UNIVERSITY OF JOHANNESBURG

FACULTY OF SCIENCE

DEPARTMENT OF BIOTECHNOLOGY AND FOOD TECHNOLOGY
B. TECH. BIOTECHNOLOGY

MODULE	PBT1YP4 PLANT BIOTECHNOLOGY
CAMPUS	**DFC**

Supplementary Examination 2019

DATE: 9 January 2019 **SESSION: Morning (8:00 – 11:00)**

EXAMINER: **MISS S. M. MOYO**

INTERNAL MODERATOR: **MS S. PELO**

EXTERNAL MODERATOR: **DR M.J. BAPELA**

DURATION: 3 Hours **MARKS 103**

NUMBER OF PAGES: (INCLUDING 1ST PAGE) 7

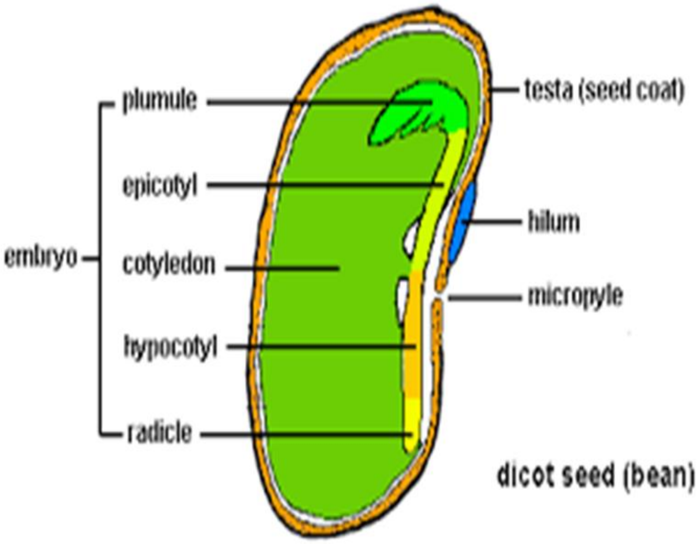
INSTRUCTIONS TO STUDENTS:

1. Answer all questions in the test answer book provided.
2. Number your answers correctly and clearly, marks will be deducted for untidy and illegible handwriting.
3. Questions may be answered in any order, but sub-sections of questions must be answered together.
4. Good luck!

QUESTION 1:

1.1 Describe the structural parts of a seed and label the diagram below

- The embryo consists of a plumule, epicotyl, cotyledons, hypocotyl, and a radicle.
- The plumule includes the young primordial leaves and growing point of the stem.
- The epicotyl is the portion of the stem above the cotyledon.
- The cotyledons are the seed leaves used for food storage.
- The hypocotyl is the portion of the stem below the cotyledons.
- The radicle is the young embryonic root and root tip.



(15)

1.2 Describe the environmental factors that affect seed germination (16)

Moisture, temperature, oxygen, and light are four environmental factors that affect seed germination

Moisture

The first step in the germination process is the absorption of water into the seed.

A seed must have an ample supply of moisture for germination to occur. Seeds fail to germinate if chemical processes that change insoluble starches to soluble sugars do not occur.

Moisture content needed for seed germination to occur ranges from 25% to 75%. For example, small grains germinate when their seed moisture content is about 50%.

Grain sorghum will germinate if their moisture content is approximately 26%. Soybeans will not germinate unless their moisture content is about 75%. Once the germination process begins, a dry period or lack of water will cause the death of the developing embryo.

Temperatures
affect both the germination percentage and the germination rate. Germination rate is lower at low temperatures.
Most plant seeds germinate at an optimum temperature range of 20 to 30°C. However, most field crop seeds can germinate at temperatures ranging from 0 to 49°C.
For example, wheat, rye, barley, and oat seeds germinate at temperatures slightly above 0°C. Field pea, alfalfa, soybean, flax, and some clover seeds germinate at 4°C.
Sorghum grain and corn seeds have a minimum germination temperature of approximately 9°C. Germination percentage may remain relatively constant if sufficient time is allowed for germination to occur.

Oxygen
is necessary for respiration to occur within a seed. Respiration converts the stored food in the seed into energy for germination.
Some seeds require less oxygen than others. For example, rice seeds germinate when covered with water, although little free oxygen is present.
Other small grains and cottonseed germinate only if a large amount of oxygen is available. Oxygen deficiency occurs if seeds are planted in flooded or compacted soil.
For this reason, the medium in which the seed is placed should be well aerated and loose.

Light
The presence or absence of light may or may not have an effect on germination. However, the light factor is not as important as a viable seed, germination medium, water, optimum temperature, and oxygen.
Seeds of some cultivars of tobacco and grass require a certain amount of light for germination.
Bermuda grass, Kentucky grass, bent grass, slender wheat grass, and Canada grass are examples of grass seeds that require adequate light for germination.
Seeds of most American cultivars of tobacco germinate in the absence of light. Seeds of pigweed and thorn apple also germinate in total darkness.

(31)

<u>QUESTION 2</u>:

2.1 Carbohydrates are defined as polyhydroxy aldehydes or ketones. They are the most abundant organic compounds formed by nature and a play very important role in plants as well as animals in different ways.
Describe the role of carbohydrates in plants (8)

- Respiration: The plants store carbohydrates and burn them for energy. This process is called cellular respiration. It breaks down the carbohydrate molecules synthesized during photosynthesis and release energy to power the plant's life and its processes.

- Structural component: Carbohydrates play structural role in plants. For example, cellulose- a polymer of beta-D-glucose is important structural component of all plant cells and makes up majority of plant cell wall.
- Energy source: Carbohydrates produce energy by metabolic pathways under the action of enzymes.
- Synthesis of other compounds: Carbohydrates provide carbon for synthesis of other organic compounds.
- Source of secondary metabolites: Carbohydrates may be the source of secondary metabolites.
- Growth factors: Plants need carbohydrates for their growth, development and synthesis of other organic compounds.
- Energy yield: Oxidation of one gram of carbohydrates yields 4-kilo-calories of energy.
- Food storage: Carbohydrates are important way of storing extra food like starch, which is an important polysaccharide, in plants is reservoir of extra food.

2.2 Describe the function of cytokinin and abscisic acid in plant development

Cytokinin
Is generally considered the second most important plant growth-regulating hormone, following auxin.
was first discovered in 1941 as the active component in coconut milk that promoted growth of plant cells in tissue culture.
Cytokinin can promote cell division and shoot growth and can delay senescence.
(4)

Abscisic acid
It was first identified in a search for an abscission-promoting hormone.
This is not the function of ABA, and as noted earlier, it functions in promoting dormancy and in sensing drought and other stresses.
ABA is derived from mevalonic acid and carotenoids and is thus similar in structure to the developmental factor from animals called retinoic acid. Transport of ABA can occur in the vascular tissues.
ABA stimulates closure of the stomatal pore, and can inhibit shoot growth.
In seeds, it promotes dormancy and stimulates the production of seed storage proteins.
It is mostly antagonistic to gibberellic acid (GA) and can inhibit the response of grains to GA
ABA is also involved in inducing gene transcription in response to wounding, which may explain why it has a role in the pathogen defence response. (5)

2.3 There are different ways by which micropropagation can be achieved. Multiplication by axillary buds/apical shoots being one of them. Describe Meristem and Shoot Tip Cultures and Bud Cultures.

Meristem and Shoot Tip Cultures

Apical meristem is a dome of tissue located at the extreme tip of a shoot. The apical meristem along with the young leaf primordia constitutes the shoot apex. For the development of disease-free plants, meristem tips should be cultured.
Meristem or shoot tip is isolated from a stem by a V-shaped cut. The size (frequently 0.2 to 0.5 mm) of the tip is critical for culture. In general, the larger the explant (shoot tip), the better are the chances for culture survival.

The most widely used media for meristem culture are MS medium and White's medium (4)

Bud Cultures

The plant buds possess quiescent or active meristems depending on the physiological state of the plant.

Two types of bud cultures are used

Single node culture:

The bud found in the axil of leaf is comparable to the stem tip, for its ability in micro propagation. A bud along with a piece of stem is isolated and cultured to develop into a plantlet. Closed buds are used to reduce the chances of infections.

Axillary bud culture:

In this method, a shoot tip along with axillary bud is isolated. The cultures are carried out with high cytokinin concentration. As a result of this, apical dominance stops and axillary buds develop. (4)

(25)

QUESTION 3:

3.1 The objective of germplasm conservation is to preserve the genetic diversity of a plant or genetic stock for its use at any time in future.
Describe the Mechanism of Cryopreservation and list steps involved in the process of cryopreservation of genetic stock. (10)

Mechanism of Cryopreservation

- The technique of freeze preservation is based on the transfer of water present in the cells from a liquid to a solid state.
- Due to the presence of salts and organic molecules in the cells, the cell water requires much more lower temperature to freeze (even up to -68°C) compared to the freezing point of pure water (around 0°C).
- When stored at low temperature, the metabolic processes and biological deteriorations in the cells/tissues almost come to a standstill.

Steps in Cryopreservation

- Grow sterile tissue cultures.
- Add cryoprotectants (pretreatment).
- Freezing.
- Storage.
- Thawing.
- Determine survival/ viability.
- Plant regeneration and growth.

3.2 Describe the Limitations of germplasm conservation in the form of seeds. (5)

- Viability of seeds is reduced or lost with passage of time.
- Seeds are susceptible to insect or pathogen attack, often leading to their destruction.
- This approach is exclusively confined to seed propagating plants, and therefore it is of no use for vegetatively propagated plants e.g. potato, Ipomoea, Dioscorea.
- It is difficult to maintain clones through seed conservation.

- Certain seeds are heterogeneous and therefore, are not suitable for true genotype maintenance.

3.3 List factors that are manipulated during the slow growth method. (4)

Factors that are manipulated:
- Temperature
- Nutrition
- Hormones Etc.
- Metabolism is manipulated to limit ageing.

(19)

QUESTION 4:

4.1 Describe these two types of DNA (i) Genomic DNA and (ii) Complementary DNA (cDNA) (4)

(i) Genomic DNA:
Total DNA isolated from the cells is called genomic DNA. It is isolated by breaking the walls of the cells by physical or biochemical methods. Finally the DNA is separated by ultracentrifugation.
(ii) Complementary DNA (cDNA):
In this case first the mRNA is isolated and then DNA is synthesized on mRNA template by the process of reverse transcription. The base sequence of this DNA is complementary to mRNA base sequence. Hence, it is known as complementary or cDNA.

4.2 Define a vector and list six (6) examples of vectors used in genetic engineering of plants. (8)

Vector is a vehicle to carry the desired gene into the genome of another organism.
There are many types of vectors like
Plasmid is a circular, single stranded and self replicable DNA molecule present inside bacteria. They help in sexual reproduction of bacteria by transfer of genetic matter from one to another. Here we use them to transfer desired gene.
Bacteriophage is a virus which attacks bacteria and inserts its gene into bacterial cell for multiplication.
Cosmid is similar to plasmid dna but can accommodate large DNA pieces.
Transposons: These are movable genes or jumping genes which move from one cell to another or plasmid to nucleus.
Yeast cloning vector: These are used to transfer the desired gene into fungi. This is similar plasmid with little modification.
Shuttle vector: These vectors have ori-gene, promoter gene for both bacteria and fungi. So it is two in one type.

4.3 Explain the function of the restriction endonuclease and gel electrophoresis in the Isolation of a desired gene (4)

A restriction endonuclease is used to cleave the source DNA into fragments.

Because the endonuclease's recognition sequence is likely to occur many times within the source DNA, cleavage will produce a large number of different fragments.
A different set of fragments will be obtained by employing endonucleases that recognize different sequences.
The fragments can be separated from one another according to their size by gel electrophoresis
The isolated genes are purified and taken for next step to fix to a vector.

(16)

QUESTION 5

5.1 Describe Electroporation and Agrobacterium-mediated transformation as a method of plant transformation (8)

Plant cell **electroporation** generally utilizes the protoplast because thick plant cell walls restrict macromolecule movement.
Electrical pulses are applied to a suspension of protoplasts with DNA placed between electrodes in an electroporation cuvette.
Short high-voltage electrical pulses induce the formation of transient micropores in cell membranes allowing DNA to enter the cell and then the nucleus.

The **Agrobacterium-mediated transformation** process includes the following steps:

- Isolate genes of interest from the source organism.
- Insert the transgene into the Tiplasmid.
- Introduce the T-DNA containing-plasmid into Agrobacterium.
- Attach the bacterium to the host cell.
- Excise the T-strand from the T-DNA region.
- Transfer and integrate T-DNA into the plant genome.

5.2 Advantages of Agrobacterium-mediated transformation (4)

- Natural method – more accepted
- Can infect intact plant cells, tissues + organs
- Can transfer large DNA fragments
- T-DNA insertion is precise
- Very stable gene transfer

(12)
