Lecture: Production of Haploids

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Dr. Gaurav Kumar
Department of Botany
Dyal Singh College
Delhi University

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Introduction

✓ Haploids are plants (sporophytes) that contain a gametic chromosome number (n).
✓ Blakeslee first described this phenomenon in *Datura stramonium* in 1922.
✓ This was subsequently followed by similar reports in tobacco (*Nicotiana tabacum*), wheat (*Triticum aestivum*) and several other species (Forster et al., 2007).
✓ The potential of haploidy for plant breeding arose in 1964 with the achievement of haploid embryo formation from in vitro culture of *Datura* anthers (Guha and Maheshwari, 1964, 1966).
The main factors affecting haploid induction and subsequent regeneration of embryos are:-

- genotype of the donor plants,
- physiological condition of donor plants (i.e. growth at lower temperature and high illumination),
- developmental stage of gametes, microspores and ovules,
- pre-treatment (i.e. cold treatment of inflorescences prior to culture, hot treatment of cultured microspores),
- composition of the culture medium (including culture on “starvation” medium low with carbohydrates and/or macro elements followed by transfer to normal regeneration medium specific to the species),
- physical factors during tissue culture (light, temperature).
Haploid techniques

a) Induction of maternal haploids

1. **In situ induction of maternal haploids:** In situ induction of maternal haploids can be initiated by pollination with pollen of the same species (e.g., maize), pollination with irradiated pollen, pollination with pollen of a wild relative (e.g., barley, potato) or unrelated species (e.g., wheat).

2. **Wide hybridization:** Wide crossing between species has been shown to be a very effective method for haploid induction and has been used successfully in several cultivated species.

Refer to the 4th slide of production technique of gynogenesis and androgenesis provided to you on your e mail/whatsapp group.
a) Induction of maternal haploids

1. In situ induction of maternal haploids:

✓ In situ induction of maternal haploids can be initiated by pollination with pollen of the same species (e.g., maize), pollination with irradiated pollen, pollination with pollen of a wild relative (e.g., barley, potato) or unrelated species (e.g., wheat).

✓ Pollination can be followed by fertilization of the egg cell and development of a hybrid embryo, in which paternal chromosome elimination occurs in early embryogenesis or fertilization of the egg cell does not occur, and the development of the haploid embryo is triggered by pollination of polar nuclei and the development of endosperm.
2. **Wide hybridization:**

- Wide crossing between species has been shown to be a very effective method for haploid induction and has been used successfully in several cultivated species.

- It exploits haploidy from the female gametic line and involves both inter-specific and inter-generic pollinations.

- The fertilization of polar nuclei and production of functional endosperm can trigger the parthenogenetic development of haploid embryos, which mature normally and are propagated through seeds (e.g., potato).

- In other cases, fertilization of ovules is followed by paternal chromosome elimination in hybrid embryos.

- The endosperms are absent or poorly developed, so embryo rescue and further in vitro culture of embryos are needed (e.g., barley).

- The ‘bulbosum’ method was the first haploid induction method to produce large numbers of haploids across most genotypes and quickly entered into breeding programs.
Bulbosum Method

Hordeum vulgare
Barley
2n = 2X = 14

×

Hordeum bulbosum
Wild relative
2n = 2X = 14

Embryo Rescue

Haploid Barley
2n = X = 7
H. Bulbosum chromosomes eliminated

• This was once more efficient than microspore culture in creating haploid barley
• Now, with an improved culture media (sucrose replaced by maltose), microspore culture is much more efficient (~2000 plants per 100 anthers)
3. **In vitro induction of maternal haploids – gynogenesis:**

- In vitro induction of maternal haploids, so-called gynogenesis, is another pathway to the production of haploid embryos exclusively from a female gametophyte.
- It can be achieved with the in vitro culture of various un-pollinated flower parts, such as ovules, placenta attached ovules, ovaries or whole flower buds.
- Although gynogenetic regenerants show higher genetic stability and a lower rate of albino plants compared to androgenetic ones, gynogenesis is used mainly in plants in which other induction techniques, such as androgenesis and the pollination methods above described, have failed.
- Gynogenic induction using un-pollinated flower parts has been successful in several species, such as onion, sugar beet, cucumber, squash, gerbera, sunflower, wheat, barley etc. (for a detailed list and protocols overview, see Bohanec, 2009 and Chen et al., 2011) but its application in breeding is mainly restricted to onion and sugar beet.
b) Induction of paternal haploids – Androgenesis:

Androgenesis – I have taken as separate lecture, so follow that.

Next slide I will continue considering that we have obtained haploids by androgenesis (Pollen and anther culture). So what next?
Diploidization of haploid plants:

- Haploids plants derived from either anther culture or pollen culture are sterile.
- These plants contain only one set of chromosomes.
- By doubling their chromosomes number, the plants can be made fertile and resultant plants will be homozygous diploid or isogenic diploid. These homozygous diploid plants show the normal meiotic separation.
- The fertile homozygous diploid plants are more important than the sterile haploid plants and can be used as pure line lines in breeding programme. Haploids plants can be diploidized by following methods.

i) Colchicine Treatment.
ii) Endomitosis.
iii) Fusion of Pollen Nuclei.
i) Colchicine Treatment:
Colchicine has been utilized widely as spindle inhibitor to induce chromosome duplication and to produce polyploid plants. The young plantlets while still enclosed within the anther are treated with 0.5% colchicine solution for 24-48 hrs. Treated plantlets are planted in the medium after thorough washing. In case of mature haploid plantlets, 4% colchicine-lanoline pastes may be applied to the axil of the leaves.

ii) Endomitosis:
Haploid cells are unstable in culture and have tendency to undergo Endomitosis, i.e. chromosome duplication without nuclear division. This property can be used for obtaining homozygous diploid plants. In this process, a small explant of stem from a haploid plant is cultured on auxin-cytokinin added medium where the segment forms the callus tissue. During callus growth, diploid homozygous cells are produced by endomitosis. Now large number of isogenic diploid plants can be obtained by organogenesis.

iii) Fusion of Pollen Nuclei:
Homozygous diploid callus or embryoids may form by the spontaneous fusion of two similar nuclei of the cultured pollen after first division. In Brassica, the frequency of spontaneous nuclear fusion in microspore is high in culture.
1. Releasing New Varieties through F1 Double - haploid System.
2. Selection of Mutants Resistance to Diseases.
3. Developing Asexual Lines of Tree Perennial Species.
5. Establishment of Haploids and Diploid Cell Lines of Pollen Plant.

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

Haploid breeding technique usually involve only one cycle of meiotic recombination. However, many agronomic traits are polygenically controlled. One cycle of recombination is usually insufficient for the improvement of such quantitative traits since linkage between Polygenes will not release all potential variations available in the cross. To overcome these disadvantages, the Chinese developed a method combining anther culture with sexual hybridization among different genotypes of anther derived plants. 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:
Mutants with resistance to disease is of prime 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. Another example of pollen-haploids in plant improvement is popular.
Importance and Implications of Anther and Pollen Culture

1. Haploids derived from anther and pollen culture are useful in cytogenetic studies.

2. Recessive phenotypic characters can be identified easily by comparing heterozygous diploid with haploid or homozygous diploid population.

3. Double haploid that are homozygous and fertile, are readily obtained, enabling the selection of desirable gene combination.

4. Culture of isolated pollen provides a novel experimental system for the study of factor controlling pollen embryogenesis of higher plants.

5. Study of meiotic behaviour of haploids valuable cubes to measure chromosome duplication within a species for understanding of phylogentic relationship between species. It also provides information for the interpretation of chromosome homology.

6. Genetic analysis could be performed on haploid population to establish inheritance patterns.

7. Use of haploids in production of monosomics, nullisomics and other aneuploids. This approach has been used in tobacco for the isolation of nullisomics, trisomics.
Next lecture: Production of Triploids