

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.



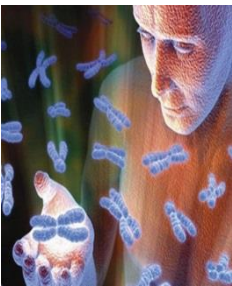
Main questions:

1. Applications of In Vitro Tissue Culture Technologies in Breeding and Genetic Improvement of Plants.
2. Techniques and advantages of microspore culture for crop improvement.
3. Pollen and Microspore culture in Plant Improvement.
4. What is a haploid plant?
5. What is a doubled haploid (DH) plant?





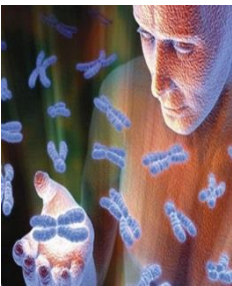
- **Applications of In Vitro Tissue Culture Technologies in Breeding and Genetic Improvement of Plants**
- **Food security is one of the most significant challenges facing the future of our planet. By 2050, the world will need nearly double the amount of food produced today to feed an expected nine billion 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 in Breeding and Genetic Improvement of Plants**



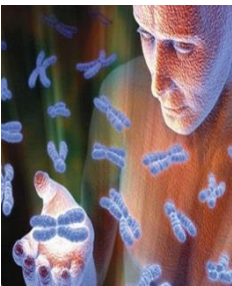
- **Crop breeding programs and improved management regimes have led to steady increases in crop yields over the past five decades. However, the rate of yield improvement has plateaued [1]. The relatively small annual incremental gains in yield (wheat yields increasing at 0.9% per year, non-compounding 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 in 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.**





- **Techniques and advantages of microspore culture for improvement.**
- Haploids/DHs are generated through parthenogenesis (Bohanec 2009), wide hybridization or chromosome elimination (Dunwell 2010; Jauhar 2003) and androgenesis or gynogenesis (Asif 2013; Dong et al. 2016).
- Of these methods, microspore culture or androgenesis is one of the most advanced and efficient tools for generating haploid and DH plants that have attracted global researcher's attention (Hale et al. 2022). Unlike anther culture, where entire anthers are cultured, microspore culture involves isolating immature microspores from the anther and culturing them under controlled in vitro conditions. In fact, the isolation of microspores provides several important benefits.



Pollen and Microspore culture in Plant Improvement



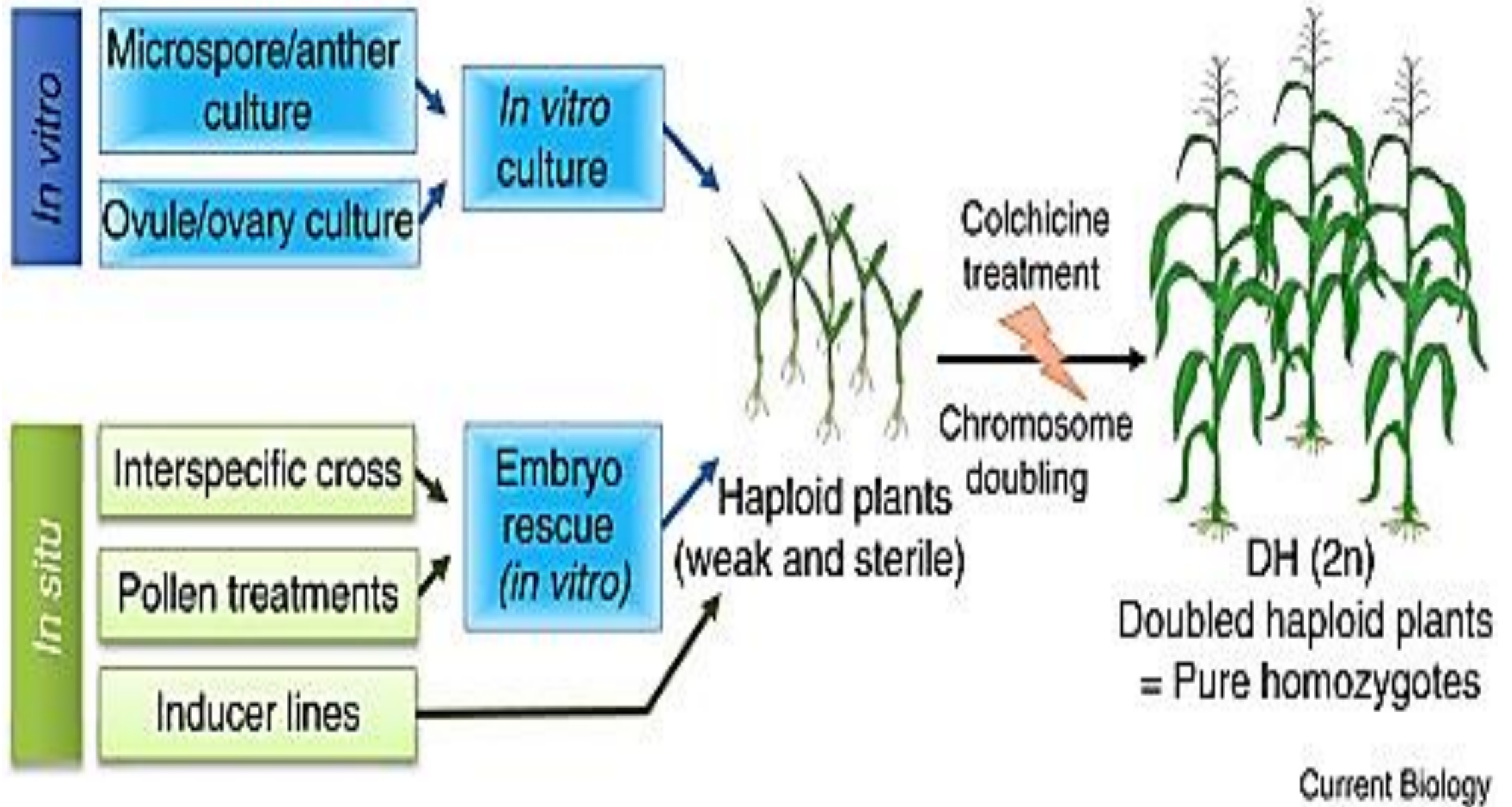
- **What are haploid plants?**
- **What is Dihaploid?**
- **What is haploid and diploid plants?**
- **What is Bulbosum technique?**

- ***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.**

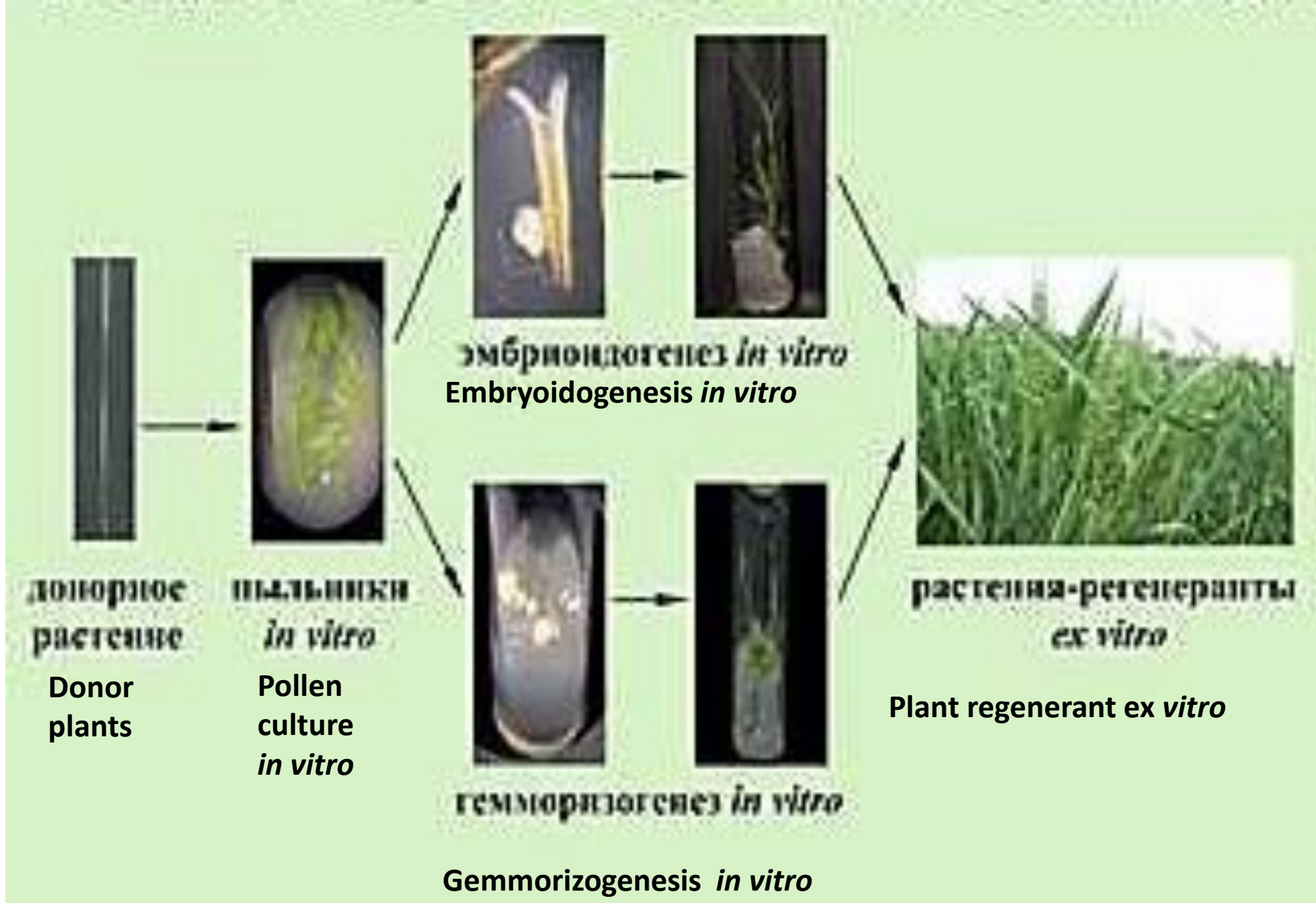
- **The most commonly used chemical agent to render haploid plantlets diploid is colchicine, which blocks cell division without blocking chromosome duplication.**
- **This treatment acts like a ‘copy–paste’ of the haploid genome into a diploid genome.**
- **Consequently, in DH plants all loci are homozygous.**
- **Chromosome doubling creates ‘pure’ homozygotes or fully inbred lines (Figure 1).**



- **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 vivo* 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.



In vitro pollen culture of wheat

- **What is Bulbosum technique?**

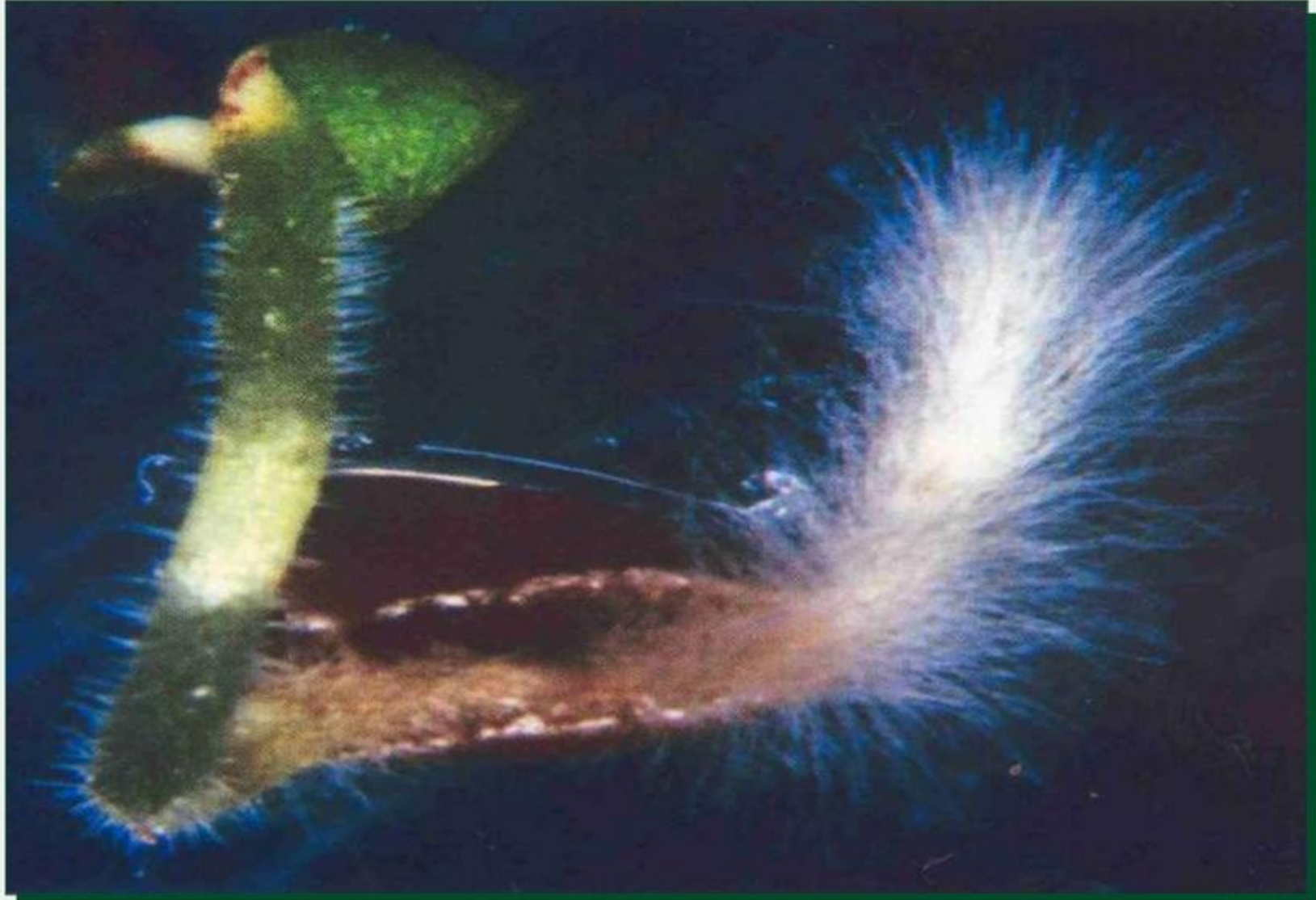
- **Another method of producing the haploids is wide crossing. In barley, haploids can be produced by wide crossing with the related species *Hordeum bulbosum*; fertilization is affected, but during the early stages of seed development the H. bulbosum chromosomes are eliminated leaving a haploid embryo.**

- **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.**

Haploid plant

(*N. tabacum*)



- **Questions for monitoring the material being studied:**
 - **1. Applications of In Vitro Tissue Culture Technologies in Breeding and Genetic Improvement of Plants.**
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 - **4. What is a haploid plant?**
 - **5. What is a doubled haploid (DH) plant?**

• **Recommended list of references:**

- 1. Шулембаева К.К., Токубаева А.А. Реконструкция генома мягкой пшеницы на основе хромосомной инженерии и отделенной гибридизации: монография. КазНУ им. аль-Фараби. - Алматы : Қазақ ун-ті, 2019. - 240 с.
- 2. Огурцов А.Н., Близнюк О.Н., Масалитина Н.Ю. Основы генной инженерии и биоинженерии. Учебное пособие. Часть 1.: Молекулярные основы генных технологий. Харьков: НТУ "ХПИ", 2018. - 288 с.
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- 4. Муминов Т.А., Куандыков Е.У. Основы молекулярной биологии : курс лекций. - Алматы : ССК, 2017. – 222 с.
- 5. Varshney Rajeev K. Plant Genetics and Molecular Biology. - London: Springer, 2018. - 298 p.
- 6. Halford Nigel G. Crop Biotechnology: Genetic Modification And Genome Editing. - London: World Scientific, 2018. - 218 p.
- 7. Glick Bernard R. Molecular biotechnology: principles and applications of recombinant DNA. - 4th ed. - Washington, 2010. - 1200 p.



GOOD LUCK!