BITS PILANI, DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2013-2014
COMPREHENSIVE EXAMINATION

COURSE NO.: BIOT F352 **5.1.2014** **MAXIMUM MARKS: 40**
COURSE TITLE: Cell & Tissue Culture Technology **DURATION: 3 Hours**

Answer Part A and Part B in separate answer books
Answer to the point; Answer all questions in the given sequence

PART –A

Q1. (a) Compare organ, tissue and cell culture on the basis of: (2)

(i) potential for scale- up
(ii) level of differentiation of cells

(b) If you use DAPI to stain your cells, what will you visualize using fluorescence microscopy? Can you detect mycoplasmal contaminations using this method? (2)

(c). Briefly describe the cytotoxicity and survival assays? (2)

Q2. (a) Why is oxidation-reduction potential a useful parameter to monitor an animal cell bioreactor? What is an ideal value for ORP? (2)

(b) What is serum? How do proteins present in serum provide a mechanical advantage in animal cell culture? What are the disadvantages of serum in culture media? (3)

©. What are the practical issues concerning the marking of storage ampoules for cell preservation and how are they mitigated? Supposing the marking on your ampoule is illegible, how will you identify the cell line? (3)

Q3 (a). List the properties of cells on the basis of which they can be separated. Describe the use of isokypnic sedimentation for cell sedimentation and factors that affect the process. (3)

(b). How are lymphocytes stimulated to divide *in vitro*? What is PHA and how does it work? (3)

P.T.O.

PART –B

Q1. (a) Being a biotechnologist, which agronomic traits would you like to improve in this part of the world (UAE), describe the strategies for the same (Be specific) (2)

(b) What is the principle of Androgenesis? Briefly describe the factors influencing the technique of androgenesis *in vitro*. (2)

(c) State various reasons for use of Meristem culture technique in generating the virus free plants. (2)

Q2. (a) Give the principle of cryopreservation? In which way the cryopreservation is made effective for plant tissue culture? (2)

(b) *In vitro* propagated plants when transferred to green house and afterwards sown in the field yields more as compared to the plants that are directly transferred into field? (2)

© Briefly explain the factors affecting the isolation of protoplasts from plant cells .What are the applications of the techniques? (3)

Q3 (a) List out the major problems a technician will face while doing the *in vitro* propagation.(1)

(b) List out the various components of Ti Plasmid, what is their basic function? Out of these what are the most important components that play a major role in the transfer process? Can you use the Ti Plasmid as such to transfer the genes into plants? Justify your answer. (4)

(c) Can all kinds of crop plants be genetically modified with Ti plasmid of *Agrobacterium tumifaciens?* If yes, how? If not, Why? Explain (2)

**********GOOD LUCK **********

BITS PILANI, DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2013-2014
TEST – 2 (OPEN BOOK)

COURSE NO.: BIOT F352 **6.11.13** **MAXIMUM MARKS: 20**
COURSE TITLE: Cell & Tissue Culture Technology **DURATION: 50 Minutes**

Answer to the point; Answer all questions in the given sequence

Q1a. In Horticulture and Forest industry tissue culture is employed for rapid propagation of elite genotypes, which technique do you think will help in generating true to type disease free planting stock, explain? [3]

b. From the plant tissue techniques you have studied till now, which technique would you think will be better in terms of the stable crop improvement program to be followed? Explain [3]

c. What would be the strategy/technique you will design to generate a variant for stress tolerance? Explain with an example, list out the steps of the technique? [3]

d .What are the reasons for of callus induction and formation from explants under *in vitro* conditions?[1]

Q2a. In culture lab, an RPMI-1640 media with phenol red was inoculated with HeLa cells on day0. On day2 it was observed that media color changes to yellow. Explain why does the media color change and how? [2]

b. What is Hayflick's limit? How does it affect cells *in vivo* and *in vitro*? [3]

c. Define aspect ratio. How does it affect surface aeration? [3]

d. Give one example of a Good's buffer. How is it different from the CO_2 system? [2]

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BITS PILANI DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
SECOND SEMESTER 2011-2012
TEST – I (CLOSED BOOK)

COURSE NO.: BIO C352 **8.3.12** **MAXIMUM MARKS: 25**
COURSE TITLE: Cell & Tissue Culture Technology **DURATION: 50 Minutes**

Answer to the point; Answer all questions in the given sequence

Q1a. Success of *in vitro* propagation of plants depends on to some critical factors /requirements, how do these factors contribute in doing so? Justify with example [4]

Ans. Appropriate tissue : checking the physiological status of the donar and objective of propagation

- A suitable growth medium -containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid or solid. Growth regulators - both auxins & cytokinins. – secondary metabolite – suspension culture , prpoper combination and conc. of growth regulators.

- Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can over run a culture
- Frequent subculturing -to ensure adequate nutrition and to avoid the build up of waste metabolite.
- A controlled environment

b. What do you think will be the important factors to be considered while setting up a tissue culture unit and a green house?

Ans. Any unit in which Tissue culture techniques are performed includes a tissue culture lab and a propagation green house .

1.Planning for a TCL requires to consider following factors; Available space, Financing, type of work to be carried out ,and required production capacity .

Carefully plan when considering the size and location of Lab.

A good location includes the following

- Isolated from foot traffic
- No contamination from adjacent areas (Driveway /Parking lot /Soil mixing /pesticide storage /shipping dock dust & chemicals from field)

Plant Nursery Unit and the Tissue Culture Laboratory should be well separated:

Basic facilities of A washing area – away from transfer area ,Media preparation , sterilization and storage area ,Explant preparation and aseptic transfer of material

Environmentally controlled incubators or culture rooms/incubation and Acclimatization room should be there and should be planned carefully. Utmost care should be taken to maintain the aseptic conditions and controlled environment.

- Green House design : A structure design of a green house must provide protection against damages from wind, rain, heat and cold. The structure should be built modularly to enable easy expansion

NB. 3rd Yr (BIOTECH)

- Covering : Covering Should prevent harmful radiation from reaching the plants . Anti – Fog properties : Accumulation of condensation and uncontrolled dripping inside the green house are avoided by a special anti fog coating.
- For the climate control indoor sensors should be there for temperature and humidity and outdoor sensors for external temperatures, humidity, wind speed and directions
- One of the important aspect in a green house is Irrigation and Fertigation System. Irrigation system consisting of drip lines, top sprinklers, Pumps, Distribution pipes.

[5]

c. What is the need of *in vitro* multiplying/ propagating the plants? [4]

- Seed Culture :Increasing efficiency of germination of seeds that are difficult to germinate *in vivo*
- Precocious germination by application of plant growth regulators
- Overcoming seed dormancy and self-sterility of seeds
  Production of clean seedlings as explants
- Overcoming embryo abortion due to incompatibility barriers
- Shortening of breeding cycle
- Production of haploid plants
- Production of virus free germplasm
- Mass production of desirable genotypes
- For generation of useful somaclonal variants
- Combining genomes to produce somatic hybrids or cybrids
- Introduction of foreign DNA to generate novel (and typically desirable) genetic combinations
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Q2a. Q2a.Briefly describe the technique of Micro propagation with problems that can be faced during each stage and solution to overcome the same. [5]

Ans. Stage -0 : Preparation or selection of Donor plant
stage I – initiation or establishment of an aseptic culture ,Multiplication ,Rooting ,Acclimatization,
Problems: stage I - problems

- contamination
- browning of the explant
- dormant buds (woodies only)

<u>problems –Stage -2</u>

- vitrification – a glassy appearance to leaves
- acclimation to stage II – it takes some time to get to a rapid growth stage
- generation of callus (potential for mutations)

Stage -3 .Poor rooting, callusing
Stage 4. – High mortality rates, desiccation

b. What do you think could be the limitations of the Micro propagation technique, List out [2]
Ans. 1. Expensive Technique 2. Contamination 3. Species Specificity 4. Variation
5. Acclimatization 6. Trained personnel

c. List out the Major components of Plant Tissue Culture media? What care you need to take while selecting and preparing the Tissue culture media? Explain [5]

Ans. Components: Macronutrients/Microelements, Micro elements, Carbohydrate source, Nitrate source, Vitamins, Activated Charcoal, GR, Agar

- Care should be taken to avoid precipitation in -GR stock should be prepared carefully and to be added after autoclaving. In the laminar flow
- Solidification of agar- conc. need to be as per the requirements
- Ph of media falls during autoclaving – to be taken care.

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