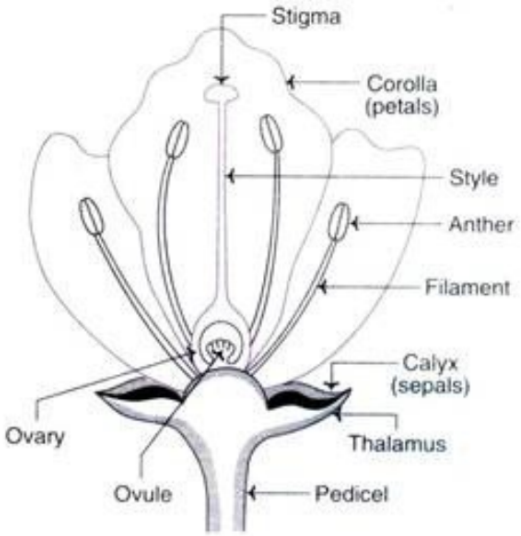
GYNOGENESIS

Haploid plants can be developed from ovary or ovule cultures. It is possible to trigger female gametophytes (megaspores) of angiosperms to develop into a sporophyte. The plants so produced are referred to as gynogenic haploids.

Gynogenic haploids were first developed by San Noem (1976) from the ovary cultures of Hordeum vulgare. This technique was later applied for raising haploid plants of rice, wheat, maize, sunflower, sugar beet and tobacco.

In vitro culture of un-pollinated ovaries (or ovules) is usually employed when the anther cultures give .unsatisfactory results for the production of haploid plants. The procedure for gynogenic haploid production is briefly described.

The flower buds are excised 24-48 hr. prior to anthesis from un-pollinated ovaries. After removal of calyx, corolla and stamens, the ovaries are subjected to surface sterilization. The ovary, with a cut end at the distal part of pedicel, is inserted in the solid culture medium.



Whenever a liquid medium is used, the ovaries are placed on a filter paper or allowed to float over the medium with pedicel inserted through filter paper. The commonly used media are MS, White's, N6 and Nitsch, supplemented growth factors. Production of gynogenic haploids is particularly useful in plants with male sterile genotype. For such plant species, this technique is superior to another culture technique.

Limitations of Gynogenesis:

In practice, production of haploid plants by ovary/ ovule cultures is not used as frequently as anther/ pollen cultures in crop improvement programmes.

The major limitations of gynogenesis are listed:

1. The dissection of unfertilized ovaries and ovules is rather difficult.
2. The presence of only one ovary per flower is another disadvantage. In contrast, there are a large number of microspores in one another.

However, the future of gynogenesis may be more promising with improved and refined methods.

Identification of Haploids:

Two approaches based on morphology and genetics are commonly used to detect or identify haploids.

Morphological Approach:

The vegetative and floral parts and the cell sizes of haploid plants are relatively reduced when compared to diploid plants. By this way haploids can be detected in a population of diploids. Morphological approach, however, is not as effective as genetic approach.

Genetic Approach:

Genetic markers are widely used for the specific identification of haploids. Several markers are in use.

i. 'a_1' marker for brown coloured aleurone.
ii. 'A' marker for purple colour.
iii. 'Lg' marker for ligule less character.

The above markers have been used for the development of haploids of maize. It may be noted that for the detection of androgenic haploids, the dominant gene marker should be present in the female plant.

Diploidizatioim of Haploid Plants (Production of Homozygous Plants):

Haploid plants are obtained either by androgenesis or gynogenesis. These plants may grow up to a flowering stage, but viable gametes cannot be formed due to lack of one set of homologous chromosomes. Consequently, there is no seed formation.

Haploids can be diploidized (by duplication of chromosomes) to produce homozygous plants. There are mainly two approaches for diploidization— colchicine treatment and endomitosis.

Colchicine Treatment:

Colchicine is very widely used for diploidization of homologous chromosomes. It acts as an inhibitor of spindle formation during mitosis and induces chromosome duplication. There are many ways of colchicine treatment to achieve diploidization for production of homozygous plants.

1. When the plants are mature, colchicine in the form of a paste is applied to the axils of leaves. Now, the main axis is decapitated. This stimulates the axillary buds to grow into diploid and fertile branches.
2. The young plantlets are directly treated with colchicine solution, washed thoroughly and replanted. This results in homozygous plants.
3. The axillary buds can be repeatedly treated with colchicine cotton wool for about 2-3 weeks.

Endomitosis:

Endomitosis is the phenomenon of doubling the number of chromosomes without division of the nucleus. The haploid cells, in general, are unstable in culture with a tendency to undergo endomitosis. This property of haploid cells is exploited for diploidization to produce homozygous plants.

The procedure involves growing a small segment of haploid plant stem in a suitable medium supplemented with growth regulators (auxin and cytokinin). This induces callus formation followed by differentiation. During the growth of callus, chromosomal doubling occurs by endomitosis. This results in the production of diploid homozygous cells and ultimately plants.

Applications of Haploid Plants

In vitro production of haploids is of great significance in plant breeding programmes. Some of them are listed below:

1. Development of homozygous lines:

It is now possible to develop homozygous lines within a span of few months or a year by employing anther/pollen culture. This is in contrast to the conventional plant breeding programme that might take several years (6-10 yrs). In this way, production of haploids is highly useful for research related to plant genetics and breeding.

2. Generation of exclusive male plants:

By the process of androgenesis to produce haploids, followed by chromosome doubling, it is possible to develop exclusive male plants. The male plants are particularly useful when their productivity and applications are much more than female plants.

3. Induction of mutations:

In general, majority of induced mutations are recessive and therefore are not expressed in diploid cells (due to the presence of dominant allele). Haploids provide a convenient system for the induction of mutations and selection of mutants with desired traits. In fact, the haploid cells can be cultured and handled in a fashion similar to microorganisms.

Mutants from several plant species that are resistant to antibiotics, toxins, herbicides etc. have been developed. When the haploid cells of tobacco plant (Nicotiana tabacum) were exposed to methionine sulfoximine (a mutagen), mutants which showed lower level of infection to Pseudomonas tabaci were produced.

4. Production of disease resistance plants:

Disease resistance genes can be introduced while producing haploids. The so developed haploids are screened for the desired resistance, and then diploidized.

5. Production of insect resistance plants:

Some varieties of rice resistant to insects have been developed e.g. Hwacheongbyeo resistant to brown plant hopper. Other varieties of rice that are resistant to pests have also been produced.

6. Production of salt tolerance plants:

The plant species with salt tolerance are needed for their cultivation in some areas. Anther cultures have resulted in some varieties of rice and wheat with good salt tolerance e.g. wheat Hua Bain 124-4.

7. Cytogenetic research:

Haploids are useful in several areas of cytogenetic research. These include

i. Production of aneuploids
ii. Determination of the nature of ploidy
iii. Determination of basic chromosome number
iv. Evaluation of origin of chromosomes.

8. Induction of genetic variability:

Besides the development of haploid mutants, it is also possible to produce plants with various ploidy levels through androgenesis.

9. Doubled haploids in genome mapping:

Genome mapping, a recent development in molecular biology, can be more conveniently achieved by using doubled haploid plant species.

10. Evolutionary studies:

A comparison of di-haploids (doubled haploids) with diploid wild plant species will be useful to trace the evolutionary origin of various plants. The close evolutionary relationship between tomato and potato has been evaluated by this approach.

Embryo Culture: Types, Nutritional Requirements and Applications

Embryo Culture is of two types. They are: (1) Mature Embryo Culture and (2) Embryo Rescue.

Embryo culture deals with the sterile isolation and in vitro growth of a mature or an immature embryo with an ultimate objective of obtaining a viable plant. Conventionally, the term embryo culture refers to the sexually produced zygotic embryo culture. There are two types of embryo culture — mature embryo culture and immature embryo culture (embryo rescue).

Types of Embryo Culture:

1. Mature Embryo Culture:

Mature embryos are isolated from ripe seeds and cultured in vitro. Mature embryo cultures are carried out in the following conditions:

i. When the embryos remain dormant for long periods.

ii. Low survival of embryos in vivo.

iii. To avoid inhibition in the seed for germination.

iv. For converting sterile seeds to viable seedlings.

Seed dormancy in plant species is a common occurrence. This may be due to chemical inhibitors or mechanical resistance exerted by the structures covering the embryo. Seed dormancy can be successfully bypassed by culturing the embryos in vitro.

Embryo culture is relatively easy as they can be grown on a simple inorganic medium supplemented with energy source (usually sucrose). This is possible since the mature embryos excised from the developing seeds are autotrophic in nature.

2. Embryo Rescue:

Embryo rescue involves the culture of immature embryos to rescue them from unripe or hybrid seeds which fail to germinate. This approach is very useful to avoid embryo abortion and produce a viable plant. Wild hybridization involving crossing of two different species of plants from the same genus or different genera often results in failure. This is mainly because the normal development of zygote and seed is hindered due to genetic barriers.

Consequently, hybrid endosperm fails to develop leading the abortion of hybrid embryo. The endosperm may also produce toxins that ultimately kill the embryo. In the normal circumstances, endosperm first develops and supports embryo development nutritionally. Thus, majority of

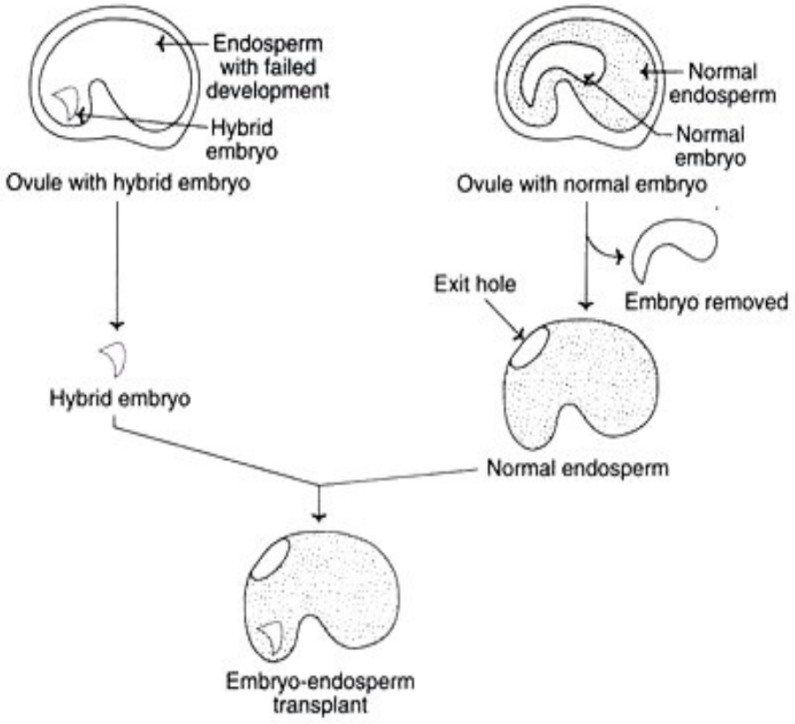
embryo abortions are due to failure in endosperm development. Embryo abortion can be avoided by isolating and culturing the hybrid embryos prior to abortion. The most important application of embryo rescue is the production of interspecific and inter-generic hybrids from wild plant species.

Culture Technique for Embryo Rescue:

The isolation of immature embryos often poses some difficulty. The aseptically isolated embryos can be grown in a suitable medium under optimal conditions. In general, a complex nutrient medium is required for culture methods involving embryo rescue. For adequate nutritional support of immature embryos, embryo-endosperm transplant is used.

Embryo-endosperm transplant:

The endosperm transplant technique used for culturing immature embryos is given in the following figures and briefly described below.



The hybrid embryo from the ovule in which endosperm development has failed is taken out by excision. Another normally developed ovule with endosperm enclosing an embryo is chosen. This ovule is dissected and the normal embryo is pressed out. This leaves a normal endosperm with an exit hole.

Now, the hybrid embryo can be inserted into the normal endosperm through exit hole. This results in embryo-endosperm transplant which can be cultured in a suitable medium. By using embryo-endosperm transplant, many interspecific and inter-generic plants have been raised e.g., hybrid plants of legumes.

Nutritional Requirements of Embryo Cultures:

There are two phases in the embryo development and the nutritional requirement varies accordingly:

1. Heterotrophic phase:

This is an early phase and the embryo is mostly dependent on the endosperm and maternal tissues for nutrient supply.

2. Autotrophic phase:

This phase is characterized by the metabolic capability of the embryo to synthesize substances required for its growth which slowly makes it independent. The critical stage is the intervening phase between the heterotrophic and autotrophic phases. The nutrient supply is highly variable at this phase which mostly depends on the plant species.

In general, the composition of the medium for culturing immature embryos is more complex than that required by mature embryos which can grow on a simple inorganic medium. Further, the transfer of embryos from one medium to another is frequently needed in order to achieve full development of embryos.

Composition of the Medium:

Some salient features of medium and culture conditions are listed below:

i. Inorganic constituents of MS, B5 or White's media are adequate.

ii. Sucrose is most commonly used energy source.

iii. Ammonium nitrate is the preferred source of nitrogen.

iv. Casein hydrolysate, rich in various amino acids is frequently used.

v. Certain natural plant extracts with embryo factor promote embryo cultures e.g. liquid endosperm of coconut milk. The embryo factor is believed to supply certain amino acids, sugars, growth regulators etc.

vi. In general, growth regulators are not required, as they induce callus formation.

vii. Embryos grow well in the pH range of 5-7.5.

viii. An incubation temperature of 24-26°C is ideal.

ix. Better growth of embryos is observed in darkness which are then transferred to light for germination.

During the culture conditions, the embryos are grown into plantlets, and then transferred to sterile soil for full-pledged growth to maturity.

Applications of Embryo Culture:

1. Prevention of Embryo Abortion:

Incompatibility barriers in interspecific and inter-generic hybridization programmes leading to embryo abortion can be successfully overcome by embryo rescue. In fact, many distant hybrids have been obtained through embryo rescue techniques. A selected list of distant plant species crossed and the resistance traits developed by employing embryo rescue technique is given in Table below.

Distant plant species crossed	*Resistance trait(s)*
Oryza sativa × *O. minuta*	Bacterial blight and blast
Solanum tuberosum × *S. etuberosum*	Potato leaf roll virus
Solanum melanogena × *S. khasianum*	Brinjal shoot and fruit borer
Brassica napus × *B. oleracea*	Cabbage aphid
Lycopersicon esculentum × *L. peruvianum*	Virus, fungi and nematodes
Hordeum sativum × *H. vulgare*	Powdery mildew and spot blotch
Triticum aestivum × *Thinopyrum scirpeum*	Salt tolerance

2. Overcoming Seed Dormancy:

Seed dormancy is caused by several factors—endogenous inhibitors, embryo immaturity, specific light and temperature requirements, dry storage requirements etc. Further, in some plants the natural period of seed dormancy itself is too long. Embryo culture is successfully applied to overcome seed dormancy, and to produce viable seedlings in these plant species.

3. Shortening of Breeding Cycle:

Some of the plants in their natural state have long breeding cycles. This is mostly due to seed dormancy attributed to seed coat and/or endosperm. The embryos can be excised and cultured in vitro to develop into plants within a short period. For instance, Hollies, a Christmas decoration plant can be grown in 2-3 weeks time through embryo cultures in contrast to 3 years period required through seed germination.

4. Production of Haploids:

Embryo culture has been successfully used for the production of haploid (or monoploid) plants e.g. barley.

5. Overcoming Seed Sterility:

Certain plant species produce sterile seeds that do not germinate e.g. early ripening varieties of cherry, apricot, and plum. Seed sterility is mostly associated with incomplete embryo development which leads to the death of the germinating embryo. Using embryo cultures, it is possible to raise seedlings from sterile seeds of early ripening fruits e.g. apricot, plum.

6. Clonal Propagation:

Embryos are ideally suited for in vitro clonal propagation. This is due to the fact that embryos are juvenile in nature with high regenerative potential. Further, it is possible to induce organogenesis and somatic embryogenesis from embryonic tissues.

In vitro pollination and fertilization:

Pollination and fertilization under in vitro condition offer the opportunity for producing hybrid embryo that not possible by conventional method. Under controlled condition aseptic transfer of pollen grain on stigma to produce hybrid embryos among plants that can’t cross by conventional method of plant breeding is called as in vitro pollination and fertilization.

In nature intergenic and intraspecific hybridization occurs less frequently, this is due to hindering factor . Factor:- Barrier to growth of pollen tube on the stigma or style Solution:- In such cases

the style or part of it can be excised and pollen grain either placed on the cut surface of the ovary. This technique is called as intraovarian pollination. Ex. *Papaver somnifera*, *Argemone mexicana*, etc. To overcome the barriers to pollen tube growth is direct pollination of culture ovules.

Different techniques:

1. Stigmatic pollination - application of pollen to the stigma in vitro condition .
2. Ovarian pollination - application of pollen to exercised ovary in vitro condition .
3. Placental pollination - application of pollen to ovules attached to the placenta in vitro condition.
4. Ovular pollination - application of pollen to excised ovules in vitro condition.

Material required:

The ovaries which are large and which contain many ovules . Other essential materials is pollen which should be viable and able to germinate . 1% calcium chloride solution that favour the growth of pollen tube.

Disinfection of material :

Flower bud are emasculated before anthesis and bagged in order to prevent the undesirable pollination the whole pistil or ovaries alone are sterilized by a quick rinse in 70% alcohol, surface sterilized with suitable agents. To collect the pollen under aseptic condition anther removed from bud or open flower. then kept in sterile Petri dish contain presterilised filter paper until their dehiscence.The dehiscence pollen is asceptically deposited on the cultred ovules, placenta or stigma depend on nature of experiment.

Culture of ovules and ovary:

Ovules-The growth of pollen tubes attached to bare ovules is often inhibited by the presence of water on the surface of ovules this water is removed by filter paper and later dried ovules covered by pollen grain ,raising seed by ovules which contain globular embryo after in vitro pollination ovules contains a single celled zygote which requires more complex growth condition . For developing subsequently embryonic stages ovules which have been self pollinated are kept on placenta until seed form. Ex.*Nicotina tabacum* , *Alliums cepa*,etc.. Ovary-this technique developed by Nitsch in 1951 who successfully reared ovaries excised from pollinated flower *in vitro* to develop into mature fruits. Ex. *Linaria macroccana*, *Tropaeohim majus* ,etc.. Culture of ovules and ovary

Purpose:

Intergenic hybridization . Intraspecific hybridization . Interspecific hybridization. Intrafamilar crossing.

Reference

Development of Haploid Production in Fruit Crops by Chandan kumar Rout, Bhagyalaxmi Pahi and Anubhav Biswa

Plant Cell and Tissue Culture - A Tool in Biotechnology: Basics and Application (Principles and Practice) by Karl-Hermann Neumann, Ashwani Kumar, Jafargholi Imani

Plant Tissue Culture, Third Edition: Techniques and Experiments 3rd Edition by Roberta H. Smith