Lab 5: HAPLOID CULTURE

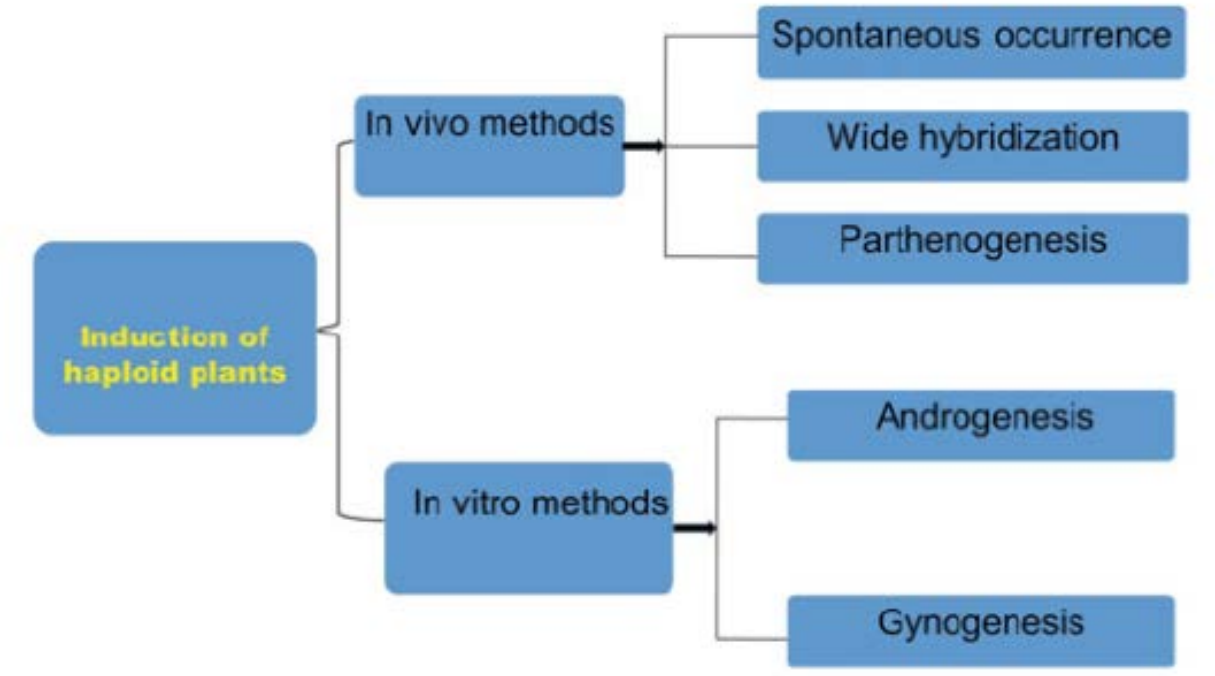
Haploids are plants (sporophytes) that contain a gametic chromosome number (n).

What is Haploid Production?

Haploid culture is an in vitro technique used to produce haploid (cells have half the number of chromosomes) plants.

Production of Haploids:

Production of Haploids are produced by most important methods currently being utilized under biotechnology programmes include: (i) anther or pollen culture and ovule culture (ii) chromosome elimination following interspecific hybridization (bulbosum technique).



In vitro methods of haploid production

1. Androgenesis ((Pollen or Anther Culture))

Pollen / Anther culture: production of haploid is through culturing anthers or microspores on artificial culture medium. The production of haploids through anther or pollen culture is called androgenesis and to-date, it has been reported in 135 species. The principle involved in the process is to stop the development of pollen

cells into a gamete and induce it in a suitable environment to develop into a haploid plant. The two types of androgenesis include:

- Direct androgenesis: the formation of an embryo directly from pollen or microspore without callus.
- Indirect androgenesis: the formation of an embryo with an intermediate callus stage.

The development of haploids through androgenesis depends on several factors that include:

1. The genotype of donor plants
2. Stage of microspore or pollen development
3. Physiological status of donor plants
4. Pretreatments of anthers.
5. Anther wall factors
6. Culture medium and the culture density
7. Effect of temperature and/or light

2. Gynogenesis

Ovule culture Haploids from Unpollinated Ovaries - Production of a haploid individual by development of an unfertilized egg cell as a result of delayed pollination is referred to as gynogenesis.

It was first reported in Hordeum vulgare (Barley) by San Norm in 1976, and later the technique was used for haploid production in wheat, maize tobacco, sunflower, sugarbeet, and other economically important plants.

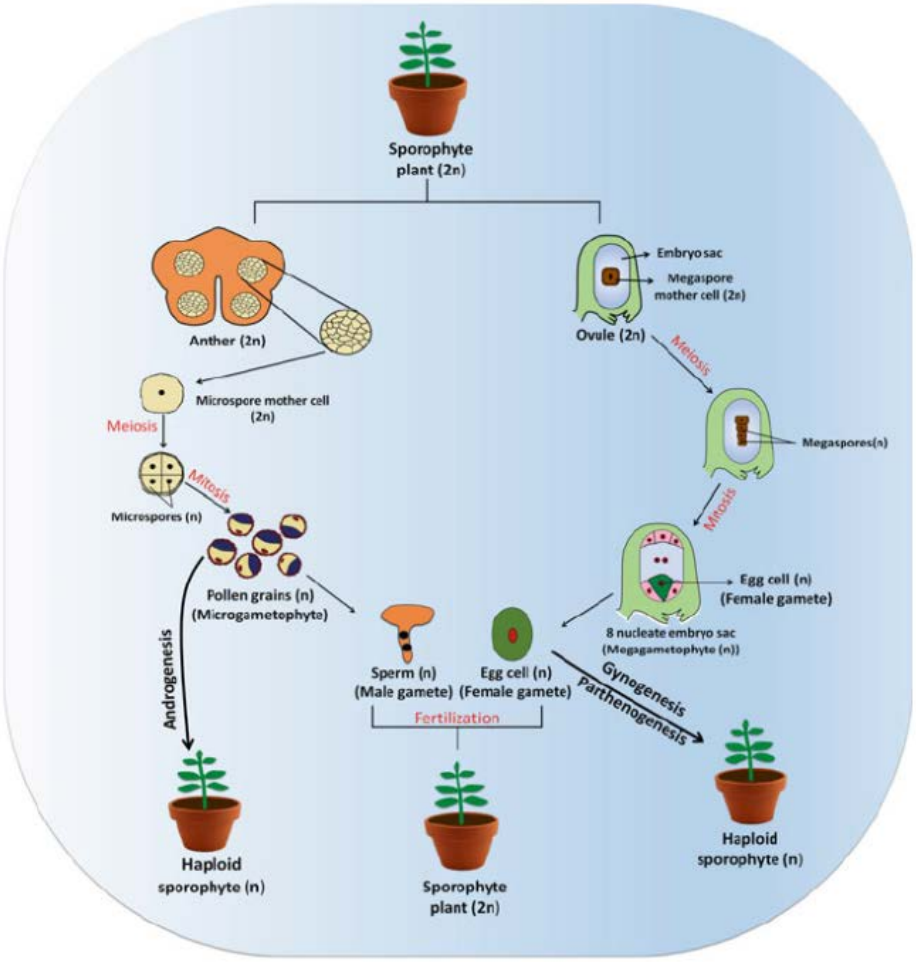
This approach is used where androgenesis is not effective in producing haploids. To-date, haploids have been produced in about 19 species (distributed to 10 families) using the approach of gynogenesis.

This method has a two-way approach, which includes:

- Direct embryogenesis
- Indirect embryogenesis that goes through callus formation followed by plant regeneration on another medium.

These methods have been very useful in quantitative trait analysis, mutational studies, identification of recessive traits, and hybrid production with particular traits.

These methods have shortened the time required for cultivar development. Future research may provide more insights into the factors critical to haploid production and expand into the production of some other species of the plants.



Diploidization of Haploids Plants:

Haploid plants obtained either from anther or ovule culture may grow normally under in vitro conditions up to the flowering stage. But viable gametes are not formed due to the absence of one set of homologous chromosomes and consequently, there is no seed set.

A simple procedure designed to achieve diploidization involves immersion of very young haploids in a **filter sterilized solution of colchicine (0.4%) for 2-4 days**, followed by their transfer to the culture medium for further growth. In this procedure

chromosome or gene instabilities are minimal compared to other methods of colchicine or chemical treatment.

The haploid plants can be treated with colchicine to obtain doubled haploids.

Uses of haploids and doubled haploids:

- Completely homozygous plants Inbred lines
- Mutation studies Breeding
- Mapping.

Application of Haploids in Plant Breeding

In Vitro production of haploids can solve some problems in genetic studies since gene action is readily manifested due to a single allelic gene present in chromosome of entire genome.

1. Releasing New Varieties through F1 Double –haploid System:

The anthers of the hybrid (F1) progeny are excellent breeding material for raising pollen-derived homozygous plants (Double –haploids) in which complementary parental characteristics are combined in one generation.

Double –haploids are also useful in studies related to inheritance of quantitative traits. Using double –haploid technique new varieties have been developed in barley, Brassica, rice, maize, rye, potato, pepper and asparagus.

2. Selection of Mutants Resistance to Diseases:

A mutant with resistance to disease is of major importance in crop improvement. Haploids provide a relatively easier system for the induction of mutations. Some examples of using anther culture technique in mutant successfully are tobacco mutants resistant to black shank disease and wheat lines resistant to scab *Fusarium graminearum*.

3. Developing Asexual Lines of Tree Perennial Species:

Chinese workers obtained pollen –derived rubber tree taller by six meters which could then be multiplied by asexual propagation to raise several clones.

4. Transfer of Desired Alien (Foreign) Gene:

Chromosomal instability in haploids makes them potential tools for introduction of alien chromosomes on genes during wider crossing programs. In rice, developing a resistance to blast requires about 12 years by conventional breeding through back crossing, but through hybridization and anther culture, this can be achieved in two years.

5. Establishment of Haploids and Diploid Cell Lines of Pollen Plant.