4.4. TISSUE CULTURE

SYNOPSIS

- The laboratory technique of growing, culturing, plant cells, tissues and organs in vitro is known as tissue culture or micro propagation.
- Information gained regarding the cell division, growth and differentiation made it possible to culture individual plant cells, tissues and organs in the laboratory.
- Plant cell and organ culture have developed rapidly and became a major biotechnological tool in agriculture ,horticulture, forestry and industry etc.,
- 'Morgan' coined the term " totipotency" to denote the capacity of cell to develop into an organism by regeneration.
- All tissue culture techniques are based on "Cellular totipotency" i.e the ability of a plant cell to divide grow and produce complete plant under controlled conditions. This property is observed only in plant cells.
- F.C. Steward first experimentally demostrated totipotency by obtaining complete carrot plant from secondary phloem tissue of root.
- The sequence of operations in tissue culture is preparation of nutrient medium, sterilization of the medium, preparation of the explants, inoculation of the explant, incubation for growth, Acclimatization of plantlets and transfer to pots.
- A nutrient medium is a mixture of various essential micro and macro nutrients, amino acids (A.A). and vitamins in required proportions.
- These nutrients are mixed in distilled water and the pH is adjusted to 5.6 to 6.0.
- For providing support during culture of plants the medium is solidified by addition of agar.
- Most commonly used nutrient medium is MS medium (Murashige and Skoog medium).
- Nutrient medium described above does not contain any growth regulators and is called basal medium.
- Basal medium is used for germinating seeds, for developing aseptic seedlings or simple callus.
- Callus can be produced on a basal medium
- For achieving complete regeneration of plants, a basal medium is to be supplimented with phytohormones auxins like IAA,NAA,2,4-D, GA,cytokinins like BA and Kinetin.
- Culture medium is poured in glass vessels and closed with non- absorbant cotton plug.This allows exchange of CO_2 & O_2.
- Medium is sterilized in an autoclave.
- Sterilization is a process by which the medium is made free from microorganisms.
- Sterilization of the medium is most commonly done in an atuoclave at 15 lbs pressure for 15 minutes at 121°C.
- Any living plant part such as axillary bud, leaf and stem segments, root and shoot tips, ovary,endosperm etc., can be used as explants.
- An explant can be obtained from root, stem, hypocotyl, cotyledons, cambium, Parenchyma, Ovules, Ovary, endosperm or anther.
- An explant may have micro organisms on it's surface .It can be surface sterilised with sodium hypochlorite and then washed with sterile water.
- Aseptic explants can also be obtained from aseptic seedlings developed from inoculated seeds.Seeds are sterilised with 0.1% mercuric chloride, rinsed in sterile distilled water and inoculated on MS medium.
- Transfer of sterilised explants on to the nutrient medium under aseptic conditions is called inoculation. It is done in a laminar air flow chamber.
- In 3-4 weeks time the cultured cells develop into undifferentiated mass of cells called callus.
- Induction of roots and shoot in the callus is called organogenesis. This is achieved using phytohormones.
- A higher proportion of auxin and low proportion of cytokinins promote root regeneration called **"rhizogenesis'.**
- A higher proportion of cytokinins and low proportion of auxin promote shoot regeneration called **'Caulogenesis'**
- Sometimes the callus develops embryos through somatic embryogenesis which are called embryoids'.
- Embryoids developed from somatic tissue are referred as **'somatic embryos'.**
- The somatic embryos are transferred to other culture media for development into complete plants.
- Acclimalization of plantlets and transfer to pots (Hardening). The plants are washed to remove culture medium and planted in plastic pots containing soilrite. Pots are covered with polythene bags and maintained in lab at room temperature for 1 to 2 weeks.
- The polythene bag provides high humidity. Polythene bags are removed and plant is transplanted to pots.
- Otherwise these embryoids can be encapsulated in sodium alginate for storage and transport.The encapsulated somatic embryos are known as '**synthetic or artificial seeds.'**

Applications of plant tissue culture

1. It is possible to produce a large number of plants within a short time and space through tissue culture.Mass propagation of plants through tissue culture is know as 'Micropropagation'. this technique is applied for propagation of ornamental plants, orchids and other exotic plants, fruits and plantation crops etc.
2. Plants regenerated through tissue culture show variations called ' somaclonal variations', which are used for crop improvement.
3. In some plants like papaya, female plants are needed in greater proportion to produce more fruits per cultivated area. Female plants are selectively produced through tissue culture.
4. Viral diseases from vegetatively propagated plants can be prevented by producing virus free plants from shoot-tip cultures.
5. Artificial or synthetic seeds are produced from somatic embryos by encapsulation with sodium alginate. These can be easily stored, germinated and transported.
6. Tissue culture of medicinal plants helps in the in vitro production of high value products of industrial and medicinal importance.
7. The production of transgenic plants by the transfer of foreign genes is completely dependent on plant tissue culture.

EXERCISE

LEVEL-I

378. Cellular totipotency was experimentally demonstracted by
1) F.C.Steward 2) Morgan
3) Muller 4) Stadler

379. Term totipotency was coined by
1) Carl Ericay 2) Nathans
3) Morgan 4) Muller

380. Capacity of a cell to develop into an organism by regeneration is called
1) Embryogenesis 2) Parthenogenesis
3) Totipotency 4) Organogenesis

381. Basal medium is devoid of
1) Vitamins 2) Aminoacids
3) Minerals 4) Hormones

382. Identify the Cytokinins added to tissue culture medium
1) IAA and NAA 2) 2, 4-D and Kinetin
3) Kinetin & Benzylamine 4) GA and BA

383. Complete eradication of micro-organisms present on the surface of any material is called
1)Inoculation 2)Incubation
3)Immunization 4)Sterlization

384. Match the following

List - I	List- II
A.Agar-Agar	i. Preparation of synthetic seeds
B. Mercuric chloride	ii. Surface sterilization of explant surface
C.Sodium hypochlorite	iii. Sterilization of seeds
D. Sodium alginate	iv. Solidification of medium

	A	B	C	D
1)	iv	i	ii	iii
2)	iv	iii	ii	i
3)	i	iv	ii	iii
4)	ii	iii	iv	i

385. Which among the plant tissue cannot be used as explant?
1) Meristems 2) Parenchyma
3) Phloem 4) Sclerenchyma

386. Identify incorrect statement among the following
1) Totipotency seen in animal cell
2) Totipotency seen in plant cells
3) 1 and 2
4) Totipotency not seen in dead cells

387. Identify the wrong statement from the following.
1) Mass propagation of plants through tissue culture is called micropropagation
2) Plants regenerated through tissue culture show somaclonal variations.
3) Viral free clones are obtained by root-tip culture of viral infected plants.
4) encapsulated somatic embroyos can be stored, germinated and transported.

388. Cellular totipotency is found in
1) Animal cells 2) Plant cells
3) Human cells 4) All of these

389. Minimal (Basal) medium helps in tissue culture upto the development of
1) Callus 2) Embryoids
3) Seedlings 4) 1 and 3

390. The factors required for the development of plants through tissue culture are
1) Aeration
2) Sterilized nutrient medium
3) Preparation of Explant 4) 1, 2 & 3

391. The final pH of the medium in tissue culture has to be adjusted to
1) 4 - 5 2) 5.6-6.0 3) 6.5-7.5 4) 8-9

392. During sterilization of the medium in autoclave the pressure and time maintained are
1) 10 lbs and 10 minutes
2) 15 lbs and 15 minutes
3) 20 lbs and 20 minutes
4) 25 lbs and 25minutes

393. Synthetic seeds are prepared using
1) Sodium hydrcxide 2)Sodium hypochlorite
3)Sodium alginate 4)Sodium chloride

394. This disinfectant solution is used in tissue culture
1) Calcium hypochlorite 2) Sodium hypochlorite
3) Hydrogen peroxide 4) Calcium chloride

395. "Hardening" refers to
1. Sterilization of explant 2. Inoculation of explant
3. differentiation of callus into embryoid
4. Acclimatization of regenerated plants.

396. The mass of cells produced during tissue culture is called
1) Thallus 2) Prothallus
3) Callus 4) Embryoids

397. The embryoids produced on callus in tissue culture are called
1) Gametic embryoids 2)Progametic embryoids
3) Somatic embroyoids 4) Zygotic embryos

398. In tissue culture the growth hormone induces root formation is
1) Cytokinin 2) GA 3) Phytochrome 4) Auxin

399. In tissue culture this growth hormone induces shoot initiation
1) Cytokinin 2) IAA 3) GA 4) ABA

400. The embryoids produced in tissue culture encapsulated with sodium alginates are called
1) Natural seeds 2) Dormant seeds
3) Artificial (synthetic) seeds
4) Condensed seeds

401. The formation of plant organs in tissue culture is called
1) Pedogenesis 2) Pseudogenesis
3) Gametogenesis 4) Organogenesis

402. Virus free plants in vegetatively propagated plants can be produced by this culture method
1) Ovary culture 2) Ovule culture
3) Shoot tip culture 4) Root tip culture

403. Soma Clonal variations are found in plants obtained through
1) Tissue culture
2) Asexual reproduction
3) Sexual reproduction
4) Vegetative propagation

404. The female plants are developed through tissue culture in
1) *Oryza* 2) *Nicotiana* 3) *Carica* 4) *Datura*

405. The tissue culture in biotechnology has got much significance because
1) Plants can be developed in short time
2) Less expenditure
3) Production of more number of plants in less time
4) All the above

LEVEL-II

406. The possibility to produce a large number of plant within short time and space.
1) SCP 2) Micro propagation
3) Bio technology 4)Mushroom cultivation

407. Female plants selectively produced through tissue culture are
1) Cucumber 2) Cannabis
3) Papaya 4) Cocas

408. For crop improvement the plants regenerated through tissue culture show these variations
1) Somaclonal variation 2) Genetic variations
3) polyploidy 4) Amphidiploids

409. seeds produced from somatic embryos by encapsulation with sodium alginate can be
1) Germinated, sowed
2) stored, germinated, transported
3) Grown quickly 4) only trans ported

410. The type of plants produced by the transfer of foreign genes
1) Virus free plants 2) Transgenic plants
3) Synthetic plants 4) Exotic plants

411. Plant cell, tissue and organ culture have developed rapidly and become a major biotechnological tool in
1) Agricultural 2) Horticulture
3) Forestry 4) All the above

412. The living part of the plant used an explant
1) Stem segment 2) Endosperm
3) Flower 4) 1,2 and 3

413. Micro propagation technique is applied for the propagation of
1) ornamental & Exotic plants
2) Orchids & Fruit plants
3) Plantation of crops 4) 1,2 and 3

414. Plant tissue culture has several applications in the fields of
1) Pharmocology 2) Forestry
3) Environment 4) All the above

415. The term totipotency was coined by
1) F.C. Steward 2) Morgan
3) Skoog 4) Murashige

416. Auxins used in nutrient culture medium are
i) IAA ii) 2-4 D iii) NAA iv) Kinetin
1) i & iii only 2) ii & iii only
3) iii & iv only 4) i, ii & iii only

417. In Laminar air flow chamber asceptic conditions are achieved by
1) α -rays 2) x -rays
3) High temperature 4) U.V. light

418. Nutrient medium is solidified by adding
1) NAA 2) Sodium hypochlorite
3) Agar 4) Explant

419. 'Soilrite' is made up of
1) Coconut endocarp + organic substances
2) Soil + organic substances
3) Cowdung + organic substances
4) Sand + organic substances

420. Assertion (A): Inoculation of explant is carried out in a steam sterilizer viz., autoclave.
Reason (R): Autoclave sterilizes the culture medium by killing the microorganisms.

421. Identify the correct sequence of events involved in culturing of tissues and production of plantlets
A) Using the laminar air flow chamber
B) Preparation of the nutrient medium
C) Using growth promoters
D) Surface sterilization of the explant
E) Callus formation
1) B,E,A,C,D 2) B,D,A,E,C
3) B,E,D,A,C 4) B,D,A,C,E

LEVEL-III

422. Grown on a basal medium, supplemented with 2,4-D, the explant produces
1) Callus and Shoots 2) Callus and Embryoids
3) Callus only 4) Callus and roots

423. If seed is used as explant, surface sterilization of the explant is done with
1) Liquid detergent 2) Sodium hypochlorite
3) Mercuric chloride 4) Distilled water

424. Explant cultured on a minimal medium produces callus in about
1) 1 to 2 weaks 2) 3 to 4 weeks
3) 5 to 6 weeks 4) 7 to 8 weeks

425. Caulogenesis in callus is promoted by
1) Auxin 2) Kinetin
3) Higher content of Cytokinin but IAA must be present in the medium
4) Higher content of cytokinin, but IAA isn't required at all

426. Encapsulated somatic embryos are called
1) Embryoids 2) Synthetic embryos
3) Artificial seeds 4) Azygotic embryos

427. For sterilisation, the culture medium is autoclaved for 15 minutes at
1) 121°C and 5 lbs pressure
2) 121°C and 15 lbs pressure
3) 121°C and 30 lbs pressure
4) 100°C and 15 lbs pressure

428. Cellular totipotency is due to
1) Active state of the cytoplasm
2) Active state of the cell
3) Incomplete differentiation of the cell
4) Presence of a genome

429. Basal medium is prepared by mixing required macro and micro nutrients in
1) Tap water 2) Filtered water
3) Distilled water 4) Water from tender coconuts

430. Which of the following provides energy for tissue growth "invitro"?
1) Macro and micronutrients
2) Amino acids and vitamins
3) Growth regulators 4) Carbohydrates

431. Consider the followings statements
I) Embryoids develop into plantlets by-passing the normal stages of zygotic embryo
II) Plantlets will be dessicated and killed if they are transferred to field conditions directly from the culture flask
III) All plantlets derived from an explant constitute a clone
The correct statements are
1) I,II,III 2) I,II 3) II,III 4) II only

432.Assertion (A): Culture tubes are plugged with non - absorbent cotton

Reason (R): Non absorbent cotton allows exchange of CO_2 and O_2 but prevents entry of microbes

433.Popular tissue culture nutrient medium is

1) MS-medium 2) GS-medium
3) Arnon's 4) Sach's

434.Read the following and identify a group containing correct sequence

I) Inoculation of explant II) Incubation of explant
III) Washing explant with detergent

IV) Washing the explant with sodium hypochlorite

V) Procuring the explant from helathy plant

1) V,III,IV,I,II 2) III,V,I,IV,II
3) II,I,V,III,IV 4) IV,V,I,III,II

435.Nutrient medium is sterilized with the help of

1) Laminar air flow chamber 2)Incubation chamber
3) Kline 4) Autoclave

436.Somoclonal variations useful for crop improvement are shown by

1. Inter specific hybrides 2. Intergeneric hybrid
3. Micropropagation 4.Mutation breeding

437.Assertion (A) : Soilrite made up of coconut shell and organic substances.

Reason (R) : Inoculation of explant carriedout by autoclave.

438.Assertion (A) : During tissue culture agar agar is added to the nutrient medium

Reason (R) : Solidified medium supports the plant Tissues during culture

439.Addition of Benzyl adenine to the nutrient medium helps in.
1) Sterilization of explant 2) Inoculation of explant
3) Initiation of shoot from callus
4) Initiation of root from callus.

440. Total duration required for both incubation and acclimatization during tissue culture experiment is about

1) 1 – 2 weeks 2) 2 – 4 weeks
3) 4 – 6 weeks 4) 6 – 8 weeks

441.When male sterile plants are used as female parents in hybridization

1) Both emasculation and Bagging are not required

2) Both emasculation and bagging are required

3)Emasculation is not required and bagging is required

4)Emasculation is required and bagging is not required

442.Assertion : (A) : During acclimatization of plant lets the pots are covered by polythene bags

Reason (R) : Polythene bag provides humidity to the plant

443.During tissue culture ' mercuric chloride ' is used for

1) Surface sterilization of explant tissue during inoculation

2) Surface sterilization of seeds during their inoculation

3) Sterilization of the nutrient medium
4) Autoclaving

444.Synthetic seed coat is made up of

1) Calcium chloride 2) Sodium alginate
3) Sodium hypochloride

4) Sodium lauryl sulphate

445.Assertion (A) : P^H of the nutrient medium is slightly alkaline

Reason(R): P^H of the nutrient medium is 5.6 to 6.0

446.In tissue culture ' Laminar air flow chamber' is used for.

1) Preparation of explants

2) Sterilization of culture medium

3) Inoculation of explants 4) Incubation of tissue

447. Assertion (A): Agar agar is used for the solidification of the medium

Reason(R): Tissue culture is based on cellular totipotency

448. The correct sequence of following stages are noticed during organ culture

a) Sterilization b) Incubation

c) Callus formation d) Inoculation

1) d-a-c-b 2) a-c-b-d

3) a-d-b-c 4) b-d-c-a

449. In tissue culture, which one of the following pairs of substances are used to induce shoot formation and root formation respectively during organogenesis : (EAMCET - 2005)

1) Hydrogen peroxide and chlorine

2) Auxins and cytokinins

3) Cytokinins and auxins

4) Ethylene and Abscissic acid