Chrysanthemum, Azadirachta) and micro-organisms (e.g. Bacillus thuringiensis, Trichoderma, nucleopolyhedrosis virus), and include living organisms (natural enemies) etc.

Qno5: What is need germplasm preservation? 2 marks

Germplasm Preservation

• Germplasm is living tissue from which new plants can be grown. It can be a seed or another plant part a leaf, a piece of stem, pollen or even just a few cells that can be turned into a whole plant.

• Germplasm contains the information for a species' genetic makeup, a valuable natural resource of plant diversity.

Qno6: Role of auxin in plants. 3 marks

Role of auxins

• Auxins stimulate cell elongation

• Influence a host of other developmental responses,

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• 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.

Qno7: Environmental conditions that causes stress in plants. 3 marks

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

Qno8: Characteristics of DNA based marker. 3 marks

• 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.

Qno9: Explain Herbicides action. 3 marks

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

Qno10: Define Herbicides. 2 marks

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A herbicide is a chemical substance used to control or manipulate undesirable vegetation, especially weeds. Herbicides are extensively used in gardening, farming, and landscape turf management.

Qno11: What are Somaclonal variations? 3 marks

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.

Qno12: Why kids are mostly affected by pesticides is compared to adults? 3 marks

Effects of Pesticides on Kids

• Children are especially susceptible to harmful effects of pesticides.

• They can easily become exposed to pesticides (via inhalation or skin contact) in schools, daycare, playgrounds, hospitals, and any other public areas, no matter how careful you are.

Qno13: How can you justify cell suspension? 2 marks

A cell suspension culture consists of cell aggregates dispersed and growing in a moving liquid media.

• It is normally initiated by transferring pieces of undifferentiated and friable callus to a liquid medium, which is continuously agitated by a suitable device.

Qno14: What are the side effects of pesticides to environment? 5 marks

Environmental effects

• Widespread use of pesticides in agriculture has experts worried due to their longterm environmental damage.

• Some pesticides can stick around for years, posing a very real threat to the ecological system and hence human health.

• Excessive and careless use of pesticides can contaminate water sources and soil, make fruits and vegetables less nutritious, and reduce biodiversity.

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• Some pesticides have also been linked to the dramatic reduction in the number of bees across the world, posing a huge threat to agriculture and food security, given that bees pollinate more than 70% of all crops.

Qno15: How ozone negatively affects the plants? 5 marks

The negative effects of ozone on plants

• Decreased rates of photosynthesis

• Leaf injury

• Reduced growth of shoots and roots

• Accelerated senescence

• Reduced crop yield

Qno16: Explain the steps for initiation and maintenance of cell culture. 10 marks

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 calluses responsive due to the presence of large no. of actively dividing cells

• Explants isolated in sterile conditions

• Ensure proper sterilization methods for particular explants.

Culture medium and conditions required

• Culture the sterilized explants on suitable autoclaved culture medium

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• Incubate cultures at 22-24 ± 2°C in light or dark

• Most calluses 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.

Qno17: Advantages of GMOs. 5 marks

It allows for more profit:

GMOs are an effective way to provide farmers a larger profit, while making them spend less time on resources.

It introduces the knowledge of genetic alterations:

• This is done through mapping genetic material for GMO crops. This way, we would get the ability to enhance crop genes and make them more beneficial for human production and consumption.

• Plants can be engineered to resist temperature or produce higher yields, which is good for regions where climate limits productivity.

It is economically efficient:

• Because GMOs are designed to resist pests, there will be no need for pesticides to be used, which means more savings.

It is known to decrease food prices:

• Advanced crops and lower costs can lead to cheaper food.

• This will certainly help families who cannot afford to buy their needed supply for everyday consumption, so starvation will be prevented.

Its products are found to be safe:

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• The precise evaluation and testing of GMOs crops and other products means they are safe for human consumption.

• In fact, research shows that they are safer compared with traditional crops.

Qno18: Describe the factors influence somatic embryogenesis. 5 marks

Factors influencing somatic embryogenesis

1. Auxin

2. Cytokinin

3. Nitrogen

4. Activated charcoal

5. Age of culture

Auxin:

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

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

Cytokinin:

• The role of cytokinin in embryogenesis is not clear. Embyogenesis in carrot cell suspension is stimulated by addition of zeatin in medium lacking auxin but inhibited by the addition of kinetin.

• Inhibitory effect of exogenous cytokinin may be due to an increase in endogenous cytokinin in growing embryoids.

Nitrogen

• The ratio of nitrogen to auxin is an important factor controlling embryogenesis. Embryo development can be initiated on White's medium with low nitrogen content only in absence of auxin. At low nitrogen concentration organic nitrogen is more suitable than inorganic nitrogen.

• Substances used as a source of nitrogen are potassium nitrate, ammonium chloride, glutamine, glutamic acid, alanine, urea etc.

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Activated charcoal

• Presence of activated charcoal in the medium helps embryogenesis in several cases.

• Activated charcoal may adsorb the inhibitory substances present in the medium.

Qno6: Define Transformation with example and how we transform the plants.

In molecular biology, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane. Transformation occurs most commonly in bacteria and in some species occurs naturally.

Plant transformation

• Plant transformation is a scientific approach whereby DNA from any organism is inserted into the genome of a species of interest.

• The inserted DNA is called a "transgene", and the resulting plant is said to be "transgenic". Transgenic plants can be obtained after transformation of single cells and the subsequent regeneration into complete, fertile plants by tissue culture protocols.

Qno19: How transformation of chloroplasts analysis? 3 marks

Analyses

• PCR – specific primers were used to assess the presence of aadA gene in resistant cell lines.

• Immunoblot analysis – using HRP-conjugated secondary antibodies.

• Southern and Northern blots were performed to look for target genes and their transcripts.

Qno8: Which tumor is used in Agrobacterium tumefaciens how we use it for plant?

Agrobacterium tumefaciens, was discovered and in the early 1970s the tumor inducing agent was found to be a DNA plasmid called the Ti plasmid.

By removing the genes in the plasmid that caused the cancer and adding in novel genes researchers were able to infect plants with A. tumefaciens and let the bacteria insert their chosen DNA into the genomes of the plants.

Qno20: Function of Gibberellins in the tissue culture of culture medium. 5 marks

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Gibberellins, although found in all plants and fungi, are unevenly distributed. The sites of synthesis are very young, unopened leaves, active buds, root tips and embryos.

• There are various gibberellin compounds with a similar gibbane nucleus but differ in the quality and position of the substituents of the nucleus. Gibberellic acid (GA3) is the most widely used compound. Gibberellins are normally used in plant regeneration.

• GA3 is essential for meristem culture of some species. In general, gibberellins induce elongation of internodes and the growth of meristems or buds in vitro.

• In its absence, the culture appears globular, due to the accumulation of nodes. Gibberellins usually inhibit adventitious root as well as shoot formation.

• During organogenesis, gibberellins are antagonistic. They seem to oppose the phenomenon of dedifferentiation.

• Thus, in in vitro cultures, they cannot be used for this purpose, but can be utilized for explants already organized (meristems, apices, buds).

Qno21: What are applications of cell culture technique in crop improvement, explain only five of them. 10 marks

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

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.

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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.

• 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.

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Qno22: Six different processes of GMOs crops. 10 marks

Cross Breeding:

• This technique has been used since the 1700s, it's when you take two sexually compatible crops and cross pollinate them to produce a hybrid.

• Some examples are the plumcot (plum and apricot), tangelos (tangerine and grapefruit), the limequat (lime and kumquat) and most famously the rabbage (cabbage and radish).

Mutagenesis

• Mutations (muta) are genetic changes that can switch, add, or delete nucleotides (those A,T,G and C bases), these genetic changes can sometimes lead to new/enhanced traits which is why plant breeders sometimes induce (genesis) these genetic changes using radiation or chemicals.

• Hermann Muller, Charlotte Auerbach and J. M. Robson founded this technique in the first half of the 20th century.

• For example, radiation was used to produce a deeper color in the red grapefruit.

Protoplast Fusion

• Sounds scary right? It's actually when you take two plant cells which have their hard cell walls removed (Protoplasts) and you add a chemical called polyethylene which allow the two cells to stick together.

• Once they are stuck together basic chemicals are added to help the two cells combine and exchange genetic information to create a hybridized plant cell (fusion).

Polyploidy

Polyploidy have more than one, and the induction of polyploidy is used by plant breeders to control reproduction. Introducing polyploidy by soaking seeds in colchcine can either make sterile crosses fertile, like the Triticale (hybrid of wheat and rye), or sterilizes crops, like watermelon, to make seedless strains.

Genome Editing

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• This process has the ability to cut, replace or insert genes within the seed cells using "molecular scissors" called nucleases—enzymes which have the ability to loosen, remove and add nucleotides. These nucleases are artificially engineered to accurately place in desired genes, or traits, into the genome of the crop.

Transgenisis:

• When genes from one crop are incorporated into another crop. Since the genetic code is readable by all living organisms, this means that the genes introduced will code for the same proteins as it did before.

• There are many ways to introduce these new genes, like using agrobacterium to carry it into the genome, or using electricity.

Qno23: Write a note on Heat stress. Heat stress can be bad for plants. 5 marks

Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development.

• Heat stress affects plant growth throughout its ontogeny, though heat-threshold level varies considerably at different developmental stages.

• Protein denaturation and aggregation

• Increased fluidity of membrane lipids.

• Enzymes inactivation

• Inhibition of protein synthesis and degradation

• Loss of membrane integrity

Qno24: Explain the physiological alternation due to biotic stress. 5 marks

Water and mineral absorption and translocation

• Plant Pathogen such as fungi that cause Damping off and root rot diseases, bacteria, most Nematodes and some viruses extensively damage roots, which directly affects the functioning of roots resulting in reduced absorption of water and minerals from the soil.

• Water & mineral absorption is also reduced by some vascular pathogen that inhibits root hair formation.

• Physical blockage in xylem vessels lead to prevention water & mineral translocation.

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• Eg. Agrobacterium tumefaciens [Crown gall disease] Plasmodiophora brassicae [Clubroot disaese]

Transpiration

• Rate of transpiration increases in plants after certain disaese infection [leaf rust, Apple scab, Downy Mildew].

• This is because, these disaeses result in destrucion of protection devices in plants like cuticle & epidermis, increase permeability of leaf cells and lead to the dysfunction of stomata resulting in uncontrolled loss of water and loss of turgor to which wilting of leaves follows.

Photosynthesis

• Photosynthesis is tremendously decreased after pathogenic infection.

This is carried out by: • Reduction in photosynthetic area :

1. Plant diseases

• Plant diseases that attack green aerial tissues [like leaf spot, blights] render harmful effects by destructing photosynthetic leaf tissue or defoliation, which result in lessening the photosynthetic area thereby reducing the photosynthesis. • eg. Complete defoliation of potato leaves after infected by blight disease.

ii. Reduction in Chlorophyll content

• Chlorosis is one of the most common symptoms of plant disaese and refers to the destruction or inhibition of chlorophyll per chloroplast subsequent to infection and usually occurs in young growing leaves. It is resulted by viral infection.

• Eg. Tobacco mosaic disaese, vein chlorosis of Vindi etc.

• It is carried out by the enzyme chlorophyllase secreted after disaese infection in leaves that causes conversion of chlorophyll to chlorophyllide and phytol.

iii. Effect of Toxins

• In some fungal and bacterial disaeses Photosynthesis is reduced because of toxins such as tabtoxins and tentoxin secreted by the pathogen itself.

• These toxin inhibit some of the enzymes that are directly involved in photosynthesis.

• Tabtoxin is not so toxic but when it hydrolyzed in the host cell, it releases. Tabtoxinine which is the active toxin molecule.

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Qno25: Write a note on Regeneration of plants in culture. Also write direct and indirect organogenesis. 10 marks

Plant regeneration

• The process of growing an entire plant from a single cell or group of cells.

• Regeneration is possible because plant cells can be made totipotent using hormones.

• Differentiated tissue: stems, leaves, roots, etc.

• Undifferentiated (embryonic) cells are totipotent: can become a whole new plant by differentiating into a whole new plant.

Indirect Organogenesis

• Formation of organs indirectly via a callus phase is termed indirect organogenesis.

• Induction of plants using this technique does not ensure clonal fidelity, but it could be an ideal system for selecting somaclonal variants of desired characters and also for mass multiplication.

• Induction of plants via a callus phase has been used for the production of transgenic plants in which

(1) The callus is transformed and plants regenerated or

(2) The initial explant is transformed and callus and then shoots are developed from the explant.

Direct Organogenesis

• The production of direct buds or shoots from a tissue with no intervening callus stage is termed direct organogenesis.

• Plants have been propagated by direct organogenesis for improved multiplication rates, production of transgenic plants, and—most importantly—for clonal propagation.

• Typically, indirect organogenesis is more important for transgenic plant production.

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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.

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?

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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.

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

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• (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

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• 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

• Inflamed kidneys

• Less developed brains and testicles

• Intestines reduced digestive enzymes

• The animal‘s life spans are shortened

• Inflamed lung tissue

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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

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.

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• 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

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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.

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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.

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.

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• 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.

VU Medical Zone effort by Amaan Khan