MBT 303

Haploids: Androgenesis and Gynogenesis

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Introduction:

Parthenogenesis: Spontaneous haploids production

Spontaneous production of haploids usually occurs through the process of parthenogenesis (embryo development from an unfertilized egg). However, occasionally, they bear the characters of male parent only, suggesting their origin through ‘ovule androgenesis' (embryo development inside the ovule by the activity of the male nucleus alone where elimination or inactivation of egg nucleus occurs before fertilization).

in situ parthenogenesis (pollen irradiation and chemical treatment) can be employed for generation of haploid plants

In vitro Haploid Production:

1. **Androgenesis**
2. **Gynogenesis**

   production of haploids via gynogenesis is more tedious, less efficient in comparison to androgenesis.

In vitro androgenesis (anther-microspore culture) is one of the most preferred techniques for obtaining haploids but, *in vitro* gynogenesis (unfertilized ovary-ovule culture) can prove to be a complementary technique in species where anther culture is inaccessible or less productive. It means that not only the microspore but, also the megaspore of angiosperms can be triggered *in vitro* to undergo sporophytic development

Androgenesis

- First *in vitro* culture of anthers of *Datura* (Guha and Maheshwari 1964,1966)

- In androgenesis, the male gametophyte (microspore or immature pollen) produces haploid plants.

- The basic principle is to stop the development of pollen into a gamete (sex cell) and force it to develop into a haploid plant or sporophyte.

- Haploid production through anther/microspore culture scores higher over other methods due to the fact that anthers harbour large numbers of haploid microspores per anther and is a potentially efficient means to generate homozygous true-breeding progeny lines in plant breeding programs.

Method for Androgenesis

(i) Anther culture

(a)Select the flower buds from an elite plant and determine the stage of microspores by acetocarmine squashes of anthers or by staining them with fluorescent dye DAPI (4,6-diamidino-2-phenylindole).

(b)Surface sterilize the selected size of buds (ca 2mm size flower bud) to initiate *in vitro* anther cultures.

(c)Dissect the buds under a stereo-microscope, using pre-sterilized Petriplates, forceps and fine needles. Discard the damaged anthers, if any, and remove the filament gently.

(d)Inoculate the anthers, bearing early-to-late uninucleate stage of microspores, in a nutrient medium and maintain the cultures in defined conditions.

(e) As the anthers proliferate, they produce embryos/callus.

(f)The callus/embryos formed can be transferred to a suitable medium to finally produce haploid plants and then diploidize them by colchicine to produce homozygous diploids.

(ii) Microspore (Pollen) culture

Haploid plants can also be produced from isolated immature pollens or microspores (male gametophytic cells):

(a) Select the flower buds from an elite plant and determine the stage of microspores by acetocarmine squashes of anthers or by staining them with fluorescent dye DAPI (4,6-diamidino-2-phenylindole).

(b) Surface sterilize the selected size of buds to initiate *in vitro* anther cultures.

(c) Extract the microspores by pressing and squeezing the buds with a glass rod against the sides of a beaker.

(d) Filter the pollen suspension to remove anther tissue debris.

(e) Wash and collect the viable and large pollen (smaller pollen do not regenerate) by filtration.

(f) Culture these microspores on a solid or liquid medium.

(g) As the microspores undergo multiple divisions, they produce multicellular and multinuclear structure.

(h) The callus/ embryos formed can be transferred to a suitable medium to finally produce a haploid plants and then diploid plants by colchicine treatment

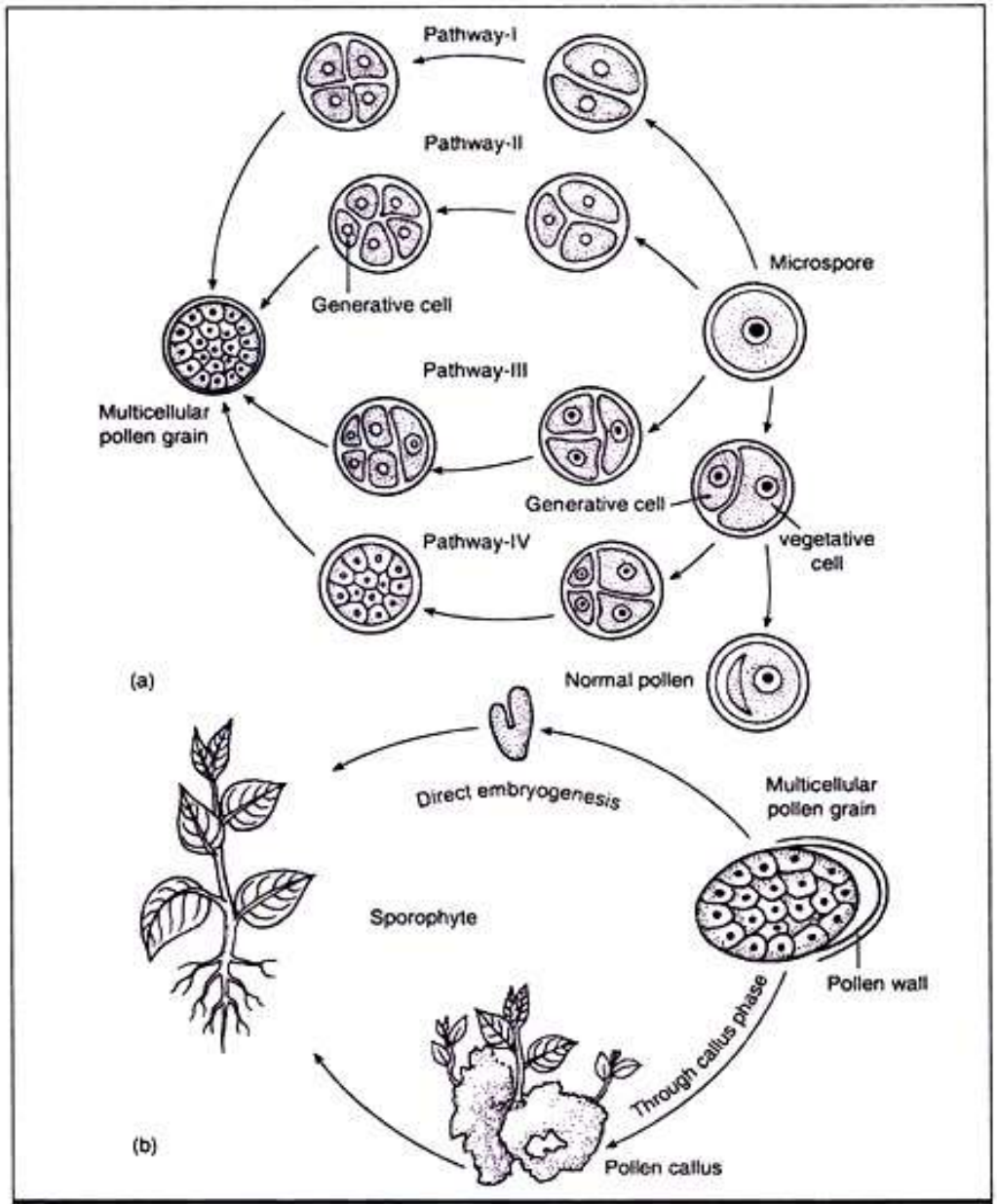

Fig. 21.4: (a) Pathways of microspore divisions leading to the formation of a multicellular pollen grain; (b) Multicellular pollen may directly form an embryo or produce sporophytes through a callus phase (after Bhojwani and Bhatnagar, 1990)

Factor Affecting Androgenesis:

(a) Stage of Pollen

(b) Physiological Status of Donor Plant

(c) Genotype of Plants

(d) Pretreatment of Anthers (3-5^{o}c for 72hr)

(e) Culture Media and density

(f) Culture Conditions

(g) Anther wall

Gynogenesis

Gynogenic development of plants from unfertilized cells of female gametophyte (embryo-sac) in ovary/ovule/young flower cultures is one of the available alternatives for haploid production. It was first reported in barley San Noeum (1976).

Method:

Ovary slice culture

a. For Ovary slice culture in Tea, unopened and unpollinated mature flower-buds (6-10 mm) size were collected early in the morning. Some of the buds were fixed in FAA (5:5:90 v/v/v Formaldehyde: Acetic acid: 70% Ethanol), for 48 h, and then stored in 70% alcohol. Later on, the appropriate developmental stage of the embryo sac was determined by histological analysis.

b. The flower buds were surface sterilized with 0.1% $HgCl_2$ for 7 minutes, followed by rinsing with sterile distilled water at least thrice.

c. Carefully dissected transverse sections of ovaries were cultured on Murashige and Skoog's media supplemented with varying concentrations of Auxins and Cytokinins.

d. Six ovary slices containing unpollinated ovules were cultured in 60 mm X 15 mm pre sterilized disposable Petriplates containing 10 ml MS medium.

e. The sealed Petriplates were subjected to various regimes of temperature and light treatments.

Ovule culture

The unfertilized ovary is surface sterilized and the ovules were dissected out and placed into culture.

Two types of ovule support systems have been developed. The filter paper support system involves culturing of the ovules on top of filter paper placed over liquid medium, whereas the vermiculite support technique demands placing the ovules on a sterile vermiculite/liquid media mixture (vermiculite support) with the micropylar side down. Unpollinated ovule culture has been used for haploid production in sugar beets and onions.

Application of Haploids:

1. Development of pure homozygous lines

2. Genetic studies

3. Gametoclonal variation

4. Induction of mutations

6. Genetic manipulation

7. Shortening of breeding cycle

Source: 1. Plant Tissue culture: Theory and Practice A revised edition by S.S. Bhojwani and M. K. Razdan

2. Open access free sources