Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (Second Year)

Subject: Botany

Course Code: BOC 103

Course Title: Plant Anatomy and Embryology

Unit: 8 - Apomixis and Polyembryony

Module Name: Concepts, Types and Practical Application of Polyembryony

Module No: 49

Name of the Presenter: Dr. Kim Rodrigues

NOTES

Concepts of Polyembryony

- Polyembryony has been defined as the occurrence of more than one embryo in a seed.
- However, the additional embryos do not always mature. They may remain arrested at very early stages or may degenerate in the course of seed development.
- Polyembryony was first time reported by Antony van Leeuwenhoek in 1719 in the seeds of orange.
- Except for a few taxa (*Citrus, Mangifera*), polyembryony occurs only as an abnormal feature.

Types of Polyembryony

- Braun (1859) has recognised four types of polyembryony in angiosperms on the basis of origin:

1. Cleavage of proembryo,
2. Formation of embryos by cells of the embryo sac other than the egg,

3. Development of more than one embryo sac within the same ovule, or
4. Activation of some sporophytic cells of the ovule.

1. Cleavage of proembryo:

- It is the common and simplest method of the development of more than one embryo in a seed.
- Two or more embryos are formed in a seed by the cleavage of the zygote or proembryo.
- This feature is of widespread occurrence in the gymnosperms, but it is rare in the angiosperms.

- Cleavage polyembryony may arise in different ways:
- In *Erythronium americanum* (Liliaceae), the first division of the zygote is normal and as a result a basal and an apical cell are formed.
- Repeated divisions of the basal cell give rise to an irregular mass of cells which is known as embryonic mass.
- Several cells at the distal end of the embryonic mass develop into separate embryos.
- This type of cleavage polyembryony has also been reported in *Limnocharis emarginata, Tulipa gesneriana, Vincetoxicum nigrum, V. officinale* and *Habenaria platyphylla*.

- In *Isotoma longiflora* (Campanulaceae) and *Exocarpus* (Santalaceae), additional embryos are developed from the suspensor cells of the proembryo.

- Cleavage polyembryony is also fairly common in orchids.
- In *Eulophia epidendraea*, there are three different modes of cleavage polyembryony:

a. The zygote divides irregularly to form a mass of cells of which those lying toward the chalazal end grow simultaneously and give rise to many embryos.
b. The proembryo gives rise to small buds/outgrowths which may themselves function as embryos.
c. The filamentous proembryo becomes branched, and each branch gives rise to an embryo.

2. Formation of embryos from cells of the embryo sac other than the egg:

- Additional embryos originate from cells of the embryo sac other than the egg cell.
- Most common source of additional embryos are the synergids.
- Depending on whether it arises from fertilized or unfertilized synergid, the embryo may be diploid or haploid.
- The fertilization of the synergids can be brought about either by the entry of more than one pollen tube into the embryo sac or by the presence of additional sperms in the same pollen tube.
- In such a situation the zygotic as well as the synergid embryos are diploid.
- Embryos arising from fertilized synergids are known in *Aristolochia bracteata, Poa alpina*.
- Embryos arising from unfertilized synergids are known in *Argemone mexicana, Phaseolus vulgaris*.

- Formation of embryos from antipodals is rather rare (*Allium odorum, Paspalum scrobiculatum, Sedum fabaria, Ulmus americana*).
- The antipodal cells may divide a few times to form proembryo-like structures. However, they fail to grow into an adult embryo.

3. **Development of more than one embryo sac within the same ovule:**

- Sometimes additional embryo sacs are present in the ovule, and fertilization of their eggs results in the formation of extra embryos.
- Multiple embryo sacs in an ovule may arise from:

a. By differentiation of more than one megaspore mother cells in the ovule as in *Hydrilla verticellata, Solanum melongena, Jussiaea repens, Casuarina montana*;

b. By activation of two or more spores out of the four megaspores formed by the reduction division of the megaspore mother cell, as in *Rosa* and *Culcitium reflexum*; or

c. From the cells of the nucellus (*Citrus, Mangifera, Opuntia*) or integument (*Limnanthes*).

- Formation of twin embryo sacs within an ovule is known in *Casuarina equisetifolia, Citrus* and *Poa pratensis*.
- The normal embryo sac develops only up to the 4-nucleate stage, and the multiple embryos are formed by aposporous embryo sacs.
- Numerous embryo sacs develop concurrently in the same ovary and their tips, carrying the egg apparatus, grow up to various heights in the style.
- After fertilization, the embryos grow downward and enter the composite endosperm in the ovarian cavity.
- All embryos but one collapse during seed development resulting into monoembryonate seeds.
- Occasionally, however, two or more embryos may mature resulting into polyembryonate seeds.
- Polyembryonate seeds – there are either two green and fully developed embryos, or there are more than two embryos of which only one is well developed and the others poorly formed and non-green.

4. **Activation of some sporophytic cells of the ovule:**

- The embryos arising from the maternal sporophytic tissue (outside the embryo sac) are called adventive embryos.
- The only maternal tissues which are known to form adventive embryos are the nucellus and the integuments.
- Nucellar cells destined to form adventive embryos can be distinguished from other cells of the nucellus by their dense cytoplasm and starchy contents.
- The inception of nucellar embryos takes place outside the embryo sac but they are gradually pushed into the embryo sac cavity where they divide and differentiate into mature embryos.
- The adventive embryos do not show synchronous development and usually only one of them attains maturity.
- Nucellar embryos can be distinguished from the zygotic embryo by their lateral position in the embryo sac, irregular shape, and lack of suspensor.
- Development of endosperm plays an important role in the origin of adventive embryos.
- Nucellar polyembryony is known to occur in *Citrus, Mangifera, Opuntia dillenii*.

Causes of Polyembryony

1. Necrohormone theory

- This theory proposed by Haberlandt (1921, 1922), states that degenerating cells of the nucellus act as source of stimulus to the adjacent cells to divide and form adventive embryos.
- But no clear evidence is available to show that adventive embryony is induced by the secretory substances of the dying cells.

2. Recessive gene theory

- In *Linum*, Kappert (1933) has shown that polyembryony is a recessive genetic character, controlled by multiple genes.

- Due to hybridization in different strains, genes recombine in an individual plant and this exhibits the phenomenon of polyembryony.
- Leroy (1947) also considers that in *Mangifera indica* polyembryony is caused by one or more recessive genes.
- Frusato et al. (1957) showed that embryo number in *Citrus* seeds may be influenced by factors like: age of the tree (increasing in older trees), fruit-set (being higher in years of higher fruit-set), nutritional status of the plant (decreasing with reduced food supply), etc.

Induced Polyembryony

- Earlier, it was considered that a specific physical and chemical environment is required for the development of the embryo which is available only in the embryo sac.
- But studies have clearly indicated that not only the ovular tissue but all the cells of a plant are capable to develop into embryos, and embryo development can also be carried out in culture medium.
- The embryos developed in culture medium are known as adventitious embryos, somatic embryos, supernumerary embryos or embryoids.
- Besides zygote and ovular tissue, embryoids can also be obtained from the cells of the root, leaf, fruit, pollen grain, etc.
- Somatic embryos have been obtained successfully in over 100 species, including wheat, paddy, soybean, apple, coffee and grapes.

Factors affecting the growth of embryoids:

- In culture medium, two important factors which affect the growth of embryoids are growth hormones and source of nitrogen.
- The requirement of growth hormones varies in different plants.
- In carrot, for the development of callus, auxin is required in ample amount (2,4-D,0.5-1 mgl^{-1}) in the medium, whereas embryos are differentiated from the

callus only when the culture medium is auxin-free or with very little amount of auxin (2,4-D, 0.01-0.1 mgl^{-1}). In *Coffea arabica*, embryos are differentiated on the callus only in auxin-free medium. Abscissic acid alone can also be used for induction of somatic embryos.

- The source of nitrogen in the culture medium also influences development of somatic embryos.
- Nitrogen supplied in the form of nitrates (KNO_3) stimulates the development of callus, but for the differentiation of embryos, nitrogen in the form of ammonium (NH_4Cl) should be supplied to the medium.

Classification of Polyembryony

Polyembryony is of two types:

1. Spontaneous: Includes instances of naturally occurring polyembryony.
2. Induced: Includes instances of experimentally induced polyembryony.

- Spontaneous polyembryony has been sub-divided by Ernst (1901, 1910) into two categories:

a. True polyembryony: Two/more embryos arising in the same embryo sac; from the zygote/embryo (*Eulophia, Vanda*), from synergid (*Sagittaria*), from antipodal cell (*Ulmus*), or from nucellus or integument (*Citrus, Spiranthes*).
b. False polyembryony: Development of embryos in more than one embryo sac in the same ovule (*Fragaria*) or placenta (Loranthaceae).

- Yakovlev (1967) proposed a classification of polyembryony on genetic basis. He distinguished two types of spontaneous polyembryony:

1. Gametophytic: Arising from any gametic cell of the embryo sac with/without fertilization.
2. Sporophytic: Arising from the zygote, proembryo or the initial sporophytic cells of the ovule (nucellus, integuments).

- Bouman and Boesewinkel (1969) put forth another classification based on the origin of the additional embryos. According to them spontaneous polyembryony should be split into four categories:

1. Supernumerary embryos arising from sporophytic cells of the parental generation (integuments and nucellus).
2. Supernumerary embryos arising from the cells of the gametophyte (by one or more embryo sacs in the same ovule).
3. Supernumerary embryos arising from the new sporophyte (fertilized egg/proembryo).
4. Supernumerary embryos arising from the male gametophyte (disputed group).

Practical Application of Polyembryony

- Adventive polyembryony is of great significance in horticulture and plant breeding.
- The adventive embryos provide uniform seedlings of the parental type, as obtained through vegetative propagation by cuttings.
- However, nucellar seedlings of *Citrus* furnish better clones of orchard stock than cuttings. This is because the nucellar seedlings have a tap root and, therefore, develop a better root system than do the cuttings. The latter have only a small lateral root system; the nucellar seedlings show a restoration of the vigour lost after repeated propagation by cuttings; the nucellar embryos are free from disease. So far nucellar polyembryony is the only practical approach to raise virus-free clones of polyembryonate *Citrus* varieties in nature (orchard stock).
- Adventive embryos are also of utmost significance in morphogenetic studies.

SUMMARY

- Polyembryony has been defined as the occurrence of more than one embryo in a seed.

- Adventive polyembryony is of great significance in horticulture and plant breeding.
- The adventive embryos provide uniform seedlings of the parental type, as obtained through vegetative propagation by cuttings.
- So far nucellar polyembryony is the only practical approach to raise virus-free clones of polyembryonate *Citrus* varieties in nature (orchard stock).
- Adventive embryos are also of utmost significance in morphogenetic studies.