Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

DISCIPLINE: «Modern Problems of Plant Genetics»

Lecture 3

In vitro culture and plant breeding. Pollen and Microspore culture in Plant Improvement

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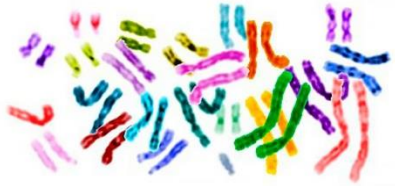
***Purpose of the lesson*:**

Acquaintance with application of *In vitro* culture technique in plant breeding. Pollen and Microspore culture in Plant Improvement.

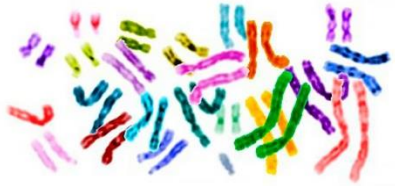
Plan of the lesson:

1. **In vitro culture technique in plant breeding.**
2. **Pollen and Microspore culture in Plant Improvement.**



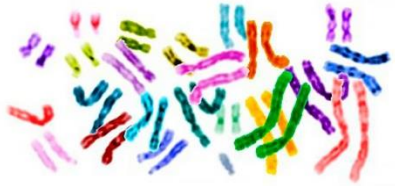
- **Applications of In Vitro Tissue Culture Technologies i Breeding and Genetic Improvement of Plants**

- **Food security is one of the most significant challenges facing the future of our planet. By 2050, the world will need nearly double the amount of food produced today to feed an expected nine billion people. To achieve this goal effectively, food production must be significantly increased sustainably on existing arable land while addressing the challenges posed by climate change.**



- **Applications of In Vitro Tissue Culture Technologies i Breeding and Genetic Improvement of Plants**

- **Crop breeding programs and improved management regimes have led to steady increases in crop yields over the past five decades. However, the rate of yield improvement has plateaued [1]. The relatively small annual incremental gains in yield (wheat yields increasing at 0.9% per year, non-compounding rates; at these rates, global production increases by ~38%) are not sufficient to meet projected demands by 2050. Therefore, producing better, higher-yielding crops is the ideal approach to be explored immediately [2].**



- **Applications of In Vitro Tissue Culture Technologies i Breeding and Genetic Improvement of Plants**

- **The genetic improvement of wheat has traditionally been achieved through sexual hybridization between related species, resulting in numerous cultivars with high yields and superior agronomic performance. Conventional plant breeding, sometimes combined with classical cytogenetic techniques, continues to be the primary method of cereal crop improvement [3]. Given the worldwide predominance of cereal grains in the human diet, cereal crops quickly emerged as prime targets for improvement by methods of haploid biotechnology.**

Production of haploid plants

- **What are haploid plants?**
- **What is Dihaploid?**
- **What is haploid and diploid plants?**
- **What is Bulbosum technique?**

- ***What is a haploid plant?***

- **Haploid plants originate from gametes (or gamete-like cells) that do not go through fertilization, but can still generate a viable individual.**

- **Therefore, haploids contain only the chromosome set found after meiosis in male (sperm cells) or female (egg cells) gametes.**

- **This chromosome set ‘n’ corresponds to only half of the chromosome set found in the fertilization product (zygote) and other somatic cells.**

- **Depending on whether the single set of chromosomes comes from the maternal or paternal side, the plant is referred to as maternal haploid and paternal haploid, respectively.**

- **What is a doubled haploid (DH) plant?**

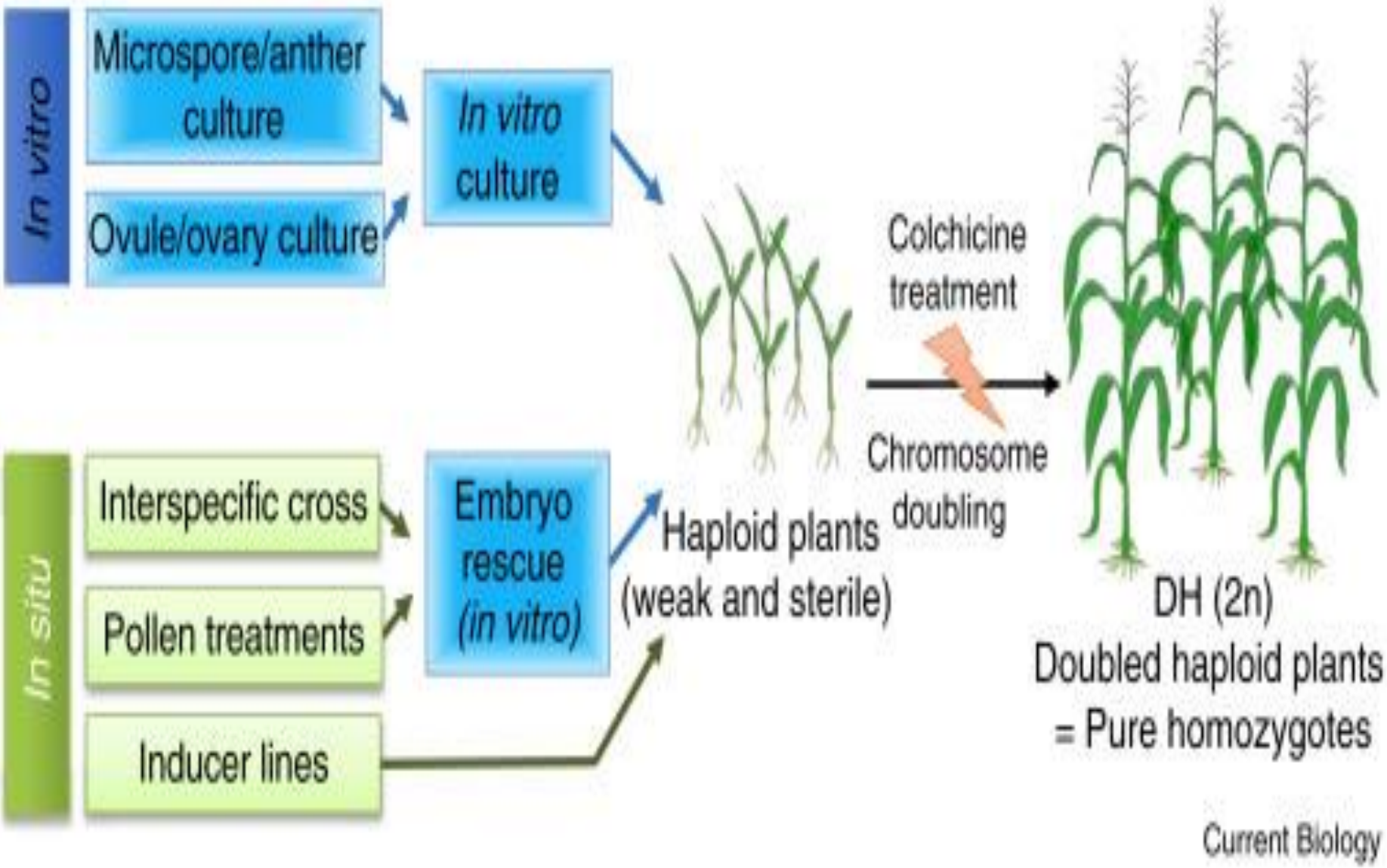
- **In a DH plant, the chromosome set of a haploid plant has been doubled spontaneously or artificially.**

- **Chromosome doubling is necessary since haploid plants are generally frail, have reduced organ size and are not fertile.**

- **The most commonly used chemical agent to render haploid plantlets diploid is colchicine, which blocks cell division without blocking chromosome duplication.**

- **This treatment acts like a ‘copy–paste’ of the haploid genome into a diploid genome.**
- **Consequently, in DH plants all loci are homozygous.**

- **Chromosome doubling creates ‘pure’ homozygotes or fully inbred lines (Figure 1).**



https://www.cell.com/current-biology/comments/S0960-9822(17)30959-4

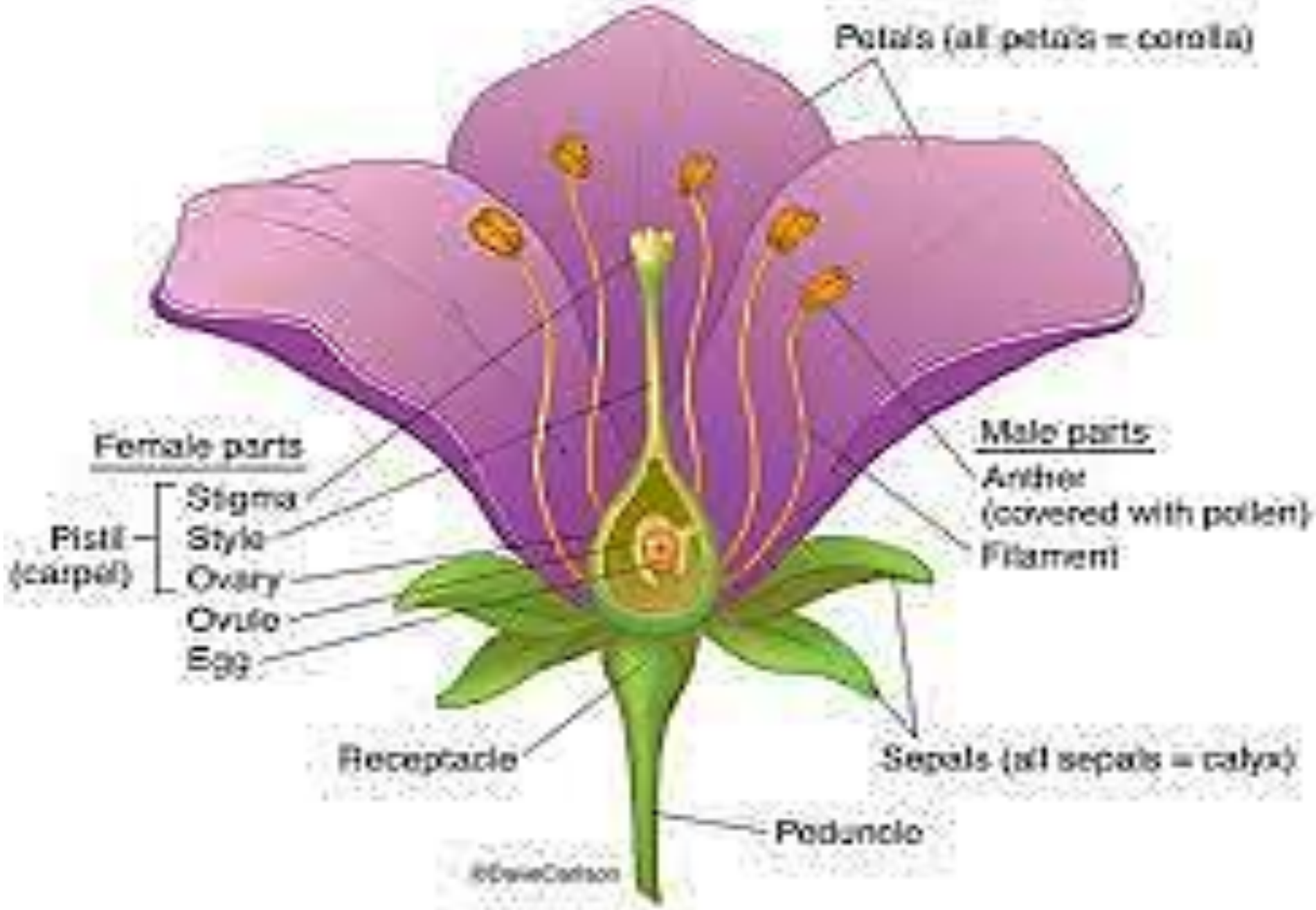
- **A doubled haploid (DH) is a genotype formed when haploid cells undergo chromosome doubling. Artificial production of doubled haploids is important in plant breeding.**

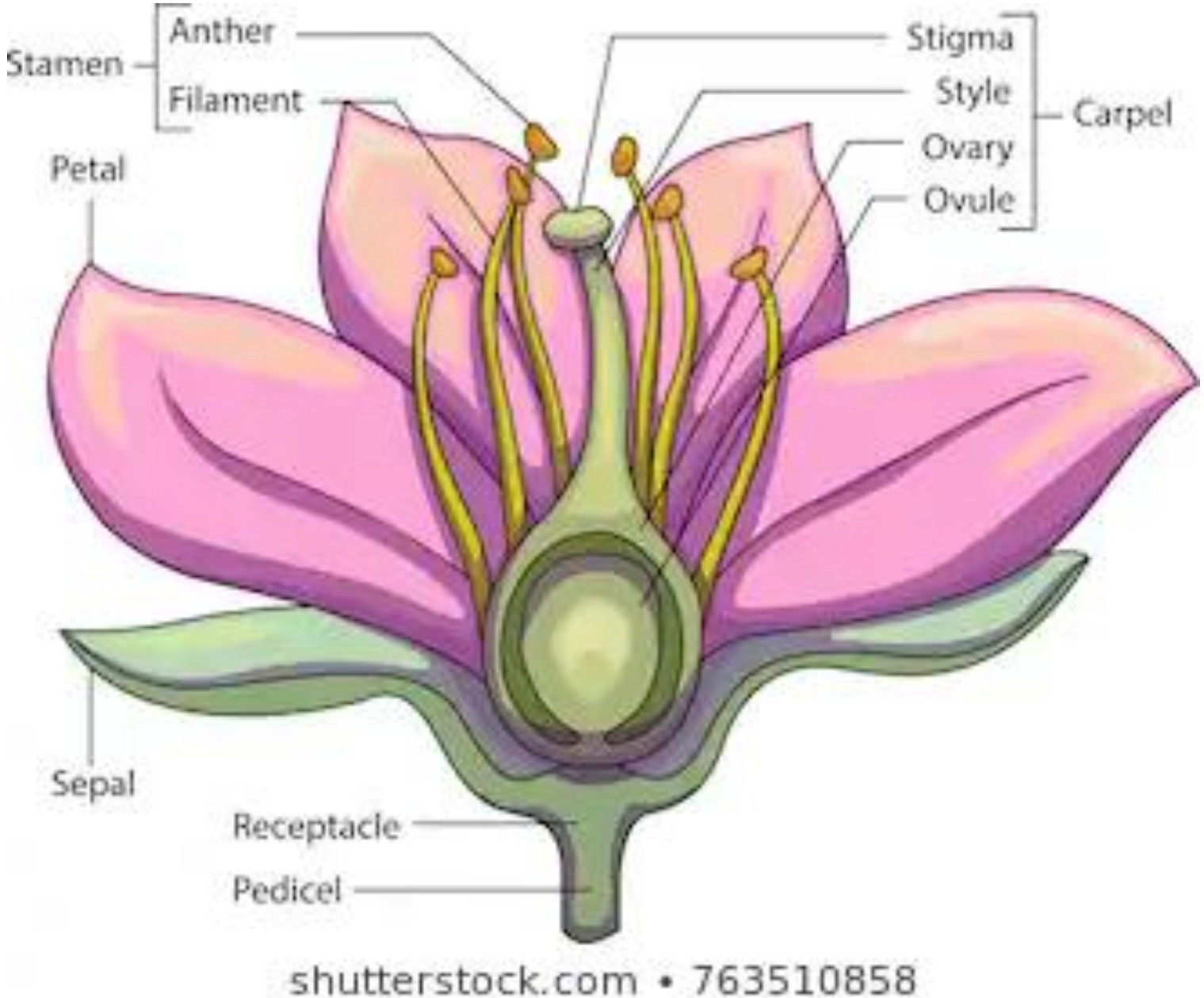
- **Haploid cells are produced from pollen or egg cells or from other cells of the gametophyte, then by induced or spontaneous chromosome doubling, a doubled haploid cell is produced, which can be grown into a doubled haploid plant.**

- **If the original plant was diploid, the haploid cells are monoploid, and the term doubled monoploid may be used for the doubled haploids.**

- **Haploid organisms derived from tetraploids or hexaploids are sometimes called dihaploids (and the doubled dihaploids are, respectively, tetraploid or hexaploid).**

- **Doubled haploids can be produced *in vivo* or *in vitro*.**
- **Haploid embryos are produced *in viv*o by parthenogenesis, pseudogamy, or chromosome elimination after wide crossing.**
- **The *in vitro* methods include gynogenesis (ovary and flower culture) and androgenesis (anther and microspore culture).**
- **Androgenesis is the preferred method.**


Petals (all petals = corolla)
Female parts
Pistil (carpel)
Stigma
Style
Ovary
Ovule
Egg
Male parts
Anther (covered with pollen)
Filament
Receptacle
Sepals (all sepals = calyx)
Peduncle


Stamen
Anther
Filament
Petal
Stigma
Style
Ovary
Ovule
Carpel
Sepal
Receptacle
Pedicel
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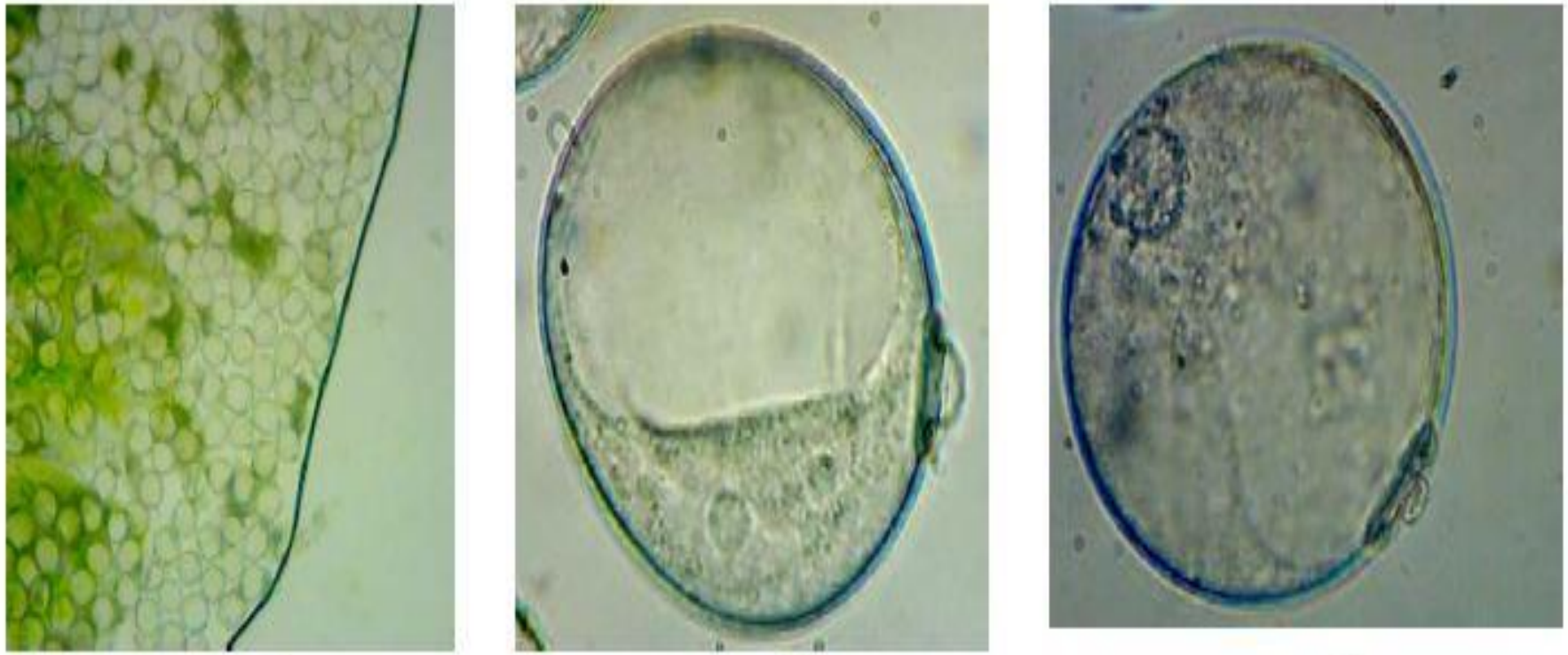
Collecting and preparing of ears

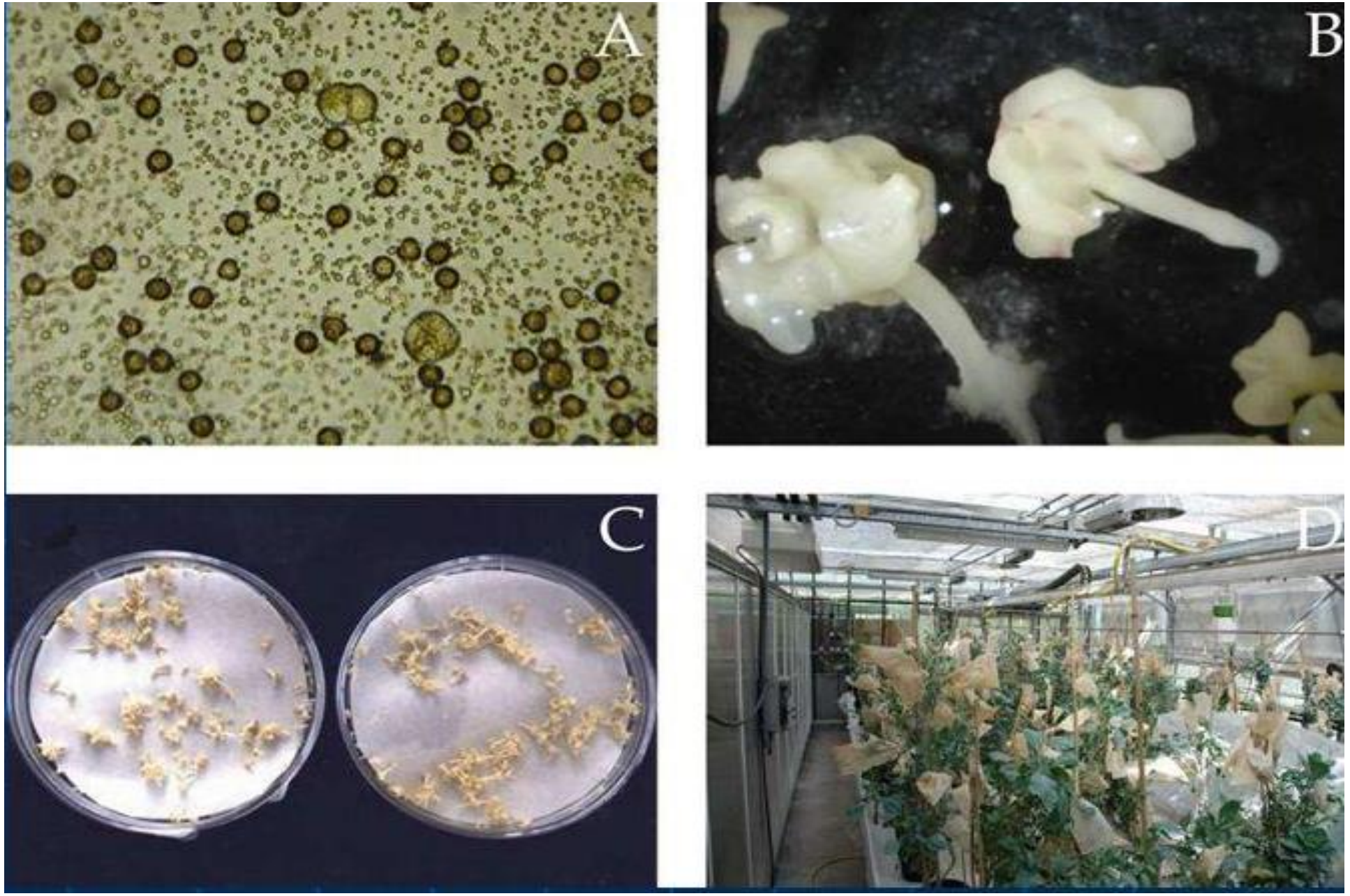


а б в

а – пыльник с микроспорами; б – средняя и в - поздняя одноядерная стадия;
Рисунок 1 - Стадии развития микроспор озимой пшеницы. Микроскоп Meiji Techno серии MT4000, увеличение на x100 и x1000

Microspore culture

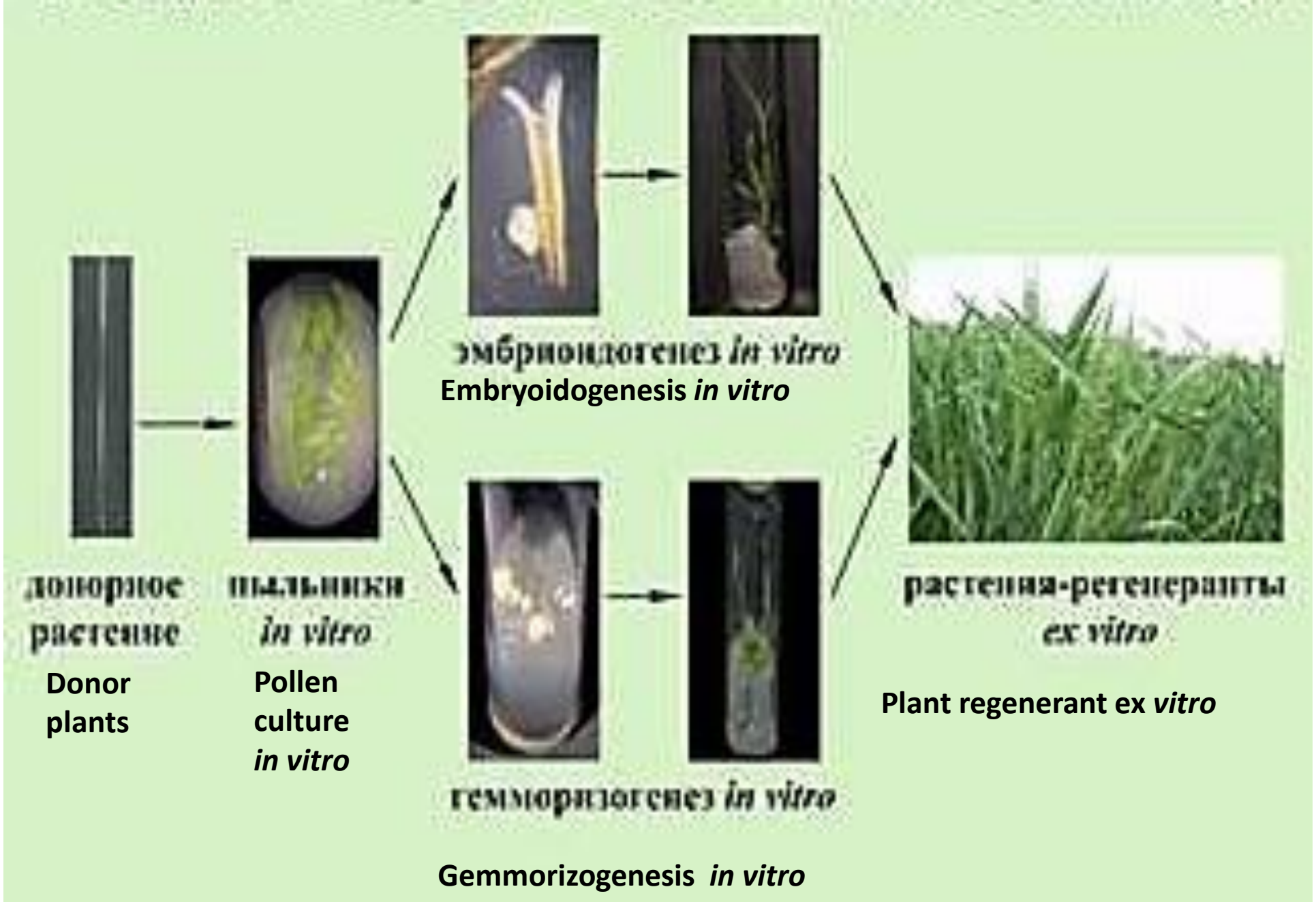
Pollen culture



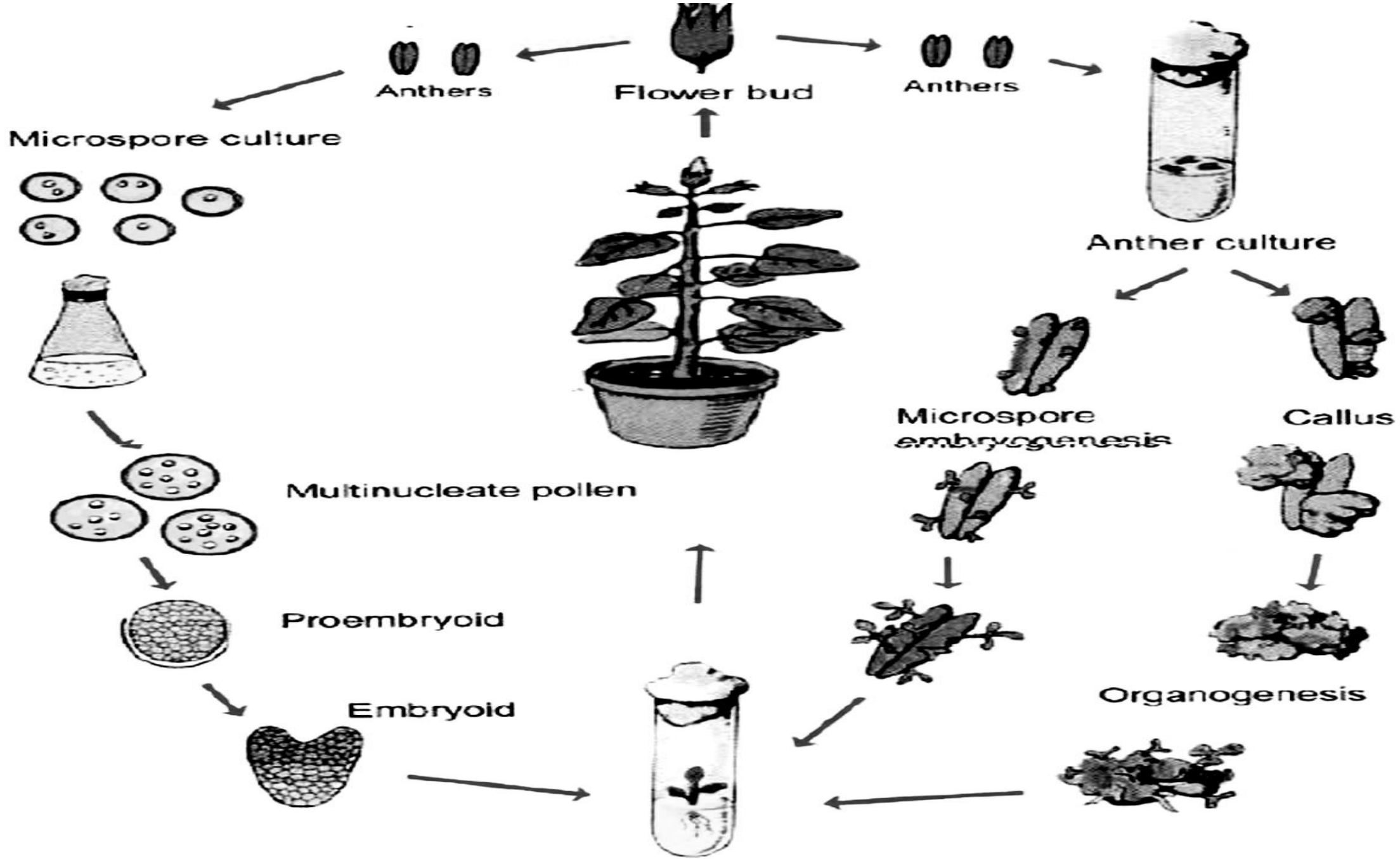
Microspore culture

Pollen culture

Formation of callus tissues on pollen



In vitro pollen culture of wheat


Anthers
Flower bud
Anthers
Microspore culture
Anther culture
Multinucleate pollen
Microspore embryogenesis
Callus
Proembryoid
Embryoid
Organogenesis

- **What is Bulbosum technique?**

- **Another method of producing the haploids is wide crossing. In barley, haploids can be produced by wide crossing with the related species *Hordeum bulbosum*; fertilization is affected, but during the early stages of seed development the H. bulbosum chromosomes are eliminated leaving a haploid embryo.**

- **Why is doubled haploid technology impactful for agriculture?**

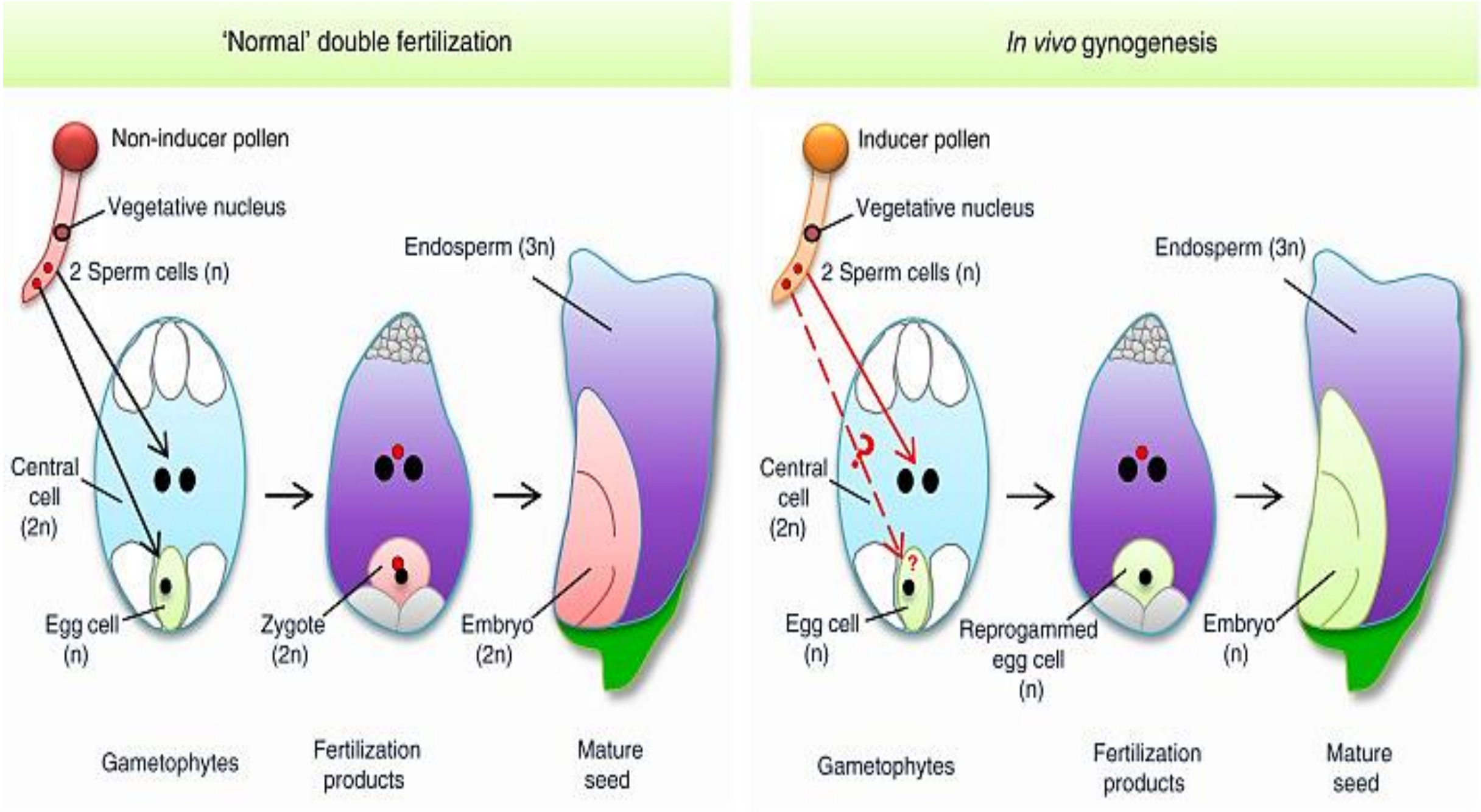
- **Doubled haploid technology comprises both the production of haploid plants and the chromosome doubling process (see Figure 1).**

- **It has become an important tool in plant breeding, since it shortens the time needed to create pure homozygous lines, which can either be released directly to farmers as cultivars or used as genitors (inbred lines) for the production of hybrid seeds.**

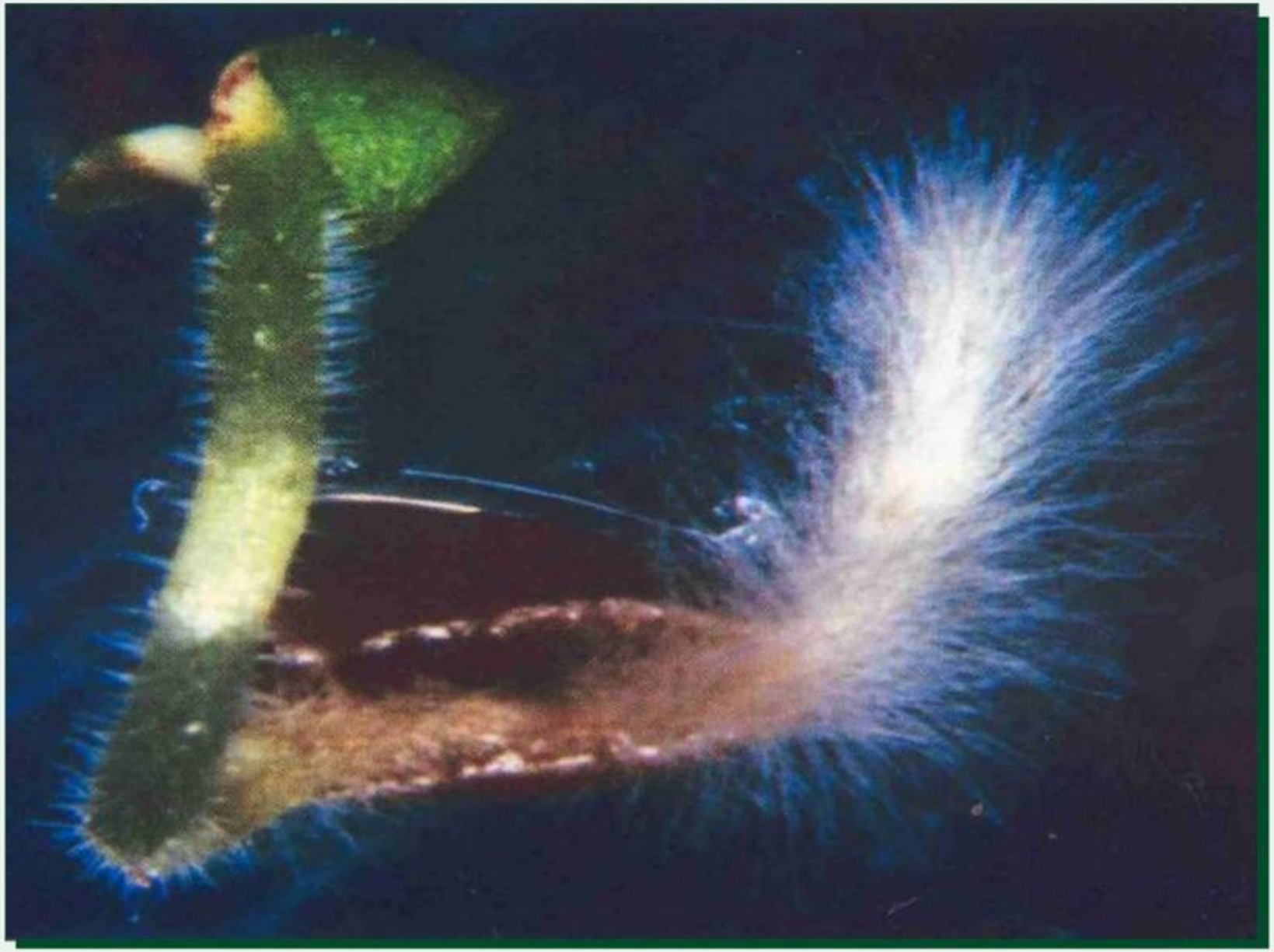
- **Why is doubled haploid technology impactful for agriculture?**

- **The primary advantage of DH plants is to possess a phenotypic stability due to the fact that all alleles are in a homozygous state.**

- **In short, DH technology increases the efficiency of plant breeding.**


'Normal' double fertilization
Non-inducer pollen
Vegetative nucleus
2 Sperm cells (n)
Endosperm (3n)
Central cell (2n)
Egg cell (n)
Zygote (2n)
Embryo (2n)
Gametophytes
Fertilization products
Mature seed
In vivo gynogenesis
Inducer pollen
Vegetative nucleus
2 Sperm cells (n)
Endosperm (3n)
Central cell (2n)
Egg cell (n)
Reprogammed egg cell (n)
Embryo (n)
Gametophytes
Fertilization products
Mature seed
Current Biology

Haploid plant ***(N. tabacum)***



- **Control questions:**

- **In vitro culture technique in plant breeding.**
- **Pollen and Microspore culture in Plant Improvement.**


GOOD LUCK!