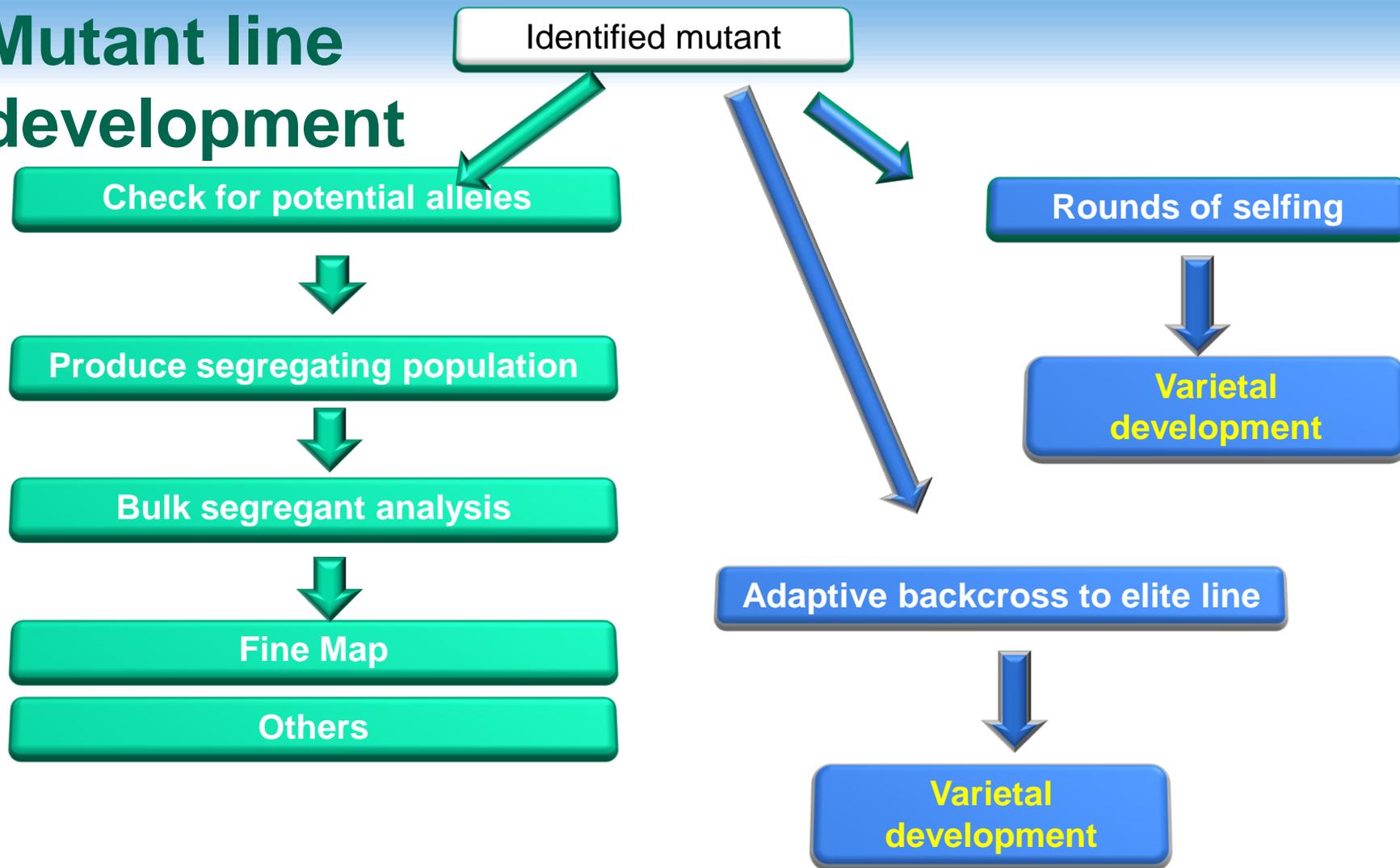


Techniques for acceleration of mutant breeding

Abdelbagi MA Ghanim



Mutant line development



Time scales in mutation breeding

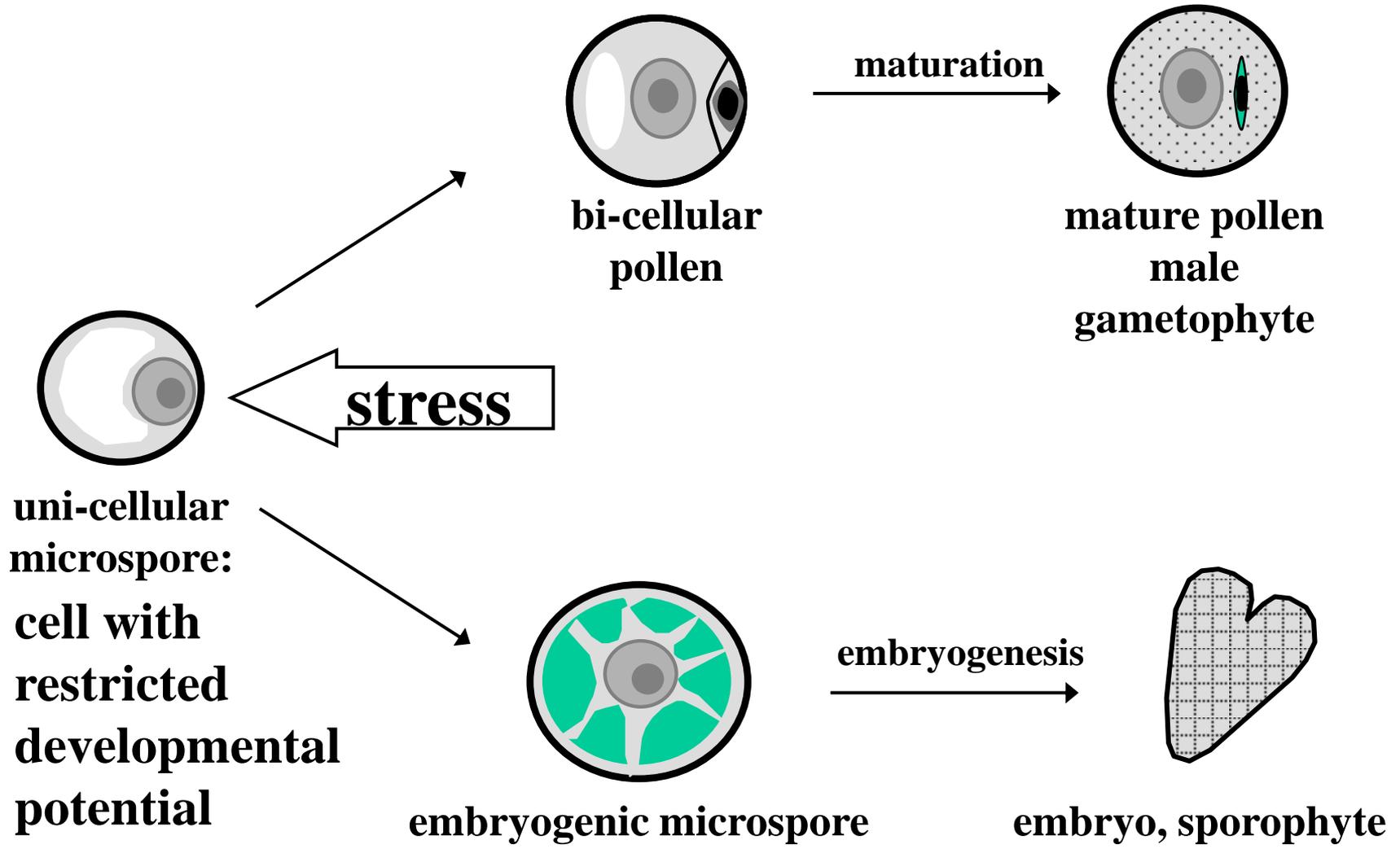
- Mutagenesis: seconds/minutes
- Mutation detection: months/years
- Mutant variety development: 10-12 years



How to speed things up

- High-throughput genotyping and phenotyping
 - still need the M2 generation
- Accelerated pre-breeding
 - Doubled haploidy
 - Rapid generation cycling
 - Markers





totipotent cell

Doubled Haploid: Practical Issues

Widely used methods:

- Anther Culture
- Microspore culture
- Ovule culture
- Irradiated pollen
- Wide hybridization (cereals)

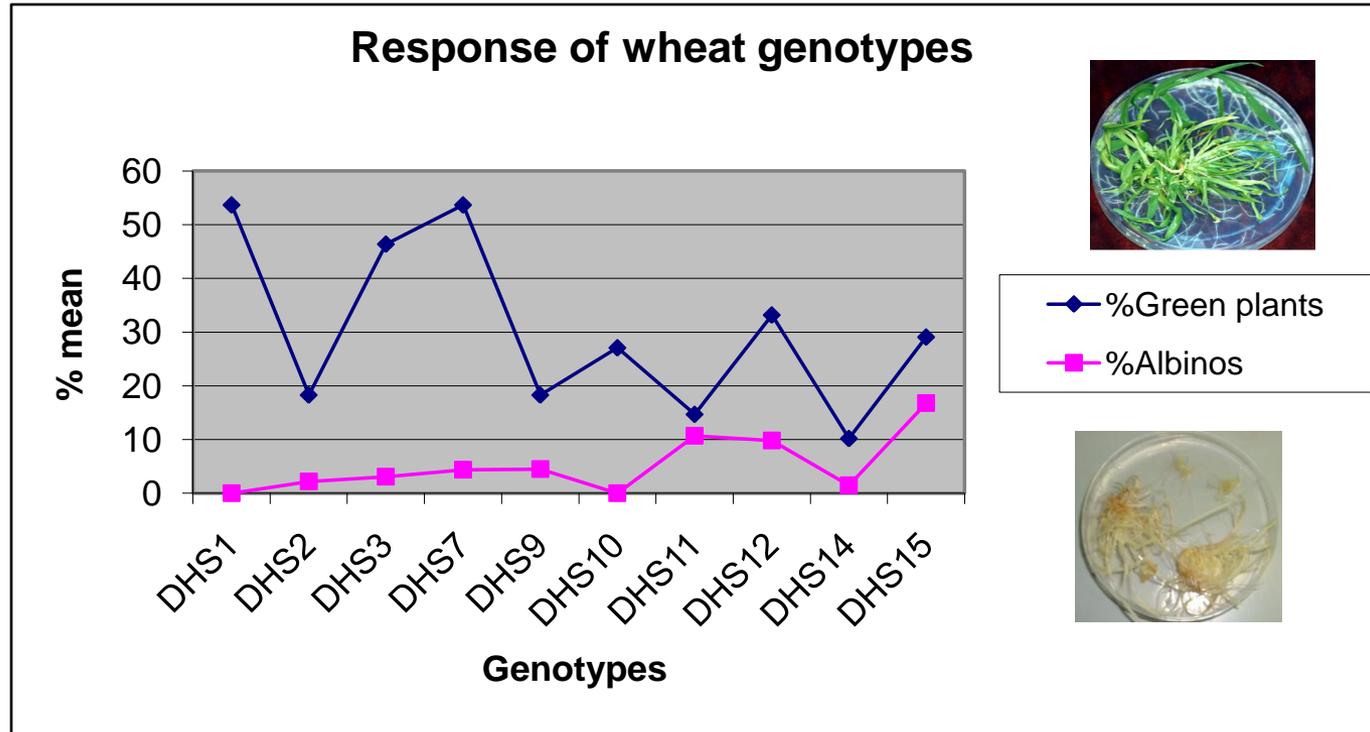


Factors influencing success of DH production by anther/microspore culture

- a) Genotype and the cytoplasm
- b) Culture conditions of donor plants
- c) Optimum stage for spike collection
- d) Pretreatment of spikes
- e) Culture media



Genotype and the cytoplasm



Culture conditions of donor plants



Growth Chamber

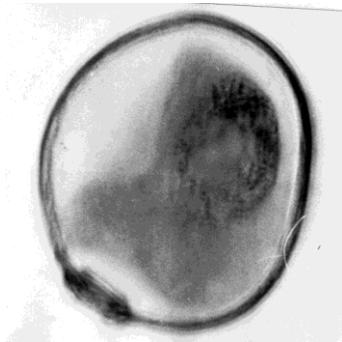
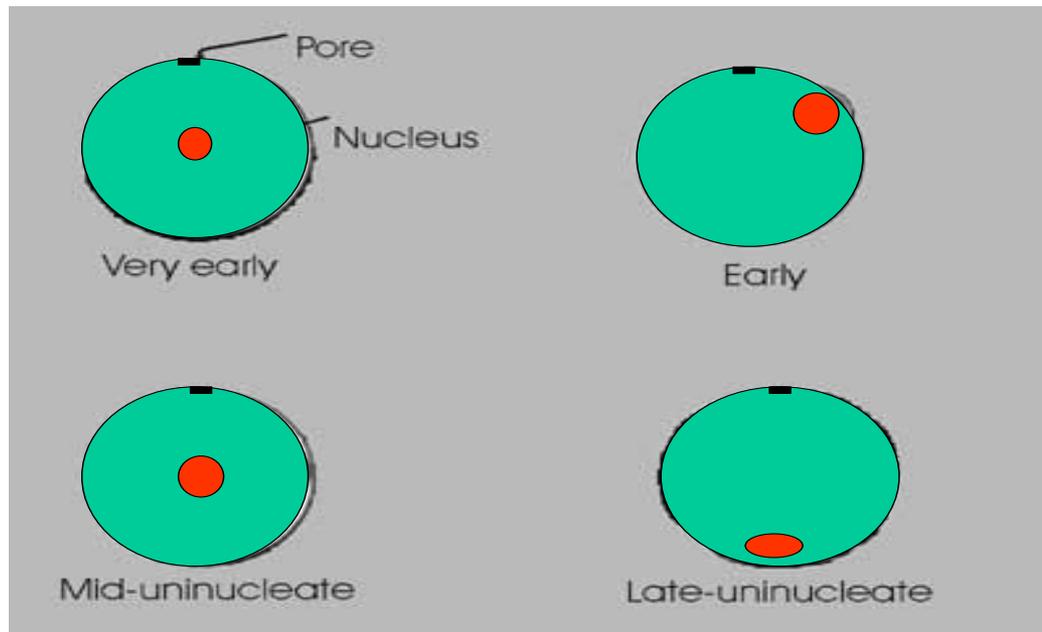


Glasshouse



Field

Optimum stage for spike collection



Pretreatment of spikes (4°C) for 2-7 days



Spike preparation



1. Select spike according to specific stage



2. Keep the spikes for each genotype separately on water tank



3. Remove flag leaf and stem

Spike sterilization



4. Wrap the spike using wet gauze and



5. Remove 80% of owns



incubate in ref for 2-3 days

6. Sterilized using 0.1% HgCl₂ or 5% chlorox for 10 min

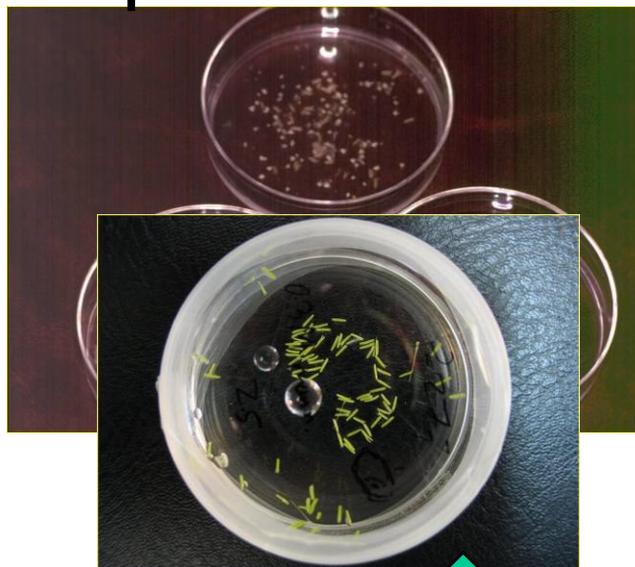


7. Remove the HgCl₂ /chlorox and wash using sterilized

Culture media



Liquid medium



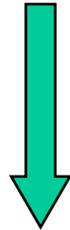
Solid medium



(35-45 days, 25 °C in dark)



Transfer of calli to
regeneration medium
(30-40 days, 25/18 °C 16/8 light/dark)



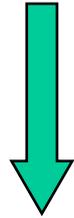
Transfer of green plants to
Plant let generation medium

(30-40 days, 25/18 °C 16/8
light/dark)





Transfer of vigorous plants to small pots



Colchicine treatment at 3-4 tiller stage
(0.5%, 5 hr, wash in running water over
night)



Harvesting of DH seeds

MS media for differentiation and 1/2 MS media for rooting



17. Green and albino plantlet

Culturing room , light and 25 °C



18. Transfer plantlet to 1/2 MS media for rooting



19. Rooting of plantlet in 1/2 MS media

Isolated Microspore Culture



Grinding spikes at 18.000 rpm

- a) Cut florets in blender cup ready to be blended;
- b) b) Blender cup assembled to the blender for blending to release microspores

DH population



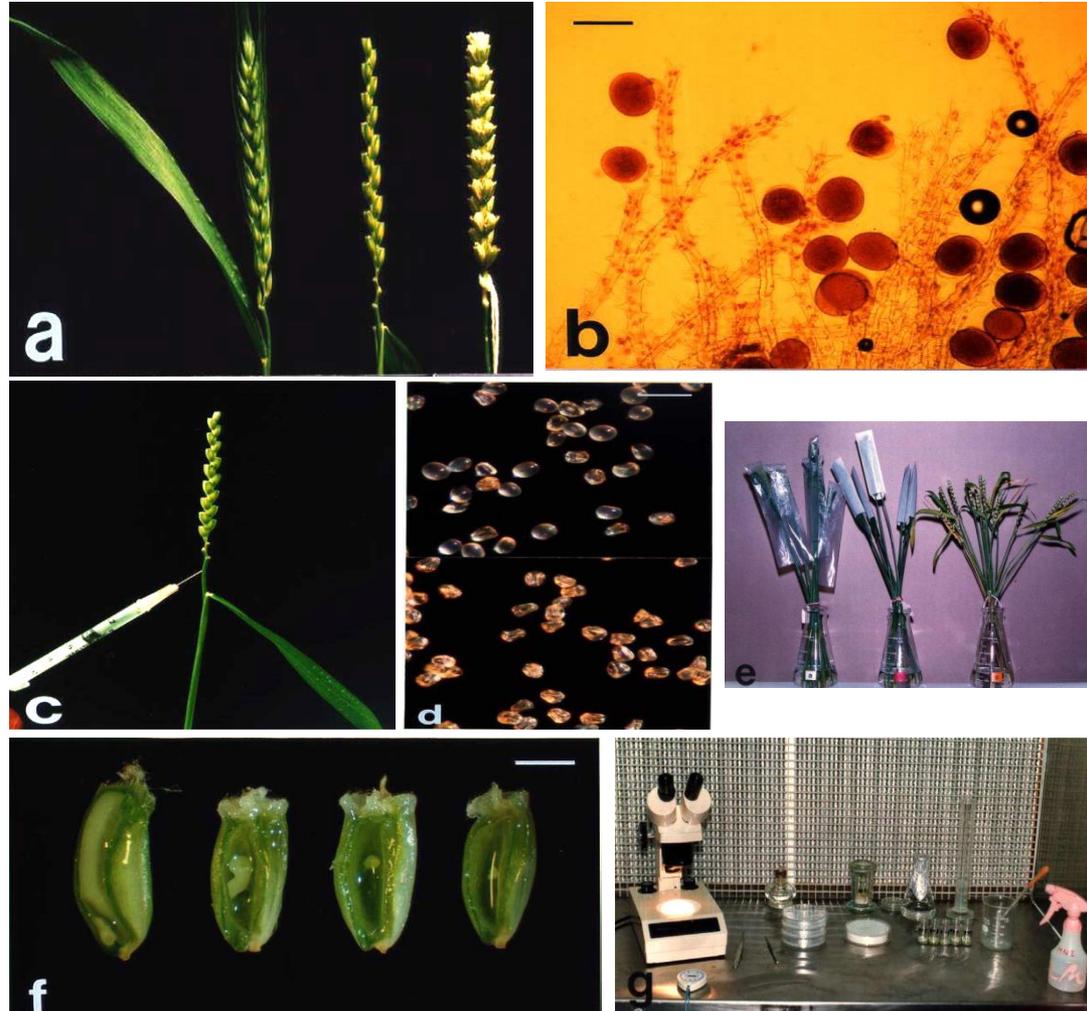
Seed increase

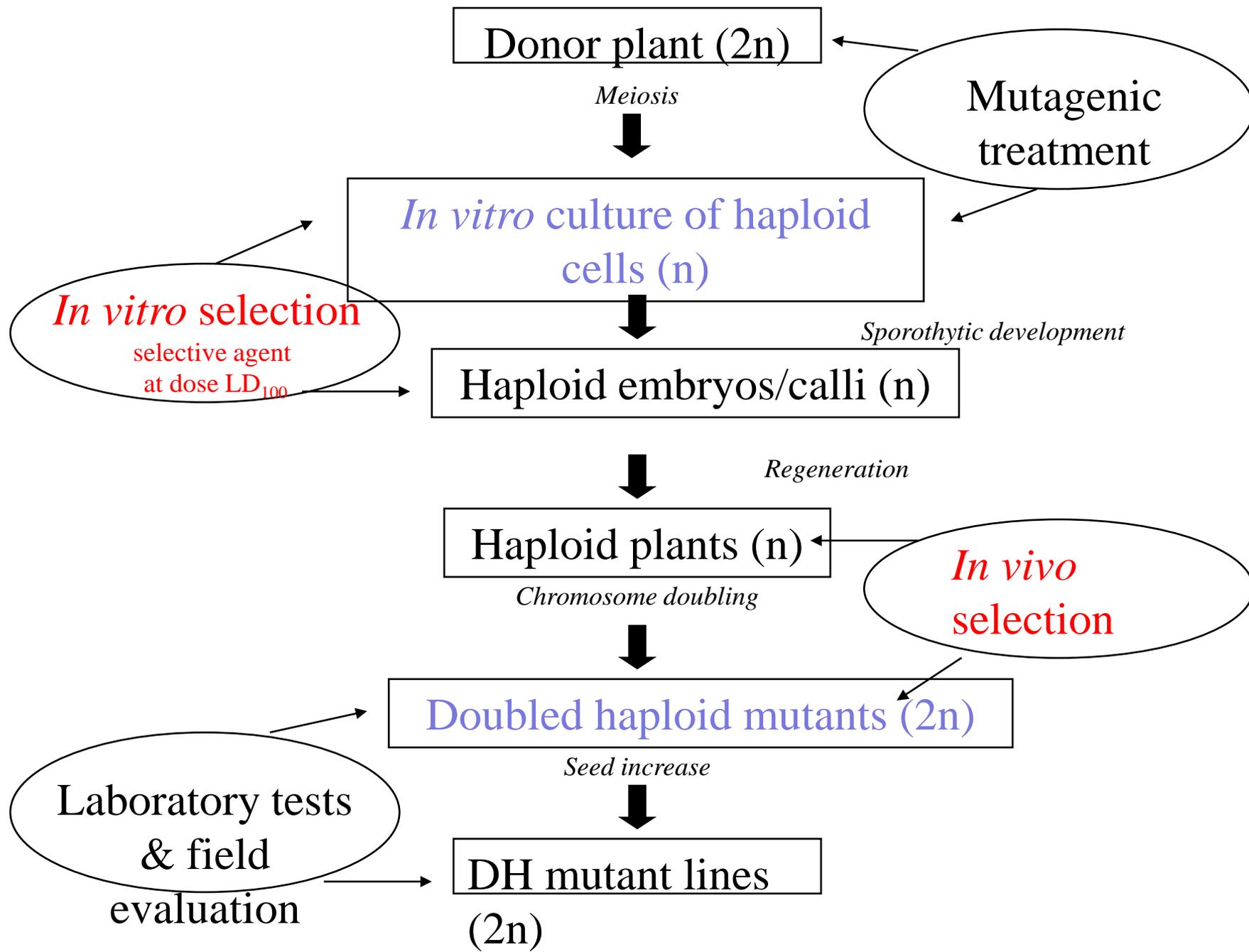


Evaluation

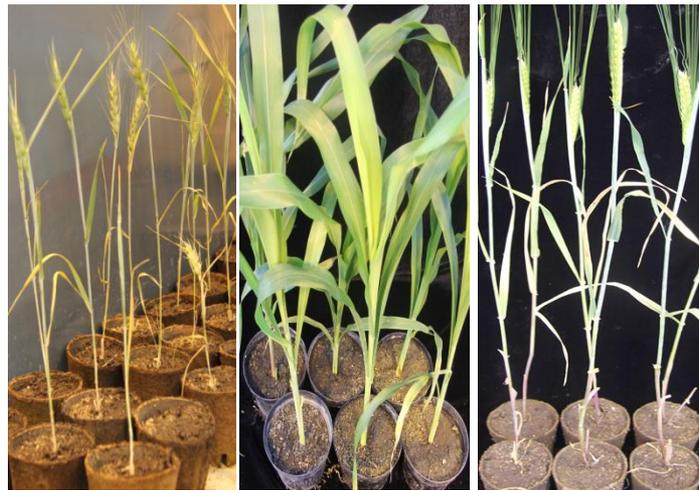
Stages in producing wheat doubled haploids through ultra-wide crosses

- a) Wheat spikes at ear emergence (*left*), emasculation (*center*) and seed setting (*right*).
- b) Germination of maize pollen on wheat stigma. Bar 0.1 mm.
- c) c) Injection of **2,4-D** into wheat spike using a syringe.
- d) d) Pearl millet pollen at collection (*upper*) and after being dried for two hours (*lower*). Bar 0.1 mm.
- e) e) Detached-tiller culture of wheat at emasculation (*left*), pollination (*center*) and seed setting (*right*).
- f) f) Wheat seeds obtained from self-pollination, and from crosses with maize, pearl millet and sorghum (*from left to right*). Bar 2 mm.





6-7 cycles per year

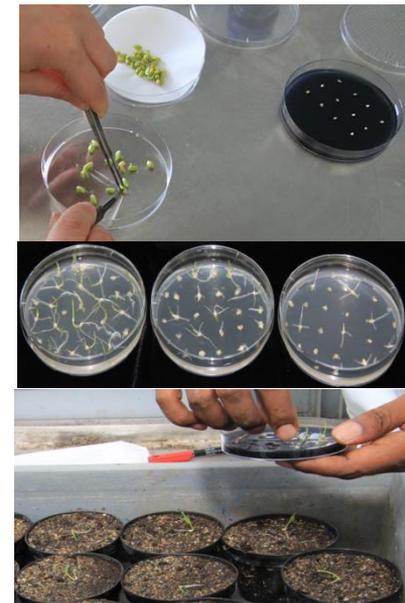
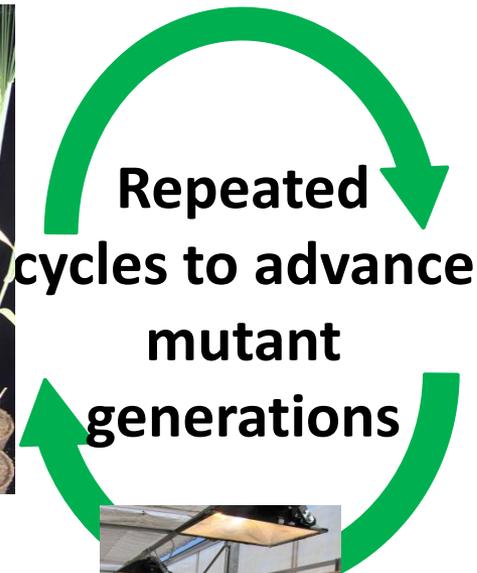


Wheat

Sorghum

Barley

Shortening the crop cycle by management techniques such as small pots, light watering and continuous light



Rescue of immature embryos to gain time

Fig. 1. Rapid cycling techniques to accelerate mutants development

THANK YOU!