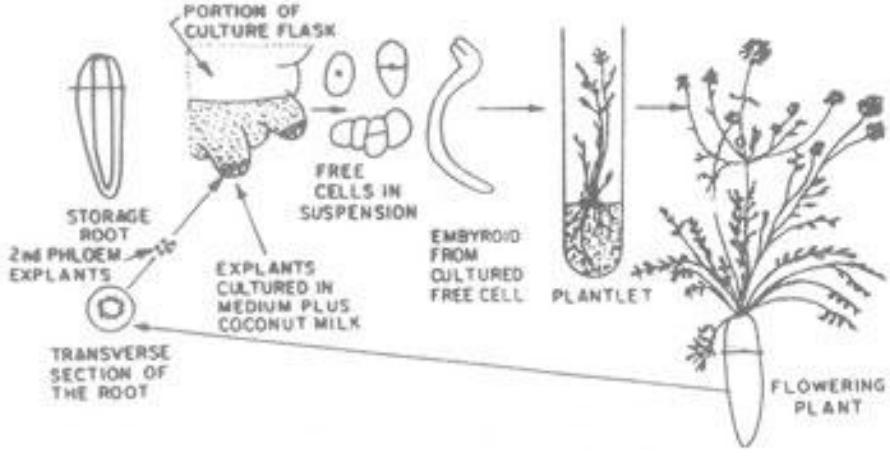
Totipotency and Morphogenesis

Totipotency

The inherent potentiality of a plant cell to give rise to a whole plant is described as cellular totipotency. This is a capacity which is retained even after a cell has undergone final differentiation in the plant body. In plants, even highly mature and differentiated cells retain the ability to regenerate to a meristematic state as long as they have an intact membrane system and a viable nucleus. This is contradicting to animals, where differentiation is generally irreversible.

For a differentiated cell, to express its totipotency, it first undergoes dedifferentiation followed by redifferentiation. The phenomenon of a mature cell reverting to the meristematic state and forming undifferentiated callus tissue is termed ‘dedifferentiation’. The phenomenon of conversion of component cells of callus tissue to whole plant or plant organs is called as ‘redifferentiation’.

The phenomenon of totipotency is demonstrated with the following experiment. Slices of the carrot root (shown on the left) were cut and small pieces of tissues were taken from the phloem region. These were inoculated into a liquid medium in special flasks, which were rotated slowly. The tissue grew actively and single cells and small cell aggregates dissociated into the medium (a single cell and some cell aggregates are drawn near the flask). Some of the cell clumps developed roots, and, when transferred to a semi-solid medium, these rooted nodules formed shoots. These plants could be transferred to soil where they developed into flowering plants. Phloem tissues taken from the roots of these plants could be used to repeat the cycle.



Morphogenesis

Biological organization of any life coordinated with several events as though a craftsman was moulding it according to a plan. In this process, the individual parts do not develop independently but all are knit together into an organised system. The biological science concerned with this dynamic and casual aspect of organic form is called "Morphogenesis". The derivation of this word is obvious, the origin of form. It attempts to expose the effects of various factors and how these factors manifest an organic form *in toto*. "Morphogenesis", a distinctive aspect of organization of life, is the crossroad where all the highways of biological exploration tend to converge", says Sinnott.

More studies have been made to understand morphogenetic problems of animals rather than plants. Recent developments in plant cells, tissues and organs of higher plants in culture, are making the science of plant morphogenesis a fruitful one. Working with plants has a number of advantages.

- In plants embryonic regions like meristem and cambium are permanently available for the study of development.
- The determinate type of development and abundance of organs such as leaves; flowers and fruits make the study possible under a wide range of environmental conditions.
- The behaviour of individual cells during development differs in plants from animals. In animals, the individual cells are free to move whereas this mobility is absent in plants and the cells are almost always attached firmly to the neighbours so that morphogenetic movements have no part in the development. This makes the study of morphogenetic problems simple in plants.
- The lesser plasticity of plant cells, their stationary habit, susceptibility to changes under environmental influences, ability to maintain polarity and differentiation and generation potential favour the study relatively simple one.

In the field of plant morphogenesis, the contributions were made by the scientists like, Hanstein on meristem, Winkler on chimeras, Haberlandt on hormones, Kuster on abnormal growth, Klebs on the effects of the environment and Goebel on the organography are noteworthy. Vochting (1878) stated in his "Organbildung im Pflanzenseich" that phenomenon of morphogenesis depends on the factors like polarity, differentiation and regeneration of individual cells and concluded that the fate of a cell is a function of its position.

Morphogenesis *in vitro*

Under normal conditions, a seed, the miniaturized sporophyte has the message to reconstitute an entire plant with similar shape, structure and function of the mother plant. All known about this phenomenon is that a complex adult multicellular organism has emerged from a relatively simple organized zygote through a sequence of mitosis. This *de novo* origin of structures and functions from a fertilized egg or zygote is a complicated phenomenon in which most of the events are not yet known in detail. This is the state in plants *in vivo* or in an entire plant. Considering the cells or organs cultured *in vitro*, the morphogenesis is still an event without many details. However, in the aspects of morphogenesis *in vitro*, significant progress has been made, after the discovery of totipotency of plant cells, phytohormones and the hypothesis of regulation of morphogenesis by the critical balance between auxin and cytokinin.

Various terms are used to define the phenomenon in *in vitro* studies. For example differentiation, de-differentiation, re-differentiation, regeneration and morphogenesis are terms with overlapping meanings. To give a clear-cut view for the usage of terms, the sharp differences among them exposed hereunder.

Differentiation

The term differentiation is used in many different senses in biology. In broad sense, it is defined as the process by which meristematic cells are converted into two or more types of cells, tissues or organs which are qualitatively different from each other.

De-differentiation

The term is used to denote the process of formation of unorganised tissues from the highly organized tissues.

Re-differentiation

The process of differentiation occurring in an undifferentiated tissue.

Regeneration

It is defined as the structuring of any part, which has been removed or physiologically isolated from the organism. In other words, genesis of an entire plant from cultured explants directly or *via* callus indirectly is called regeneration.

Morphogenesis

Attainment of biological organization or form is termed as morphogenesis. Under *in vitro* conditions this can be achieved by two routes: *de novo* origin of organs, either shoots or roots from the cultured tissues precisely termed as organogenesis and *de novo* origin of embryos with distinct root and shoot poles on opposite ends from the somatic cells or cells cultured *in vitro*, otherwise called as somatic embryogenesis

Figure 1. Stages of callus induction

The historical background, achievements and the causes for the two routes are discussed below.

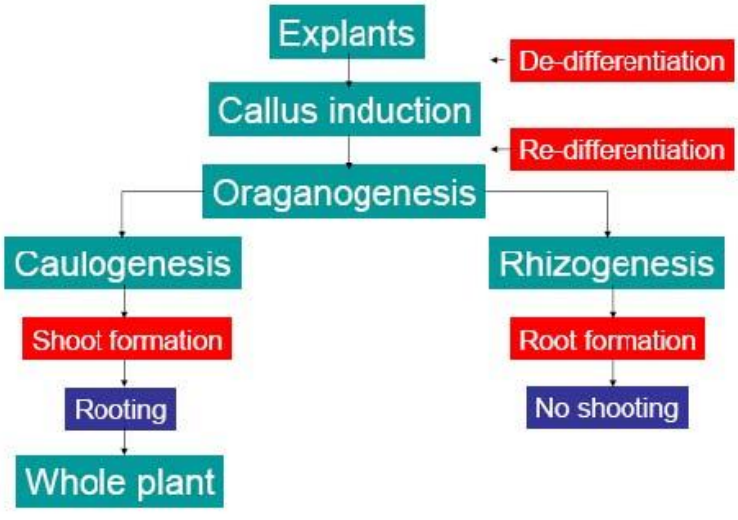
Organogenesis

In plant tissue culture, organogenesis means genesis of organs like shoots, roots, leaves, flowers, *etc*. The earliest report on induction of shoot organogenesis *in vitro* was by White (1939) using a tobacco hybrid; and the first observation of root formation was reported by Nobecourt (1939) using carrot callus. Till late 1950s, the basic regulatory mechanism underlying in organogenesis was not identified. Skoog and Miller (1957) were responsible to recognize the regulatory mechanism as a balance between auxin and cytokinin. As per their finding, a relatively high level of auxin to cytokinin favoured root formation and the reverse favoured shoot formation. Using this concept, it has now become possible to achieve organogenesis in a large number of plant species by culturing explants, calli and cell suspension in a defined medium.

In organogenesis, the shoot or root may form first depending upon the nature of growth hormones in the basal medium. The genesis of shoot and root from the explants or calli is

termed as caulogenesis (caulm = stem) and rhizogenesis (rhizo = root) respectively.

Figure 2. Pathways of *in vitro* organogenesis



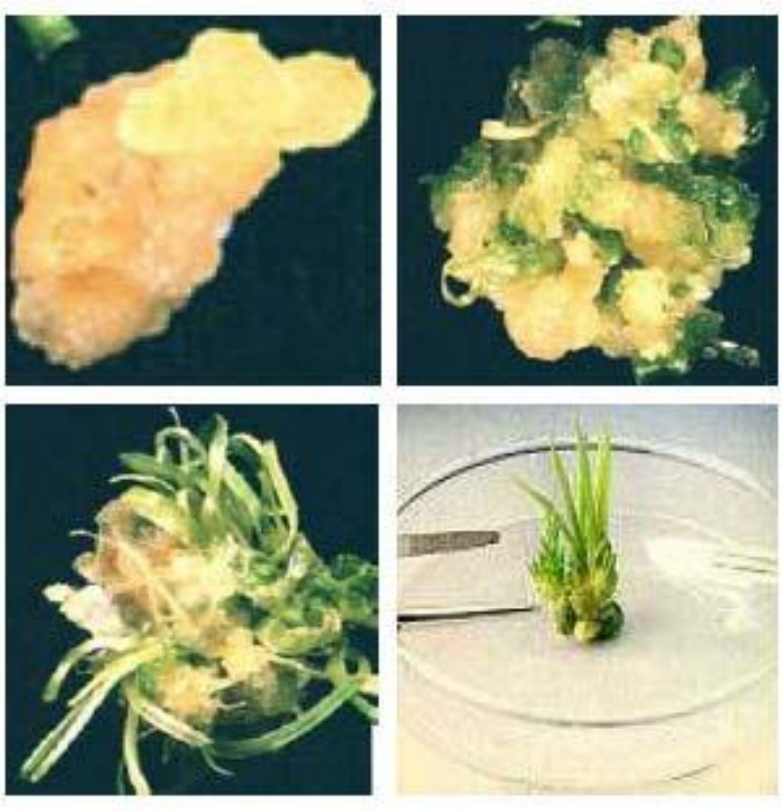
Events during organogenesis

It is a general rule that the organ formation would be through a process of differentiation in the undifferentiated mass of parenchyma. Most of the parenchymatous cells are highly vacuolated and with inconspicuous nuclei and cytoplasm, sometimes with lignification. In this group of cells, regions showing random cell division would occur, leading to radial files of differentiated tissues. These scattered cell division regions would form regions of high mitotic activity resulting in the formation of meristematic centres, otherwise termed as meristemoids. These meristemoids may be either on the surface of the calli or embedded in the tissue. Continued cell division in these meristemoids would produce small protuberances on the surface of the calli, giving nodular appearance to the tissues. From the meristemoids, the primordia of organs by repeated mitotic activity form either shoot or root. This was discovered by Torrey in 1966.

The meristemoids consist of a spherical mass of small isodiametric meristematic cells with dense cytoplasm and a high nucleo-cytoplasmic ratio. Normally, callus tissues accumulate starch and other crystals before organogenesis, but the substances disappear during meristemoid formation. During the initial stages of meristemoid formation, the cytoplasmic protrusions enter the vacuoles thus distributing the vacuoles around the periphery of each cell or dispersed throughout the cytoplasm. The nucleus is in the centre with maximum possible size. Thus cells in the meristemoids resemble the cells of highly active meristem in

an intact plant.

Figure 3. Stages of organogenesis

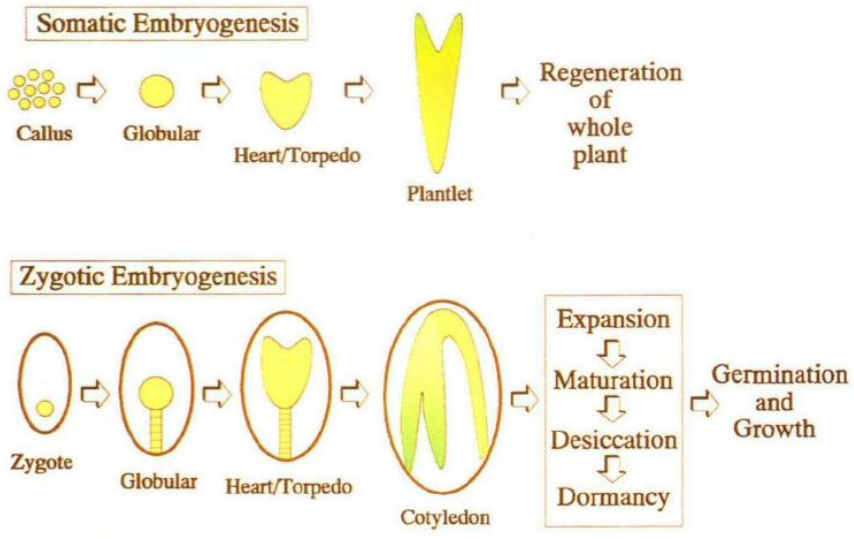


Embryogenesis

An embryo is defined as a plant in its initial stage of development. Each embryo possesses two distinct poles, one to form root and the other shoot, and is the product of fusion of gametes. In some plant species, embryos are produced without the fusion of gametes and termed as asexual embryogenesis or adventitious embyrony.

In an intact plant this type of embryogenesis may occur in sporophytic tissues like integuments and nucellar tissues or from unfertilized gametic cells. Apart from the normal course of embryo formations *viz*., zygotic embryogenesis and adventitious embryony, instances of embryo formations from the tissues cultures *in vitro* were reported. This phenomenon termed as somatic embryogenesis was first observed by Steward and his co-workers (1958) in suspension cultures of carrot followed by Reinert (1959). Since then, a number of reports of embryo formation have been published.

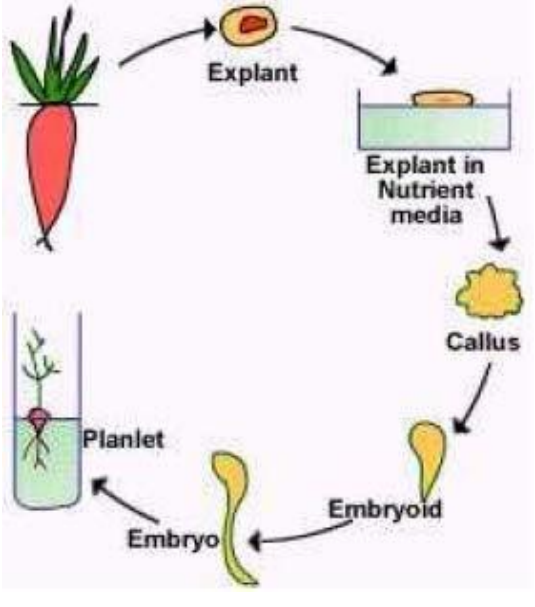
Figure 4. A comparison of somatic and zygotic embryogenesis



Morphologically and developmentally, somatic and zygotic embryos are most similar from the globular stage through the torpedo stage. Somatic embryos do not experience desiccation or dormancy, but rather continue to grow into fully differentiated plantlets.

Somatic embryogenesis or embryogenesis *in vitro* produces embryo like structures resembling the zygotic embryos in structure and morphogenetic potential. Despite this resemblance, the ontogeny of an embryo like structure from somatic cell differs from that of zygotic embryo, where the origin is from a single cell.

Figure 5. Pathway of somatic embryogenesis



Embryoid is generally used to denote the embryo like structure from cultured tissues. These embryoids possess bipolarity, no vascular connection with the mother tissue and origin from a single cell or a group of cells.

Theories on embryogenesis

Several theories have been proposed to explain the phenomenon of somatic embryogenesis, of which the following are considered important.

Cell isolation theory: Steward and his co-workers proposed this theory in 1964. According to them, the embryo producing cells are isolated from the neighbouring cells in a cell mass. The isolation of cells, favours the embryogenesis. The isolation of cell may be induced by the constraints in the surrounding cells, due to physical and physiological separation of cells. In most cases, the connection of plasmodesmata was severed. But this generally appears to be secondary to the induction process.

Differentiation theory: This theory states that the embryos would not be produced from the differentiated cells of the explants. The cells of explants have to undergo de-differentiation to form callus. Then the cells of callus will produce embryos. In other words, de-differentiation in cells is a prerequisite for the production of somatic embryos *in vitro*.

That the embryos can be formed directly from the epidermal cells of the stem or hypocotyl indicate the possibility of embryo formation without de-differentiation. The need for differentiation depends on the explant material used during primary culture. Epidermal cells of the stem, hypocotyl and young embryos may begin embryo development without going through a callus stage, while cortical cells and cells of xylem and phloem explants require de-differentiation. This theory was proposed by Halperin in 1970.

Intercellular communication and cytodifferentiation theory: According to this theory, cytodifferentiation in cells due to intercellular communication induces embryo formation. The cytodifferentiation is regulated by diffusion gradients of nutrients, endogenous plant growth regulators and gaseous factors like O2, CO2 and ethylene. The changing microclimate in the culture environment affects intercellular communication and in turn cytodifferentiation. This concept was proposed by Street (1973).

Explant physiology and culture environment theory: This concept was developed by Street in 1976. He is of the view that the embryogenesis is a dependent phenomenon on the

explant and the culture environment. Explants like flower buds, young embryos and parts of young seedling are most responsive to produce somatice embryos, but not from those of mature plants. Apart from the explant physiology, culture environment is also a factor influencing the embryogenesis. For example, highly embryogenic callus culture can be maintained non-embryogenic if the medium is supplemented with high level of auxin and the same may be induced to produce embryos when transferred to auxin free medium.

Pre-determination theory: This was proposed by Tisserat *et al*. (1979). It states that the embryo production potential is pre-determined phenomenon in the cells and the *in vitro* culture provides the opportunity for embryogenesis. In other words, embryogenesis from a cell is an inherent one which is facilitated to produce embryos by optimal culture environment.

Pre and induced embryogenic determined cell theory: Though the embryogenesis is pre-determined one there are instances of non-formation of embryos directly from the explants. In these cases, an intervening callus stage comes between the primary explant and the embryos. The cells in the calli are induced to produce embryos by the manipulation of medium with relevant growth regulator. Based on this, the above theory was proposed by Sharp and his co-workers. According to this theory, there are two types of embryogenic cells: pre-embryogenic determined cells (PEDC) and induced embryogenic determined cells (IEDC).

In pre-embryogenic determined embryogenic cells, embryogeny is determined prior to mitosis while induced embryogenic determined cells the embryogeny is induced by providing suitable mitogenic substance i.e., the embryogeny is induced in the cells of callus by the application of plant growth regulators. Thus in the callus, embryogenic precursor cells or embryogenic mother cells are formed which then develop into embryogenic cells. Later these cells undergo polarised cell divisions typical of normal embryogenesis by forming globular, heart and torpedo shaped embryos.

Events during embryogenesis

In 1959, Reinert made the remarkable claim that following a succession of changes of the nutrient media, root derived callus tissue of *Daucas carota* produced normal bipolar embryos. The changes made or observed in the nutrient medium were as follows: maintenance of callus in White's medium with high level of auxin (IAA at 10 mg/litre) and subculturing of callus for several months on White's basal medium with additives like

vitamins, amino acids, amides and purines. As a result of these manipulations, the calli showed small protuberances on the surface. Histological sections of these calli showed centres of organised development. These tissues with organised centres, on transfer to auxin lacking but coconut milk containing medium produced embryoids and from embryoids, whole plants.

Figure 6. Stages of somatic embryogenesis

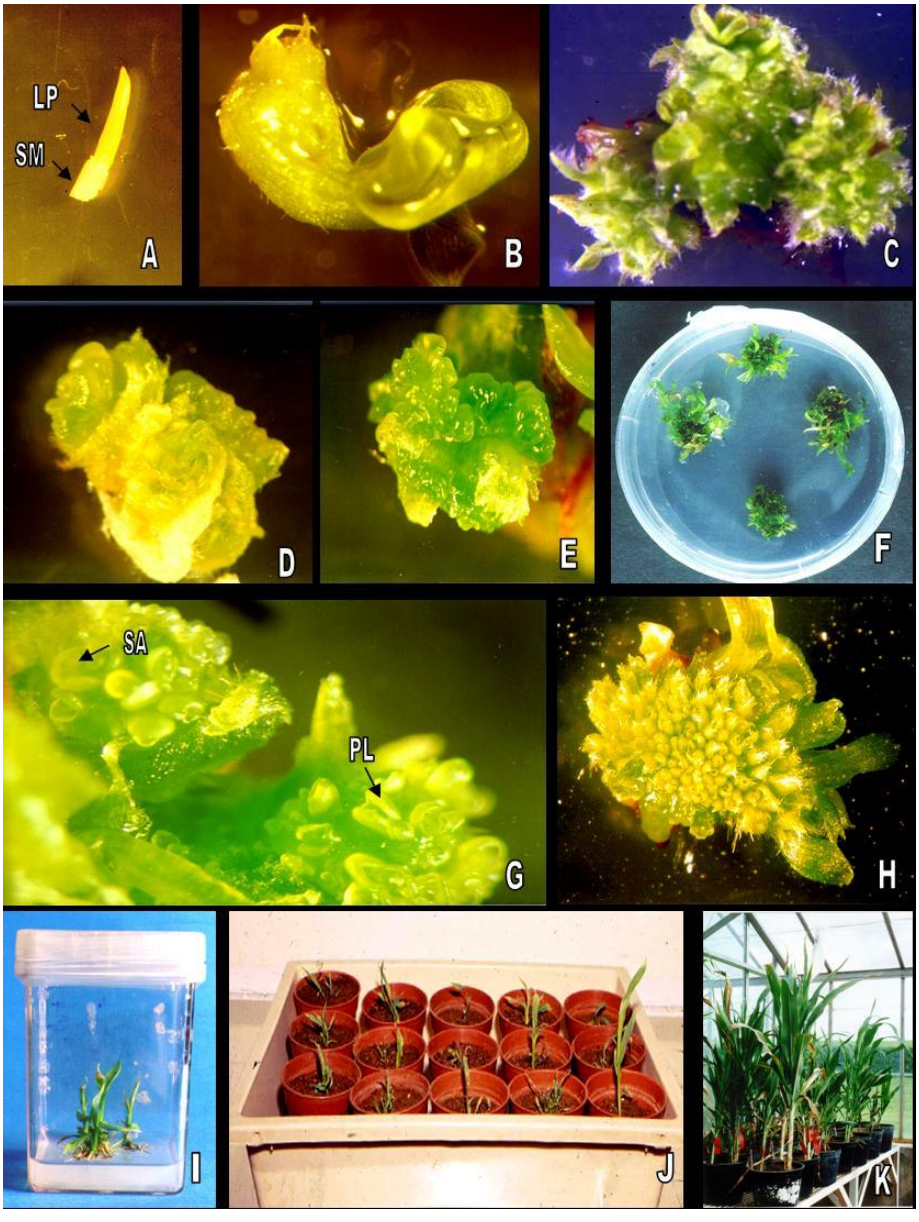
Patterns of embryogenesis

Two general patterns of embryogenesis *in vitro* are identified. Origin of embryos directly from the tissue cultured *in vitro* (direct embryogenesis) and origin of embryos *via* callus stage (indirect embryogenesis).

Differences between direct and indirect embryogenesis

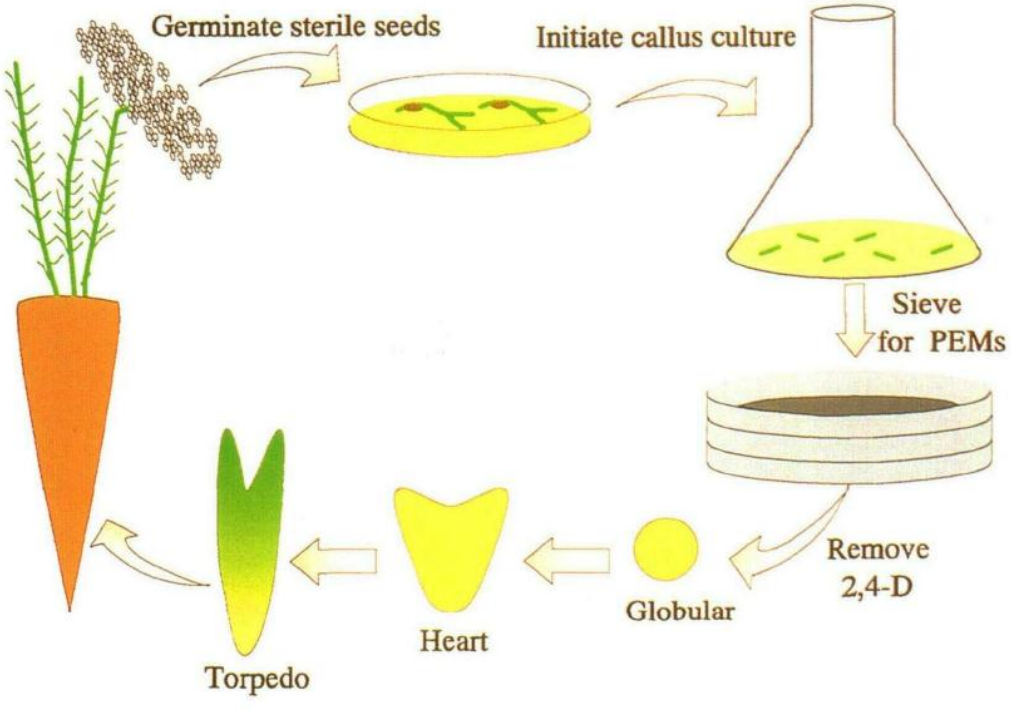
Direct embryogenesis	Indirect embryogenesis
Embryos form from the explants directly	Embryos arise from the callus induced from the explants
A promoting substance to induce the embryo formation is needed	Auxin is need to induce callus, and cytokinin is needed to induce differentiation
The embryogenic nature of a cell is predetermined	The embryogenic nature of a cell is induced in the culture
The origin of embryos mostly from individual cells; sometimes from a group of cells	The origin may be either from single cells or from a group of cells called pro-embryonal complex

Figure 7. Direct somatic embryogenesis in sorghum



(A) One day old isolated shoot apices with primordial leaves *LP = leaf primordia*; *SM = shoot meristem*. (B) One- week old explant showing bulged meristem portion and expanded primordial leaves. (C) Three- week old meristematic mass showing multiple buds and leaf initials. (D) Individual buds (shown in Fig.1C) producing 2-8 translucent tissue strata. (E) Each of the tissue stratum giving rise to many somatic embryos. (F) Meristematic clumps showing differentiating buds. (G) Germinating somatic embryo's showing shoot apex (*SA*) surrounded by a pair of primary leaves *(PL). (*H) Differentiation of somatic embryos into platelets. (I) Plantlets with well formed roots in magenta box. (J) Acclimatization of plantlets in the growth chamber. (K) Regenerated plants in greenhouse.

Figure 8. Indirect somatic embryogenesis in carrot



Once induction of embryogenic determined cells have been achieved, there appears to be no fundamental difference between indirect and direct somatic embryogenesis. In both processes, embryoids may arise from one or more of a group of determined cells. There are close homologies between direct and indirect embryogenesis and between single cell and multiple cell initiation of embryoids. The differences observed among these may be attributed to differences in the neighbouring cells and the mode of determination of embryogenic nature.

Questions

1. Cell differentiation in animals is ……..

a) Irreversible b) Reversible

c) Both a & b d) None of the above

2. Cell differentiation in plants is ……..

a) Irreversible **b) Reversible**

c) Both a & b d) None of tha above

3. The inherent potentiality of a plant cell to give rise to a whole plant is described as ……..

a) Cellular totipotency **b) Morphogenesis**

c) Organogenesis d) None of the above

4. For a differentiated cell, to express its totipotency, it should undergo dedifferentiation followed by redifferentiation

a) Dedifferentiation b) Redifferentiation

c) Both a & b d) None of the above

5. The phenomenon of a mature cell reverting to the meristematic state and forming undifferentiated callus tissue is termed as ……..

a) Dedifferentiation b) Redifferentiation

c) Both a & b d) None of the above

6. The phenomenon of conversion of component cells of callus tissue to whole plant or plant organs is called as ……..

a) Dedifferentiation **b) Redifferentiation**

c) Both a & b d) None of the above

7. The phenomenon of totipotency is demonstrated with the experiment involving ……..

a) Beetroot b) Onion

c) Carrot d) None of the above

8. The phenomenon of morphogenesis depends on the factors like……..

a) Polarity b) Differentiation

c) Regeneration of individual cells **d) All the above**

9. …….. concluded in "Organbildung im Pflanzenseichconcluded that the fate of a cell is a function of its position.

a) Vochting b) Haberlandt

c) Hanstein d) Klebs

10. Under *in vitro* conditions this can be achieved by …….. .

a) *de novo* origin of organs b) *de novo* origin of embryos

c) Both a & b d) None of the above

11. Organogenesis means genesis of organ(s) like …….. .

a) shoots b) roots

c) leaves and flowers **d) All the above**

12. The earliest report on induction of shoot organogenesis *in vitro* was by …….. using a tobacco hybrid.

a) White b) Nobecourt

c) Skoog d) Miller

13. The first observation of root formation was reported by …….. using carrot callus.

a) White **b) Nobecourt**

c) Skoog d) Miller

14. The regulatory mechanism ie., balance between auxin and cytokinin is recognized by……..

a) White **b) Nobecourt**

c) Skoog & Miller d) Murashigee

15. The relatively high level of auxin to cytokinin favoured …….. during organogenesis

a) Root formation b) Shoot formation

c) Both root and shoot formation d) None of the above

16. The relatively high level of cytokinin to auxin favoured …….. during organogenesis.

a) Root formation
b) Shoot formation
c) Both root and shoot formation
d) None of the above

17. The genesis of shoot from the explants or calli is termed as ……..

a) Caulogenesis
b) Rhizogenesis
c) Organogenesis
d) None of the above

18. The genesis of root from the explants or calli is termed as ……..

a) Caulogenesis
b) Rhizogenesis
c) Organogenesis
d) None of the above

19. The event of organogenesis is discovered by ……..

a) Torrey
b) Skoog
c) Guha
d) None of the above

20. The somatic embryogenesis was first observed by ……..

a) Steward
b) Skoog
c) Reinert
d) None of the above

21. The various theories of somatic embryogenesis are ……..

a) Cell isolation theory
b) Differentiation theory
c)Intercellular communication and cytodifferentiation theory
d) All the above

22. The cell isolation theory was proposed by ……..

a) Steward
b) Halperin
c) Street
d) Tisserat

23. The differentiation theory was proposed by ……..

a) Steward
b) Halperin
c) Street
d) Tisserat

24. The Intercellular communication and cytodifferentiation theory was proposed by ……..

a) Steward
b) Halperin
c) Street
d) Tisserat

25. The explant physiology and culture environment theory was proposed by ……..

a) Steward
b) Halperin
c) Street
d) Tisserat

26. The pre-determination theory was proposed by ……..

a) Steward
b) Halperin
c) Street
d) Tisserat

27. Members of …… family readily form somatic embryos in culture

a) Umbelliferae
b) Orchidaceae
c) Leguminosae
d) None of the above

28. High concentration of …… favours shoot initiation.

a) IAA
b) Kinetin
c) GA_3
d) None of the above

29. High concentration of …… favours rooting.

a) Auxin
b) Cytokinin
c) Gibberellic acid
d) None of the above

30. In somatic embryogenesis …… is required for induction of embryonic cells and maintenance of proliferative growth..

a) Auxin
b) Cytokinin
c) Gibberellic acid
d) None of the above

31. Embryo formation can be induced by transferring the callus to a medium lacking ……

a) Auxin
b) Cytokinin
c) Gibberellic acid
d) None of the above

32. …… is used most successfully to obtain rapid growth of somatic embryos into plants.

a) Auxin

b) Cytokinin

c) Gibberellic acid

d) None of the above