Department of Biotechnology
Guru Ghasidas Vishwavidyalaya, Bilaspur (CG)
M.Sc. Biotechnology III Semester Examination, 2013-14

AS-2247
(LBTM: 303 Plant Biotechnology)

Model Answer

Q1. Multiple choice question answer

i. (c)
ii. (b)
iii. (a)
iv. (a)
v. (d)
vi. (c)
vii. (b)
viii. (d)
ix. (a)
x. (c)

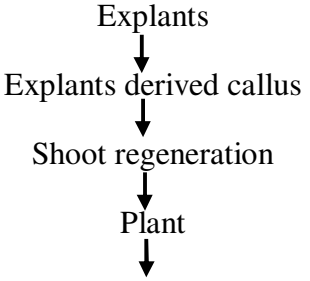
Q2. Descriptive type question answers

(i) Answer: The variability generated in the somaclones by the use of tissue culture cycle is called somaclonal variation. This term was 1st coined by Larkin and Scowcroft (1980). The plants arise from the tissue culture methods should be exact copies or clones of parental plant which is commonly called somaclone as it arises from the somatic cells. However phenotypic variants were frequently observed among the regenerated plants. These were usually dismissed earlier as tissue culture artifacts which now termed as somaclonal variation. This may be found naturally due to some environmental impact or it may also induced by using physical / chemical treatments.

Two different methods were commonly used for induction of somaclonal variation for getting somaclonal variants i.e.

a. Without in vitro selection
b. With in vitro selection

a. Without in vitro selection



Transfer to the field

↓

Screening for desirable traits

↓

Agronomic trials

(A flow chart for without selection)

b. With in vitro selection

Explants

↓

Explants derived callus

↓

Multiplication of callus

↓

Small pieces of callus growing in media containing some selection pressure
(eg-antibiotics, metabolic inhibitor, abiotic stress)

↓

Selection of tolerant calli

↓

Regeneration from tolerant calli

↓

In vitro testing of the putative variants

↓

Transfer to the field

↓

Screening for desirable traits

↓

Agronomic trials

Factors affecting the somaclonal variant induction (explain all the points in 2-3 lines)

a. Genotype
b. Explants source
c. Culture condition
d. Duration of culture
e. Culture condition
f. Concentration of selection pressure
g. Treatment time period
h. Selection method
i. Regeneration of explants
j. Agronomic analysis

Advantages: The following are the disadvantages associated with somaclonal variation

i. Development of new varieties : Development of new varieties having enhance agronomic traits either in form of quality/ quantity
   e.g.: scented geranium variety i.e. "Valvet rose", *Ipomea batatus,* cv. Scarlet
ii. Development of disease resistance: Development of varieties against different pathogens
   e.g.: in rice against *Helimenthosporium oryzae*, in potato against *Phytopthera infestans*
iii. Development of stress resistance: Development of varieties against cold stress, salt stress, heavy metal stress
   e.g.: Tomato, Potato, Egg plant

Disadvantages: The following are the disadvantages associated with somaclonal variation

i. Uncontrolled and unpredictable variation
ii. The variation is culture dependent
iii. The variation obtained is not always stable and inheritable
iv. The variations obtained are not always favorable for selection

(ii) short notes:

(a) **Answer:** A cell suspension culture consists of cell aggregates dispersed and growing in moving liquid media. It is normally initiated by transferring pieces of undifferentiated callus to a liquid medium which is agitated during incubation. It has also been started from sterile seedling or imbibed embryos.

Methods:

Explants sterilization
Callus induction
Transfer of friable callus to the liquid medium
Repeated subculture in the liquid medium (up to the level of the requirement)

Types (Explain each type)

i. Batch culture
ii. Continuous culture
    a. open
    b. close

Growth measurements:

i. Cell number count
ii. Fresh weight
iii. Dry weight/PCV

Advantages:

Resistance variety development
Large scale clonal propagation
Secondary metabolite isolation
Study of growth and developmental pattern, etc

Limitation:

Well equipped tissue culture laboratory
No universal protocol
High rate of contamination etc.

(b) **Answer** Plant nutrition is a difficult subject to understand completely, partially because of the variation between different plants and even between different species or individuals of a given clone. In case of plant Carbon and oxygen are absorbed from the air, while other nutrients are absorbed from the soil. The plant nutrients are of two types i.e. Micro and macro nutrients. Those nutrients which required comparatively large amount is known as macronutrients. The macro nutrients are N, P, K, S, Mg and Ca. Write down the function of each and in which for it is being used in tissue culture media like:

Nitrogen: It is an essential component of all proteins and Nucleic acid. In plant Tissue culture media this nitrogen is used in form of NO_3^- salt. Nitrogen deficiency most often results in stunted growth and chlorosis.

Likely explain about the other macronutrients

(iii)Answer: Protoplasts are plant cells without cell wall and can be isolated by using enzymes like cellulases, pectinases) from leaf, seedling, calli, pollen grains, embryo sacs etc. The protoplasts regenerate cell wall, undergo cell division, and form callus. The callus can also be subcultured. Some of the examples of plant species that have been regenerated from protoplasts are--- *Cucumis sativus, Capsicum annum, Ipomoea batata, Glycine max, Chrysanthemum* sp.

Source: Both in vivo and in vitro material can be used for the isolation of protoplast. The in vivo materia include leaf, petiole, root and the in vitro material include callus/ suspension culture

Isolation methods: Protoplast can be isolated by two methods i.e. Mechanical method and enzymatic methods.

Mechanical method: This method is now occasionally used but remain historically important. This method was given by Klercker (1982). Generally, protoplasts were isolated from highly vacuolated cells of storage tissues (onion bulbs, scales, radish root, mesocarp of cucumber and beet root).The procedure is as follows:

Kept the cells in a plasmolysed solution (hypertonic solution)

↓

Cut the plasmolysed tissue with a sharp edged knife

↓

Deplasmolysed the tissue by putting in hypotonic solution

↓

Protoplast were released to the isolating mi medium containing the define conc. of osmoticum

Demerits: During this process most of the cell get damaged, and also required a strong expertise. The principal deficiency of this approach is that the protoplasts released are few in number; mechanical isolation is thus only of historical importance now.

Enzymatic method: In this method protoplast are usually isolated by treating tissues with mixture of cell wall degrading enzyme. Cocking (1960) used a concentrated solution of cellulose enzyme (prepared from cultures of the fungus Myrothecium verrucaria to degrade cell walls and demonstrated the possibility of large-scale protoplast isolation from higher plants. Further progress in enzymatic isolation of protoplasts was achieved as soon as cellulase and macerozyme enzymes became available commercially in 1968. There are two types of enzymatic isolation, i.e. sequential step and single step. The sequential approach involves initial incubation of macerated plant tissues with pectinases which, in turn, are then converted into protoplasts by a cellulase treatment. In single step method plant tissues are plasmolysed in the presence of a mixture of pectinases and cellulases, thus inducing concomitant separation of cells and degradation of their walls to release the protoplasts directly. Most workers use the one step method because it is less time-consuming and reduces the chances of microbial contamination by eliminating some steps.

Once the protoplast was released to the isolating solution then the protoplast was purified by combination of filtration followed by centrifugation. Then the protoplast were culture in the defined medium using any suitable culture technique

Factors affecting protoplast isolation: The factors affecting the protoplast isolation are as follows:

i. Genotype
ii. Physiological state of tissue/ material
iii. Enzymes
iv. Osmotic condition

Culture: (Explain each in short/diagrammatically))

i. Liquid droplet method
ii. Agar culture
iii. Feeder layer
iv. Co-culturing method
v. Hanging droplet method

Viability: After isolation of protoplast the viability of the protoplast should also be checked by using any suitable method as follows (explain each in short)

i. FDA staining : living cells were stained
ii. Phenosafranin staining: dead cells were stained
iii. Cyclosis: streaming movement of cytoplasm
iv. Phase contrast microscopy
v. CFW staining : represents the onset of cell wall

Applications in crop improvement: (give suitable examples)

a) Various biochemical and metabolic studies,
b) Fusion of two somatic cells to create somatic hybrids,
c) Fusion of enucleated and nucleated protoplasts to create Cybrids (cytoplasmic hybrids)
d) Genetic manipulation.
e) Drug sensitivity.

(iv) Answer: A genetically engineered plants is generated in a laboratory by altering its genetic makeup. This is usually done by adding one or more genes to a plant's genome using genetic engineering techniques. Most genetically modified plants are generated by the biolistic method (particle gun) or by *Agrobacterium tumefaciens* mediated transformation. The objective of this type of modification is mainly for resistance development (biotic/abiotic), quality and quantity improvement and nutritional value enhancement. Till date number of genetically modified plants are found like:

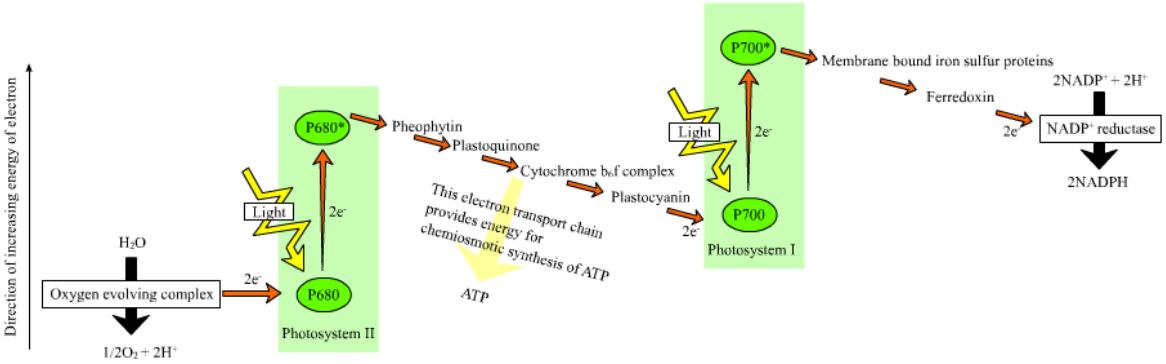
Example-1 **Bt brinjal:** It is a suite of transgenic brinjals (also known as an eggplant or aubergine) created by inserting a crystal protein gene (*Cry1Ac*) from the soil bacterium *Bacillus thuringiensis* into the genome of various brinjal cultivars. The insertion of the gene, along with other genetic elements like promoters, terminators and an antibiotic resistance marker gene into the brinjal plant is accomplished using *Agrobacterium* mediated genetic transformation. The Bt brinjal has been developed to give resistance against lepidopteron insects, in particular the Brinjal Fruit and Shoot Borer (*Leucinodes orbonalis*)(FSB). Mahyco, an Indian Seed Company based in Jalna, Maharashtra has developed the Bt brinjal. Explain Bt brinjal in the following head

- Development (Diagrammatically)
- Effective against pest
- Attempted commercialization in India
- Controversy

Example-2 ……. Likely explain the examples of other GMP as above mentioned headings

(v) Answer: Photosynthesis is the process in which the light energy is converted in to the chemical energy i.e. synthesis of carbohydrate. This photosynthesis mainly take place in two steps i.e. Light dependent step where the reducing power NADP(H) and ATP were synthesized with the help of sunlight using the photosynthetic apparatus (explain the structure of photosynthetic apparatus including the photosystems).

In Light dependent reaction of photosynthesis after photolysis of water the e- released from the H_2O molecules are drained to Yz complex which successively transfer to the PS-II subsequently the electron move through a number electron accepter and PS-I finally the electron is being used to reduce the $NADP^+$ to NADP (H) which is the final electron accepter. As the electron released from water never return back to the source of origin the movement of electron is known as noncyclic electron transport. Also here the electron moves in a Zig Zag manner so the path/ scheme of this type of electron movement is known as Z-Scheme. The details of the electron flow is as below (Explain the movement of electron in details along with steps where the ATPs were generated as the diagram given below)



(vi) short notes

(a) Answer: Photolysis means lysis or dissociation of water molecules. Water is oxidized according to the following chemical reaction ($2\ H_2O \rightarrow O_2 + 4\ H+ + 4\ e^-$. This equation indicates that four electrons are removed from two water molecules, generating an oxygen molecule and four hydrogen ions. Water is a very stable molecule. Oxidation of water to form molecular oxygen is very difficult, and the photosynthetic oxygen-evolving complex is the only known biochemical system that carries out this reaction. Photosynthetic oxygen evolution is also the source of almost all the oxygen in Earth's atmosphere. The chemical mechanism of photosynthetic water oxidation is not yet known, although many studies have provided a substantial amount of information about the process. The protons produced by water oxidation are released into the lumen of the thylakoid, not directly into the stromal compartment. They are released into the lumen because of the vectorial nature of the membrane and the fact that the oxygen-evolving complex is localized on the interior surface of the thylakoid. These protons are eventually transferred from the lumen to the stroma by translocation through ATP synthase. In this way the protons released during water oxidation contribute to the electrochemical potential driving ATP formation. It has been known for many years that manganese (Mn) is an essential cofactor in the water-oxidizing process and a classic hypothesis in photosynthesis research postulates that Mn ions

undergo a series of oxidations which are known as *S states,* and are labeled S0, S1, S2, S3, and S4 that are perhaps linked to H_2O oxidation and the generation of O_2.

(b) Answer: In the linear electron transport chain, the oxidized Rieske protein (**FeSR**) accepts an electron from plastohydroquinone (QH_2) and transfers it to cytochrome *f*. Cytochrome *f* then transfers an electron to the blue-colored copper protein plastocyanin (PC), which in turn reduces oxidized P700 of PSI. In the cyclic part of the process the plastosemiquinone transfers its other electron to one of the *b*-type hemes, releasing both of its protons to the lumenal side of the membrane. The *b*-type heme transfers its electron through the second *b*-type heme to an oxidized quinone molecule, reducing it to the semiquinone form near the stromal surface of the complex. Another similar sequence of electron flow fully reduces the plastoquinone, which picks up protons from the stromal side of the membrane and is released from the *b6f* complex as plastohydroquinone. The net result of two turnovers of the complex is that two electrons are transferred to P700, two plastohydroquinones are oxidized to the quinone form, and one oxidized plastoquinone is reduced to the hydroquinone form. In addition, four protons are transferred from the stromal to the lumenal side of the membrane. By this mechanism, electron flow connecting the acceptor side of the PSII reaction center to the donor side of the PSI reaction center also gives rise to an electrochemical potential across the membrane, due in part to H^+ concentration differences on the two sides of the membrane. This electrochemical potential is used to power the synthesis of ATP. Present the cyclic flow of electron diagrammatically

(vii) Answer: Stress mean growth in an unfavorable condition. In case of plant like humans, stresses can originate from the surrounding environment (called abiotic, or nonliving stresses) or, they can come from living organisms that can cause disease or damage (called biotic stresses).

Abiotic Stress: Number of abiotic stresses are affecting the growth and development of the plants which are as below:

Water Stress: One of the most important abiotic stresses affecting plants is water stress. A plant requires a certain amount of water for its optimal survival; too much water (flooding stress / submerged stress) can cause plant cells to swell and burst; whereas drought stress (too little water) can cause the plant to dry up, a condition called desiccation. Either condition can be deadly to the plant. Therefore genetically engineered plants having the resistance gene offer the opportunity to overcome the stress (write down the Examples of gene used to develop resistance variety).

Likely explain the other abiotic stresses given below

Temperature Stress:

Salt stress:

Metal stress:

Pesticide/Herbicide Stress

Biotic Stresses

Biotic stresses cause damage to plants via living organisms, including fungi, bacteria, insects, and weeds. Viruses, although they are not considered to be living

organisms, also cause biotic stress to plants. There are number of biotic stresses which affect the growth and developments of the plants are as below:

Insect stress: Insects cause large annual losses of crop yield. Insecticides to control insects are mainly chemically synthesized and they have a negative or adverse effect on host as well as on environment apart from the cost incurred in their use. Therefore GMP offers the best opportunity to overcome this stress. There are two way for development of insect resistance variety i.e. by Bt mode and non Bt mode. In Bt mode the resistance was developed by using the endotoxin (Crystal protein) gene (*cry*) of *Bacillus thuringiensis.* In Non Bt mode the resistance variety were developed by using metabolic inhibitors like Protease inhibitor (ex- Cowpea trypsin inhibitor, α-amylase inhibitor. (Write down the Examples of gene used to develop resistance variety)

Likely explain the other abiotic stresses given below

Viral stress:

Fungal and bacterial stress:

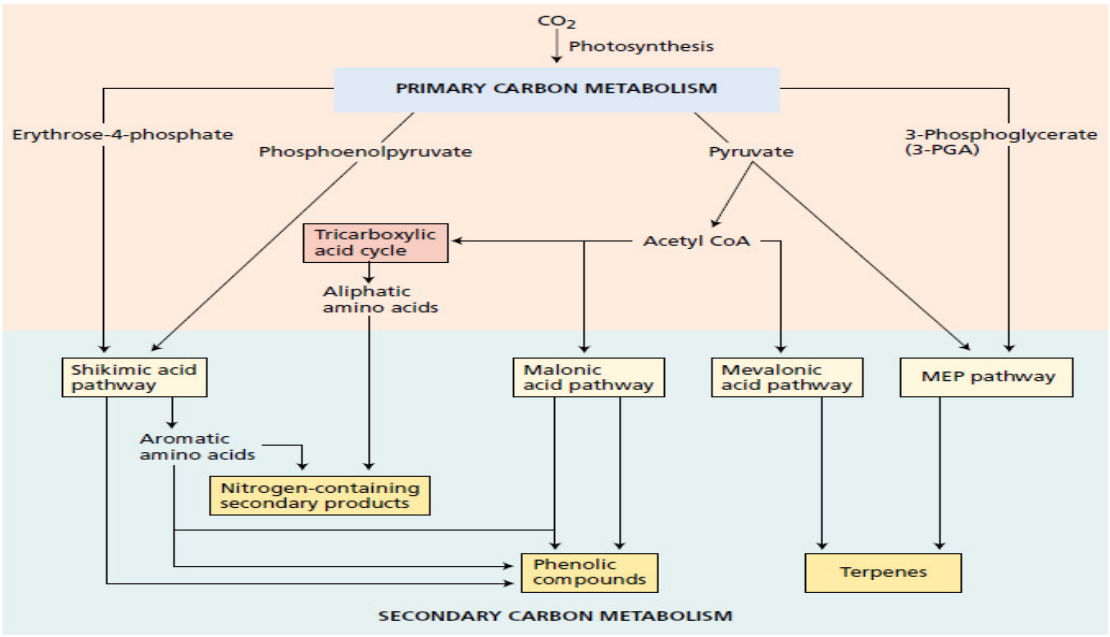
Nematode stress:

(viii) Answer: Secondary metabolites are those chemical compounds in organisms that are not directly involved in the normal growth, development or reproduction of an organism. In this sense they are "secondary". Secondary metabolites, are found in only specific organisms, or groups of organisms, and are an expression of the individuality of species. Plants are valuable sources of a vast array of chemical compounds belong to a rather broad metabolic group, collectively referred to as secondary metabolites. Secondary metabolites are produced for easily appreciated reasons, e.g. 1. As toxic materials providing defense against predators. 2. As volatile attractants towards the same or other species. 3. As coloring agents to attract or warn other species.

The building blocks for secondary metabolites are derived from primary metabolism. The number of building blocks needed is surprisingly few. The most important building blocks employed in the biosynthesis of secondary metabolites are derived from:

1. Acetyl coenzyme A (acetyl-CoA)
2. Shikimic acid
3. Mevalonic acid
4. 1-deoxyxylulose-5-phosphate
5. Amino acids

The basic path way for secondary metabolite biosynthesis is give as below (Explain the Shikimate pathway in details)


CO_2
Photosynthesis
PRIMARY CARBON METABOLISM
Erythrose-4-phosphate
Phosphoenolpyruvate
Pyruvate
3-Phosphoglycerate (3-PGA)
Tricarboxylic acid cycle
Acetyl CoA
Aliphatic amino acids
Shikimic acid pathway
Malonic acid pathway
Mevalonic acid pathway
MEP pathway
Aromatic amino acids
Nitrogen-containing secondary products
Phenolic compounds
Terpenes
SECONDARY CARBON METABOLISM