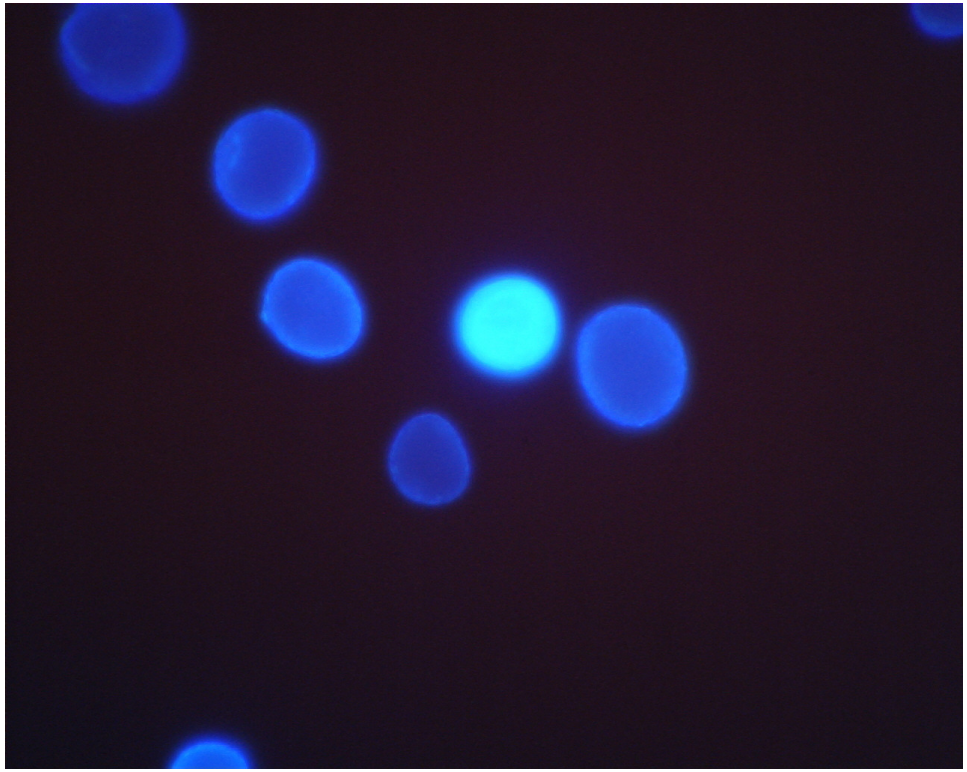
Generation of doubled haploid plants through microspore regeneration



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Why Fytagoras?

- Over 15 years experience in DH technology
  - Protocol development, Protocol implementation, Consultancy
  - Basic scientific research and scientific publications
- Worked for over 8 years on exclusive basis on DH of vegetable crops for one of Holland's leading breeding companies.
- Development of numerous successful DH protocols and commercial implementation
- Ongoing development of DH protocols for different crops (also ornamentals), for several Dutch and foreign seeds companies
- Great track record in (confidential) contract research and in-company instructions

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Why Fytagoras?

- Experience on a broad range of crops, amongst them some Solanaceae.
- We worked on more than 7 ornamental crops, and more than 5 vegetable crops (such as pepper, and egg plant)
- Barley, rice, and tobacco as model crops
- We developed and implemented an efficient protocol for pepper, where many others failed
- Establishment of different basic approaches in the development of DH plants, which increases the success rate considerably

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Why successful ?

- **On-going research program** (in cooperation with Leiden University) on the fundamentals of microspore regeneration: evolutionary aspects, metabolism aspects, genes and transcriptions factor, cell signaling aspects, which benefits **protocol development**
- Less trial and error, but instead focus on crucial physiological and cellular processes which are related to the division of microspores, and so the production of a DH plant
- Chemical compounds/hormones only, when we assume that they are relevant for the development of microspores

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Why successful ?

- **Systematic approach:**

  - growth conditions

  - single flower treatment

  - energy status of cells

  - stress

  - dedicated treatments

- **supporting techniques** on cells (upgrading, imaging, cell sorting)

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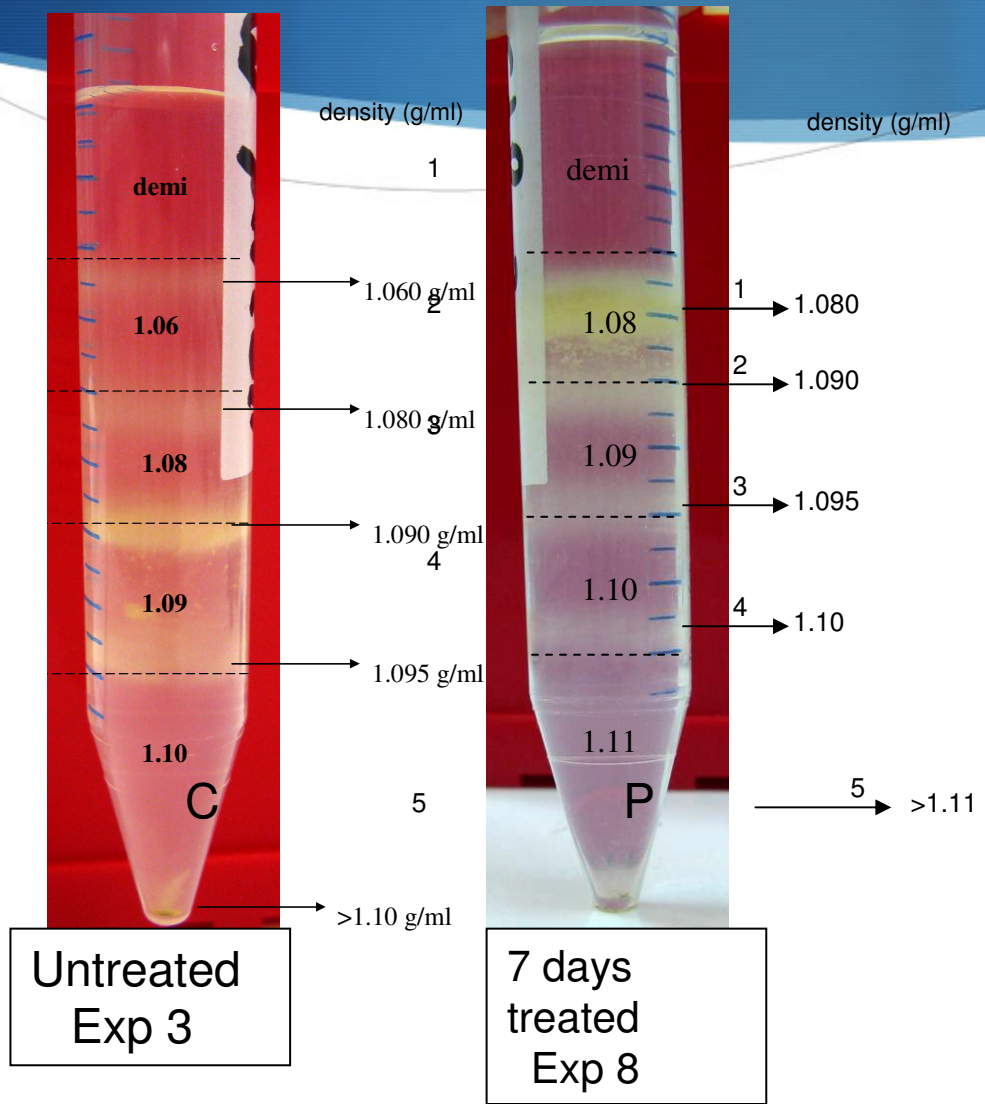
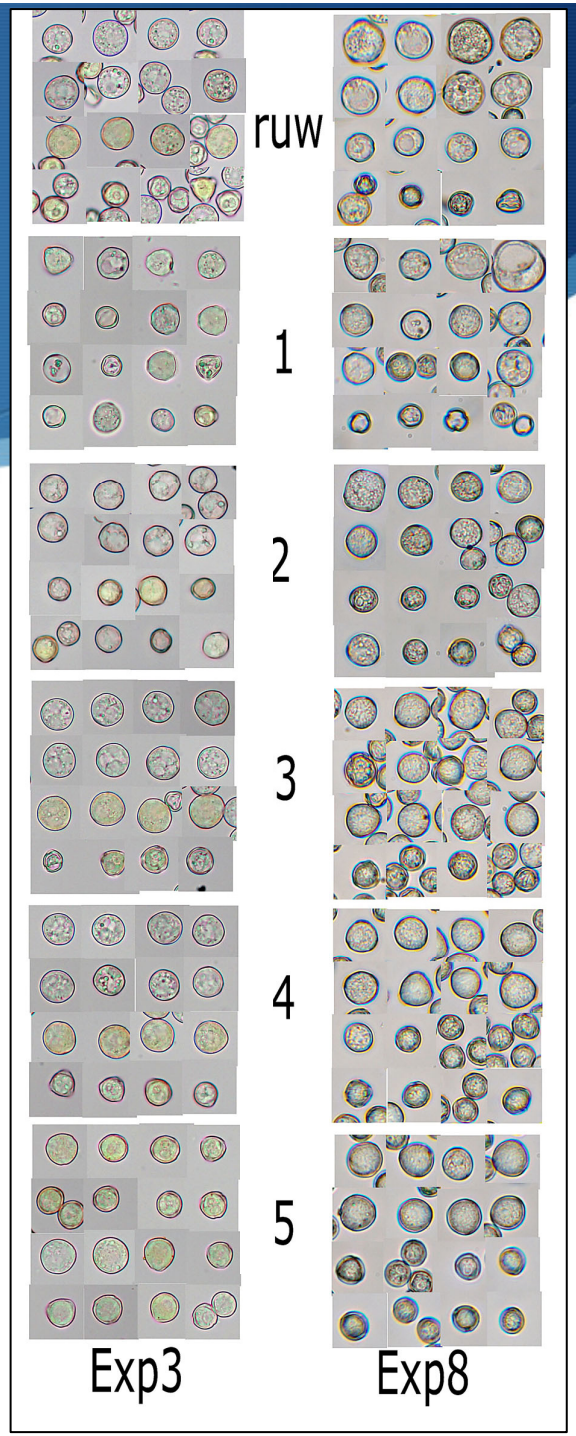
Treatment of microspores with Percoll for upgrading microspores

Percoll gradient in g/ml

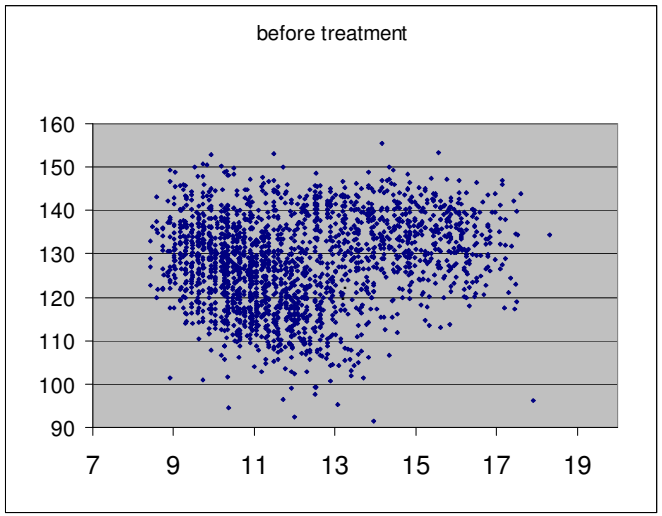


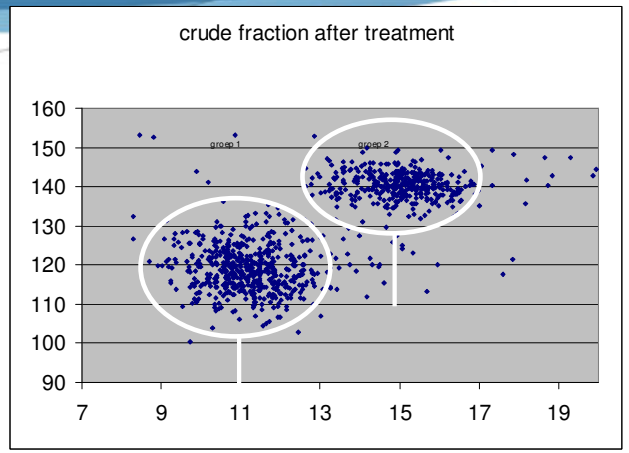
Untreated Exp 3

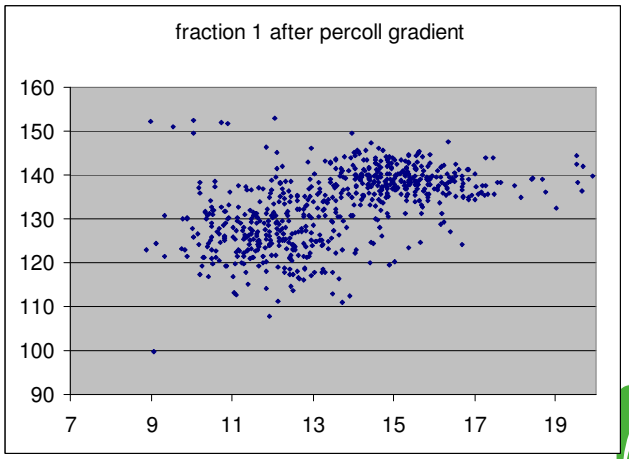
7 days treated Exp 8



Visualization of treatment by image analysis







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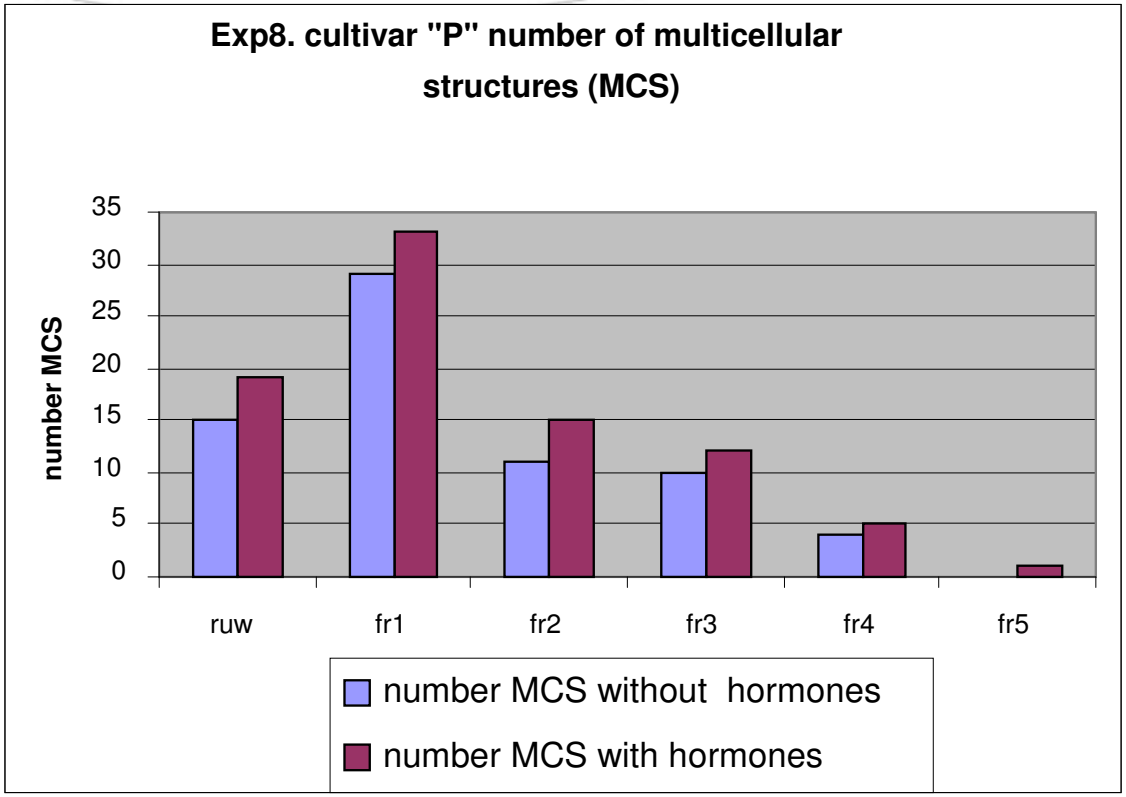
Treatment of upgraded cells



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System for staging development of microspores

- Flower age, and size
- Anther color
- Microspore morphology
- Supporting techniques to use optimal cells

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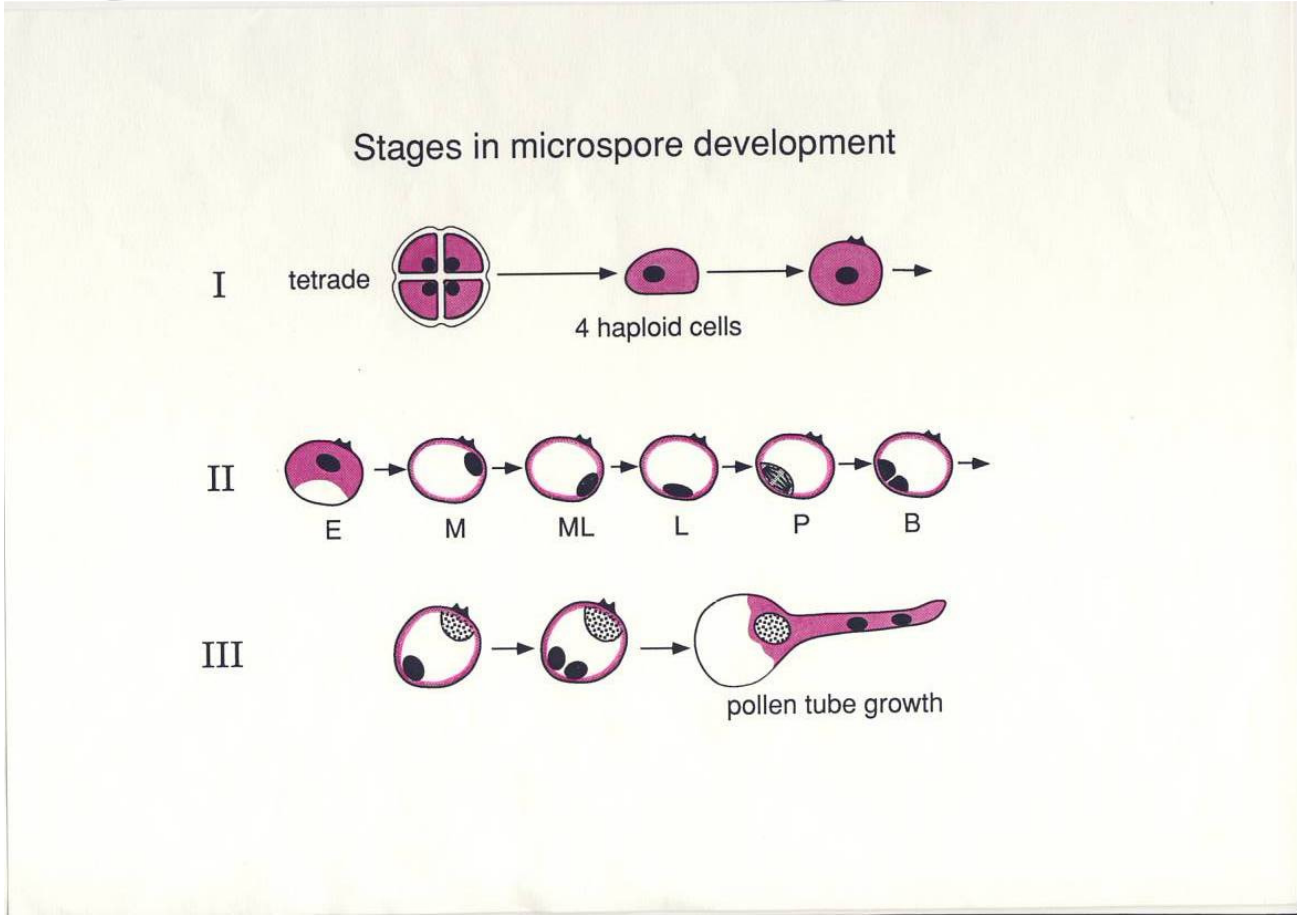
Stages in microspore development



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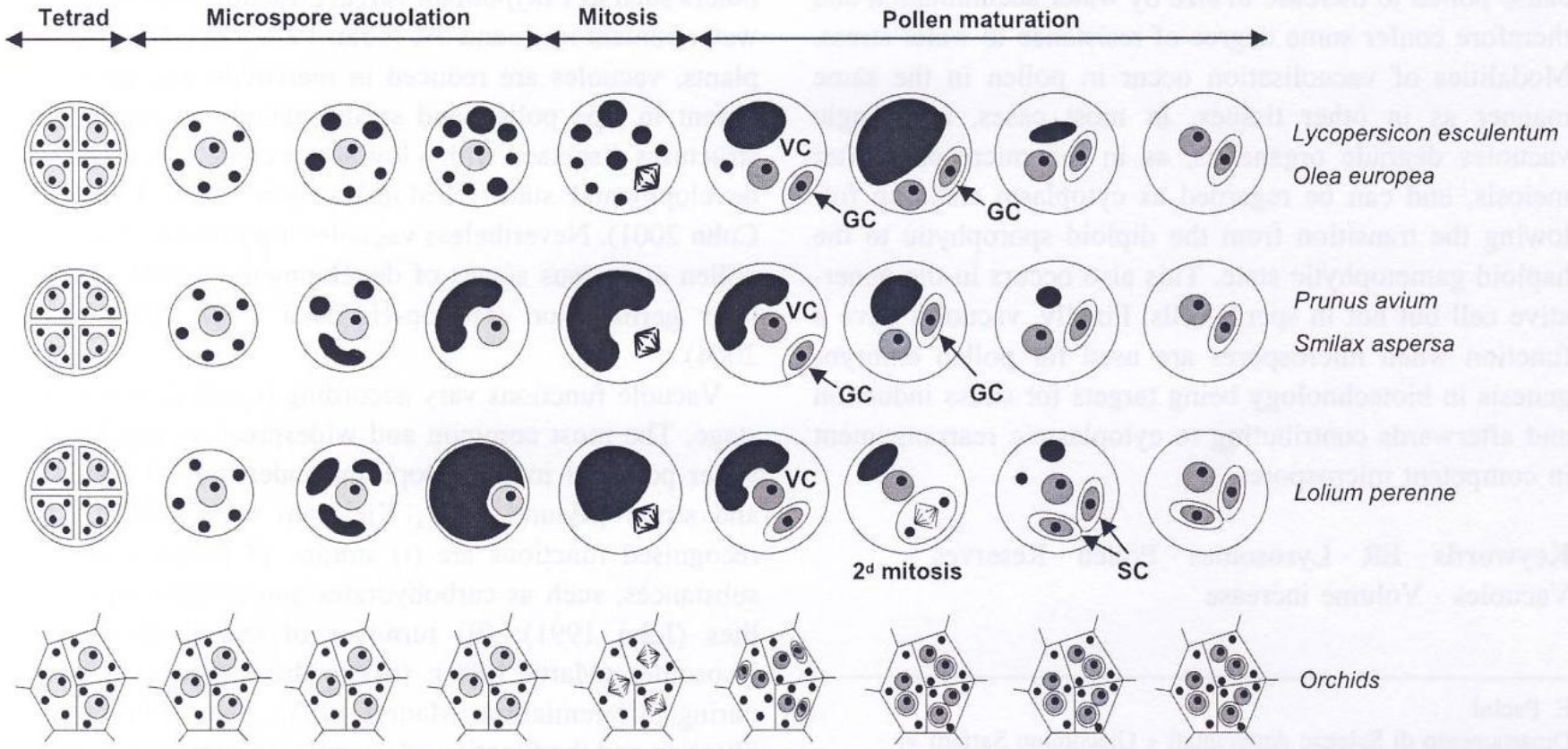
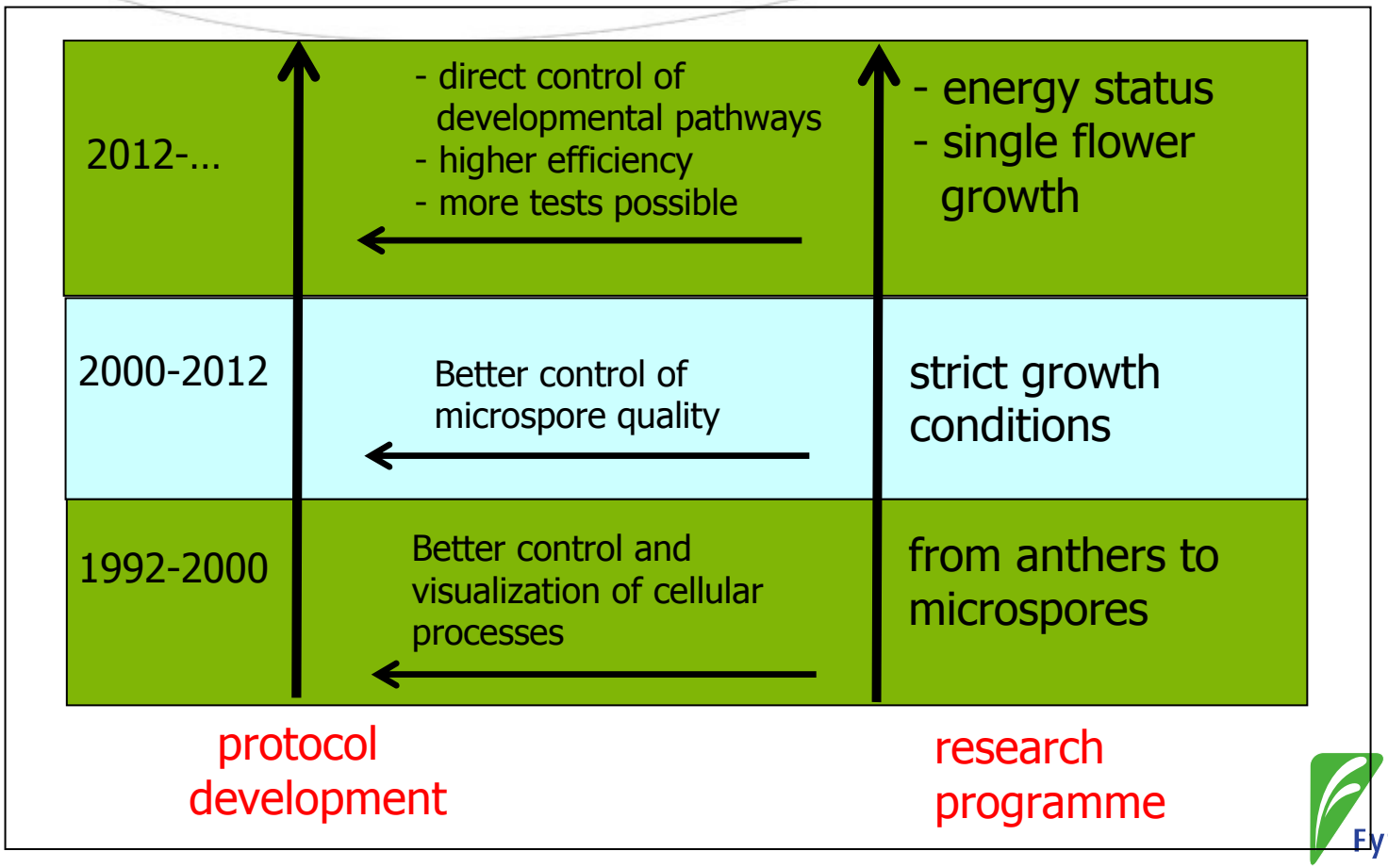
Stages of microspores



Different approach Fytagoras



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Critical steps in DH technology

- Donor plants; stage of development
- Pretreatment; induction of cell division (many different possibilities)
- Culture; formation of multi-cellular structures; embryos
- Formation of plants
- Implementation of the protocol
- Adaptations of the protocol for all varieties

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Different approach Fytagoras

- Formation of DH plants by regeneration of microspores
- Project is divided in 3 steps
- All activities are done at facilities of Fytagoras
- Delivery is a working protocol, or on request DH plants
- Implementation (support) in your laboratory

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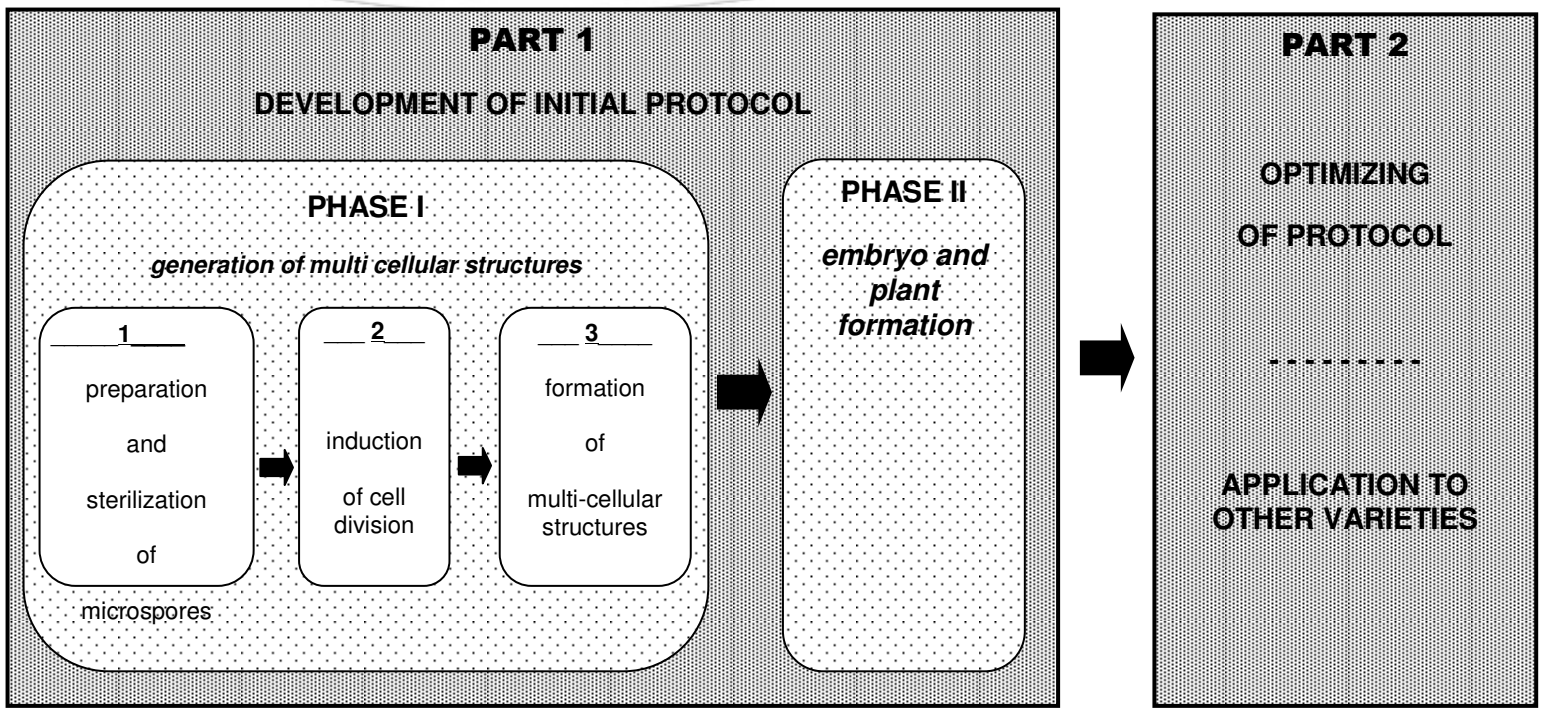
Different approach Fytagoras



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Different approach Fytagoras

- **PART 1: From microspores to doubled haploid plants**
  - ***Phase 1 Induction of multi-cellular structures***
    - ***Step 1*** *Selection of plant material, technical aspects concerning the preparation of microspores, determination of developmental stages, and characterization of microspores*
    - ***Step 2*** *Pretreatment and induction of cell division*
    - ***Step 3*** *Cultivation and formation of multi-cellular structures*
  - ***Phase 2 Embryo and plant formation***
- **PART 2 Optimizing of the procedure and implementation**

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Growth facilities

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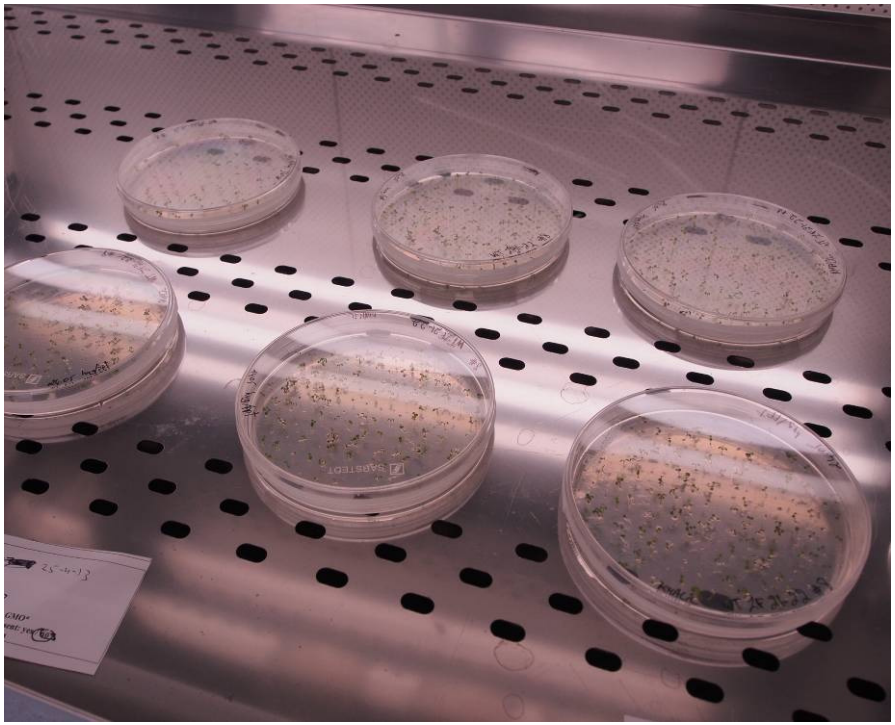
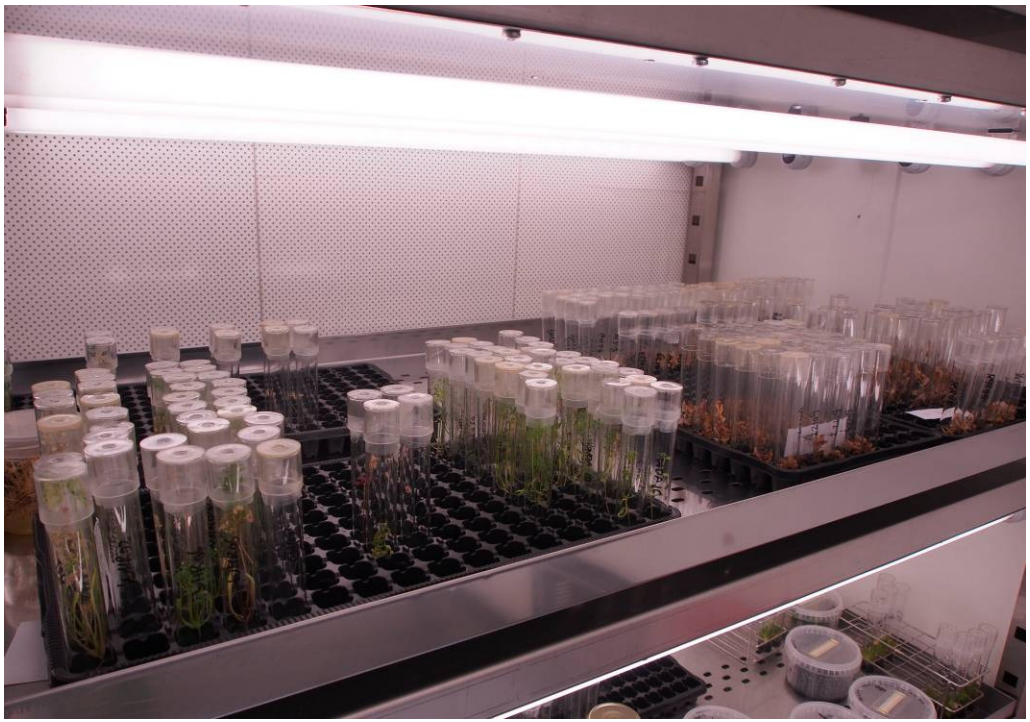
Pictures from our laboratory

Tissue culture



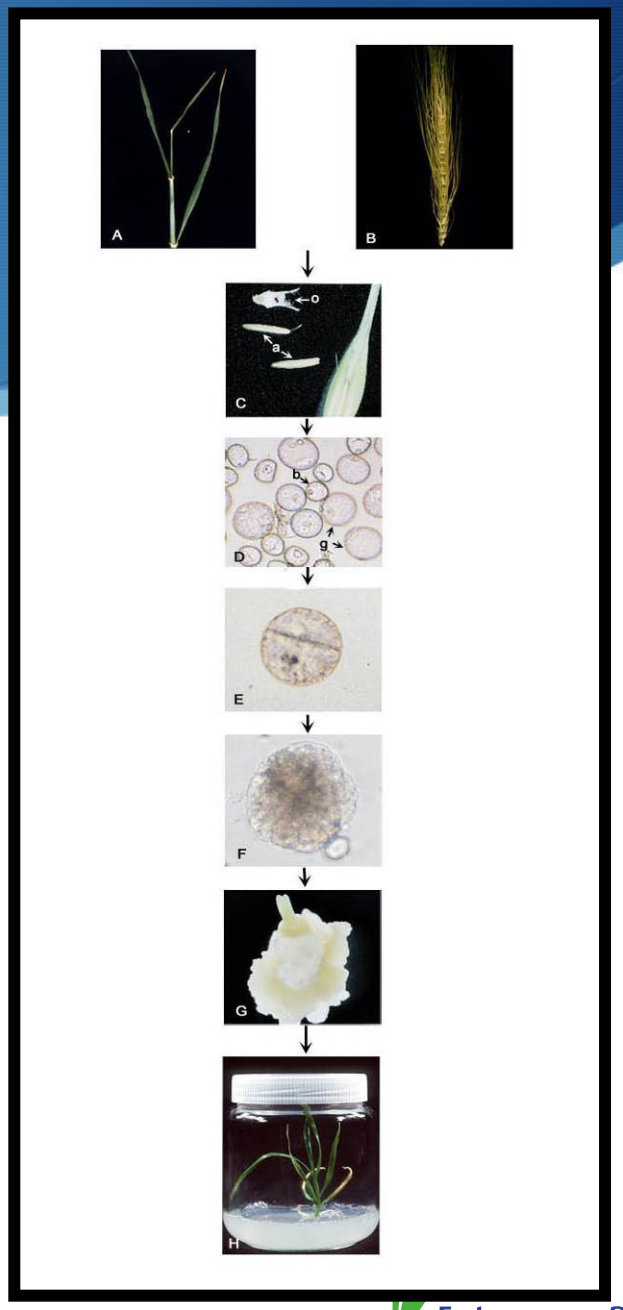


Different approach Fytagoras

1. Donorplant

2. Induction of celldivision and growth of multicellulair structures

3. Formation of plants



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