Present and future plans of the sunflower "Doubled Haploid" project

C. C. Jan[1], Lili Qi[1], Brent Hulke[1], Xuelin Fu[2]

[1]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58102
[2]North Dakota State University, Fargo, ND 58105

Doubled haploid technique in breeding

Advantages:

1. Shorten the breeding time to ½ of the conventional
2. All the lines are completely homozygous

- Canola – microspore culture
- Corn – inducing corn pollen
- Wheat – inducing corn pollen
- Rice – anther and microspore culture
- Tobacco – anther and microspore culture
- Barley – anther and microspore

Anther culture and field studies on haploids

by Robert Jonard and Antoine Mezzarobba, 1989

- Wild *Helianthus* species, interspecific hybrids, and cultivated
- For cultivated sunflower - anthers collected between meiotic diad and tetrad, pretreatment at 35 C for 12 days, culture at 35 C for 12 days in dark
- Solid initiation medium containing half-strength MS medium, vitamins of Morel and Wetmore with B-12 and a mixture of amino acids, 120 g/l sucrose, pH at 5.9, plus NAA and BAP at 0.5 g/l
- Liquid embryo transfer medium – Monnier's macro and micro, vitamins of Morel and Wetmore with a mixture of amino acids, 15g/l sucrose, pH at 5.9
- Produced 8 haploids, 68 diploids, and 15 aneuploids from over 2,000 anthers of 8 cultivated genotype
- The success is low and could be improved

Highly efficient doubled-haploid production in wheat via induced microspore embryogenesis by Weiguo Liu, Ming Y. Zhang, Enrique A. Polle, and Calvin F. Konzak, 2002

- Tiller collected at mid to late-uninucleate stage
- Pre-treat tillers using 2-HNA at 33 C for 72 hours
- Microspore isolation by blending, filtration, suspension, centrifugation
- 20 to 80 DH plants were obtained from one spike of 8 genotypes
- Similar approaches will be used for sunflower microspore culture

This project was conceived by the sunflower industry as a means to provide an important tool for sunflower breeders to speed up the time to develop elite inbred sunflower lines.

A psotdoc, Xuelin Fu, with tissue culture experience arrived on Sept. 27.

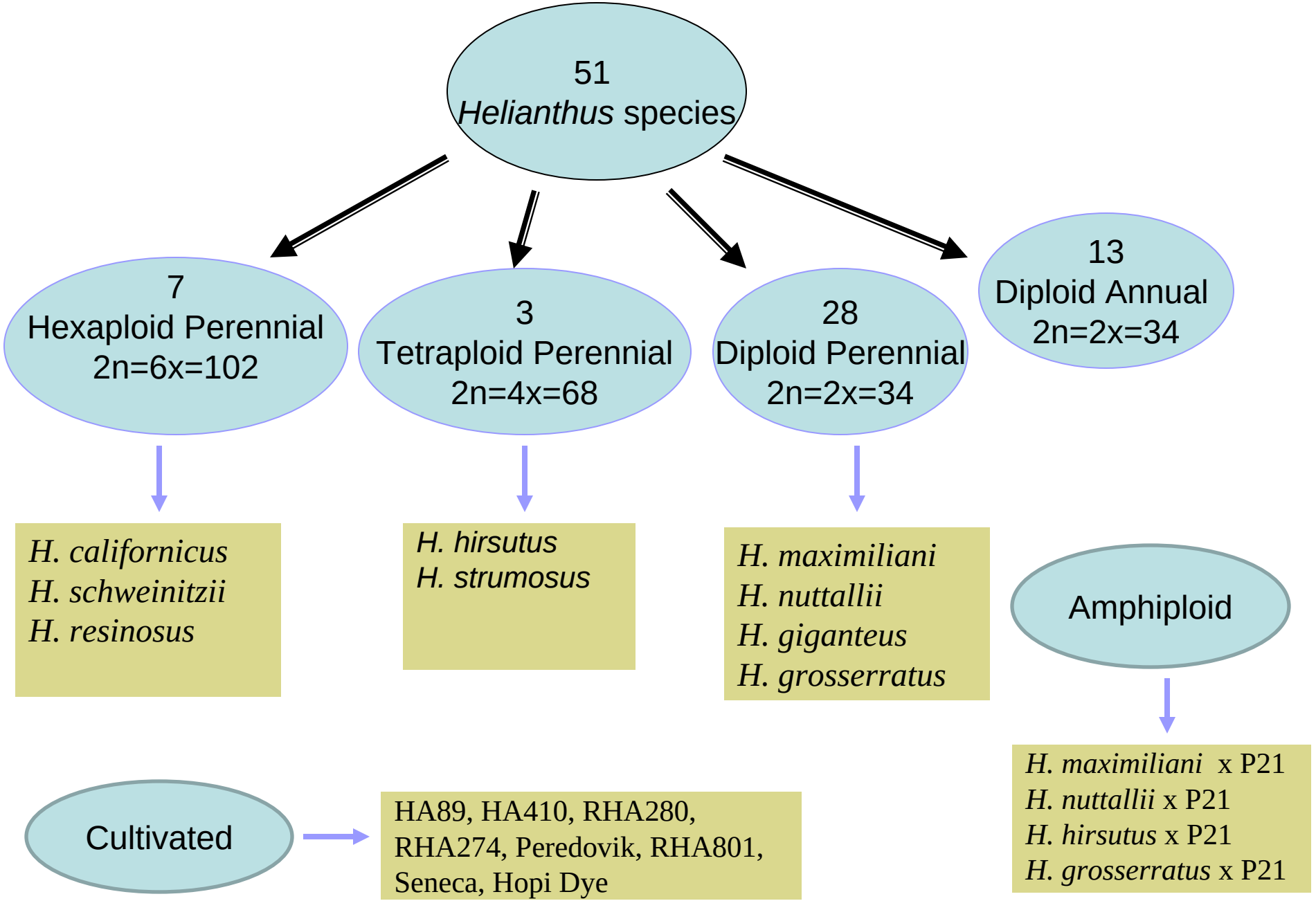
Objectives:

To develop efficient procedures of producing doubled haploid sunflower plants

1. Anther culture
2. Microspore culture
3. Foreign pollen as haploid inducer

Available plant materials

- Interspecific amphipliods have been produced for evaluation and maintained in the greenhouse.
- Wild perennial *Helianthus* accessions and some F1 hybrids of wild x cultivated have been maintained in the greenhouse.
- Embryo rescue techniques have been developed for saving the immature hybrid embryos from premature abortion.
- A large number of cultivated lines available for immediate planting.


51
Helianthus species
7
Hexaploid Perennial
2n=6x=102
3
Tetraploid Perennial
2n=4x=68
28
Diploid Perennial
2n=2x=34
13
Diploid Annual
2n=2x=34
H. californicus
H. schweinitzii
H. resinosus
H. hirsutus
H. strumosus
H. maximiliani
H. nuttallii
H. giganteus
H. grosserratus
Amphiploid
H. maximiliani x P21
H. nuttallii x P21
H. hirsutus x P21
H. grosserratus x P21
Cultivated
HA89, HA410, RHA280,
RHA274, Peredovik, RHA801,
Seneca, Hopi Dye

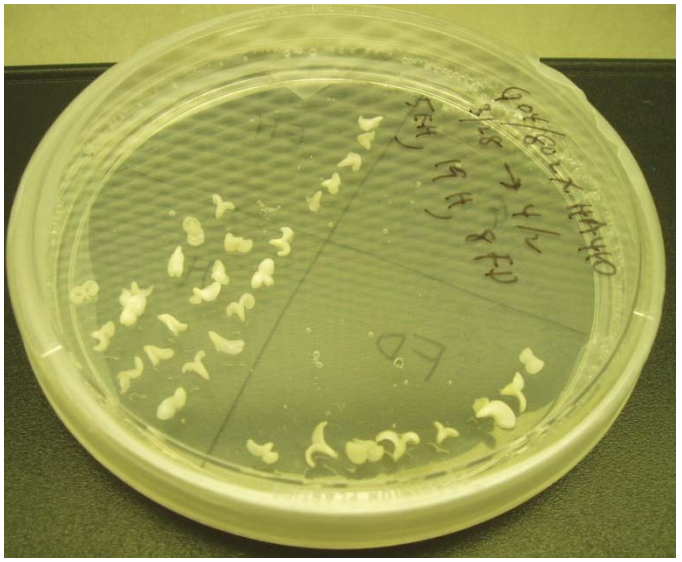
Embryo rescue

H. californicus x HA 410

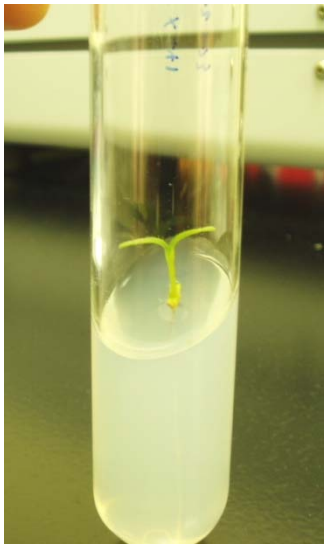
↓

F_1 hybrids

5-day-old F_1 hybrid seeds



F_1 hybrid embryos in high sucrose medium



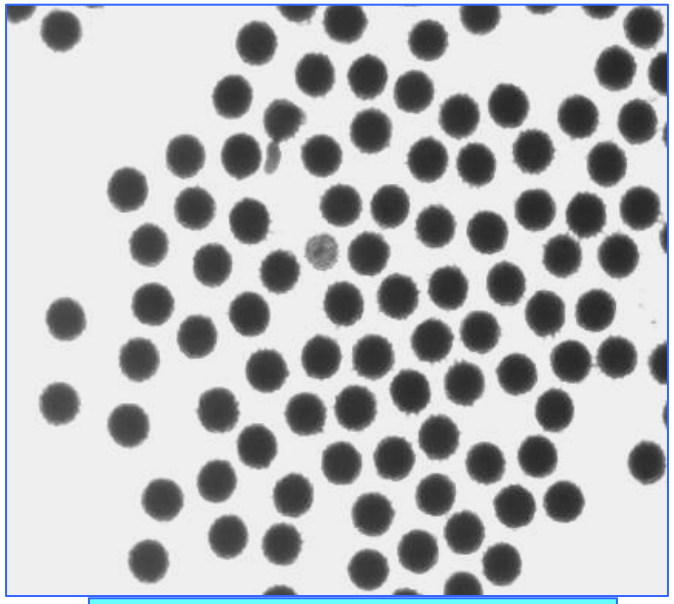
Transfer to test tube

Seedlings grown in test tubes

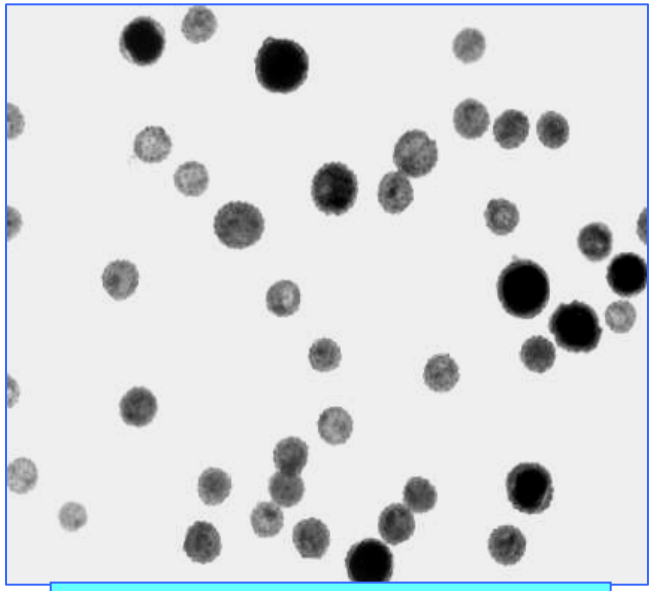
Transfer to Jiffy-7

Transfer to small pots for chromosome counting

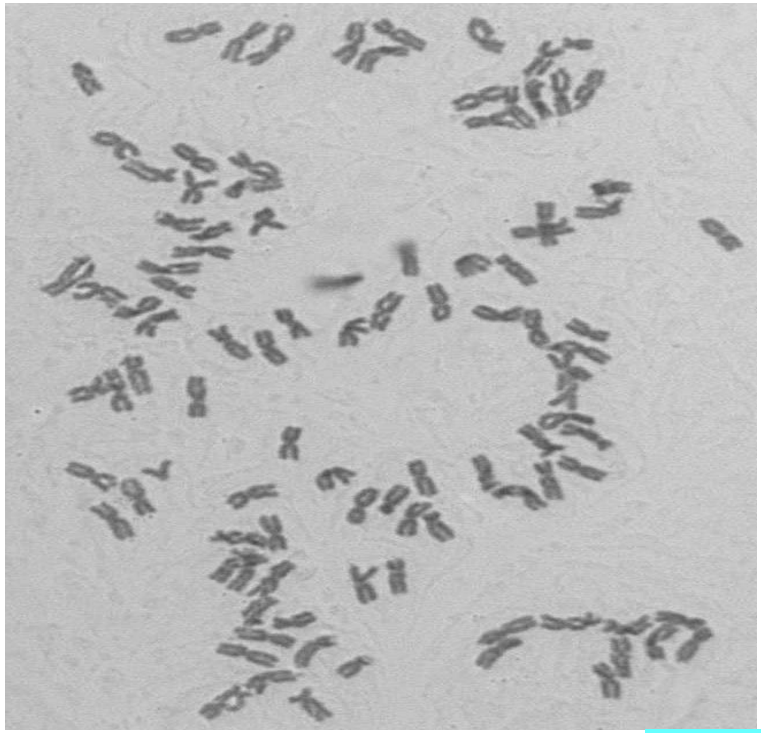
Established technique for pollen stainability evaluation



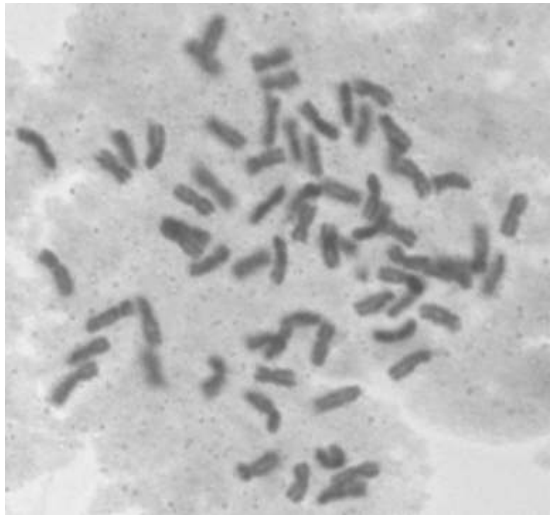
Fertile pollen grain with high stainability



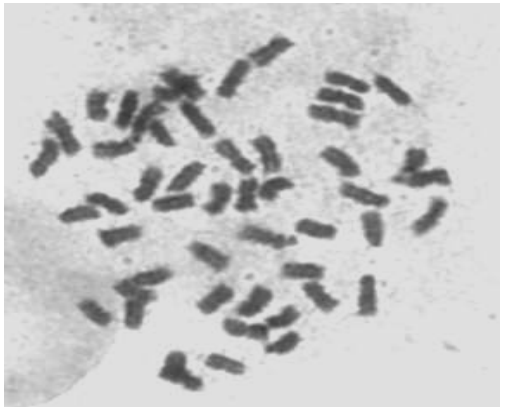
Poor pollen grain with reduced stainability



2n=102

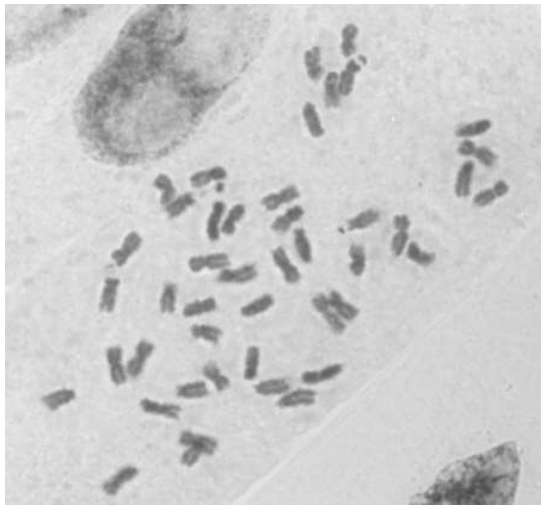


2n=68

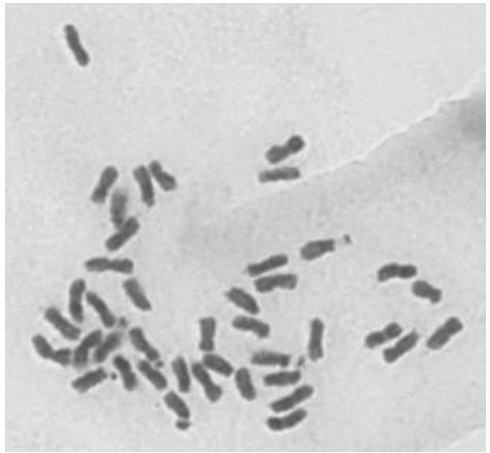


2n=51

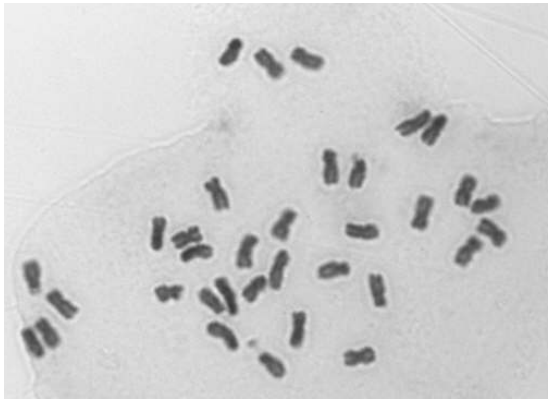
Established technique for chromosome counting



2n=45

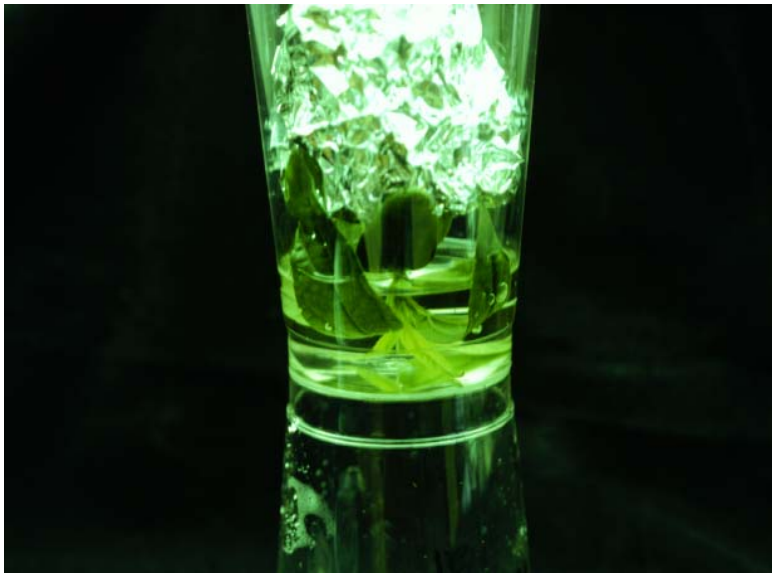


2n=41

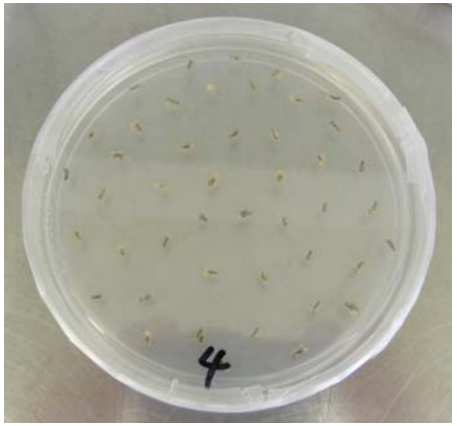


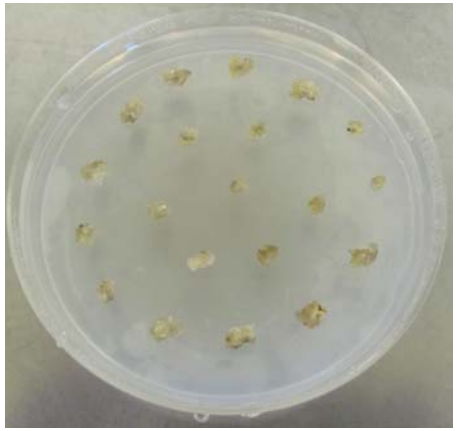
2n=34

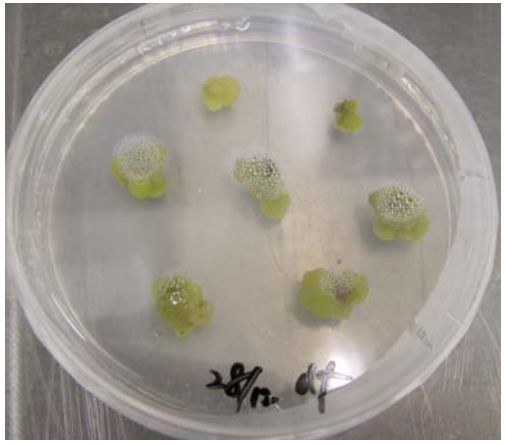
Colchicine treatment for chromosome doubling



Progress - anther culture and new source plants







Summary and Plan

1. We thank the sunflower industry and the NSA for recognizing the need of doubled haploid in sunflower breeding and their support of this project.

2. We have hired the best we could find for this project.

3. Plant materials are established in the greenhouse for all the approaches.

4. The two co-PIs are growing and collecting sources of foreign pollen to be tested as haploid inducers.

5. Real progress will be reported in 2012.

6. Questions?