Somatic Embryogenesis in *Abies fraseri* (Fraser fir):

Optimizing Levels of Abscisic Acid, Polyethylene Glycol & Maltose for Maturation

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What is Embryogenesis?

embryogenesis

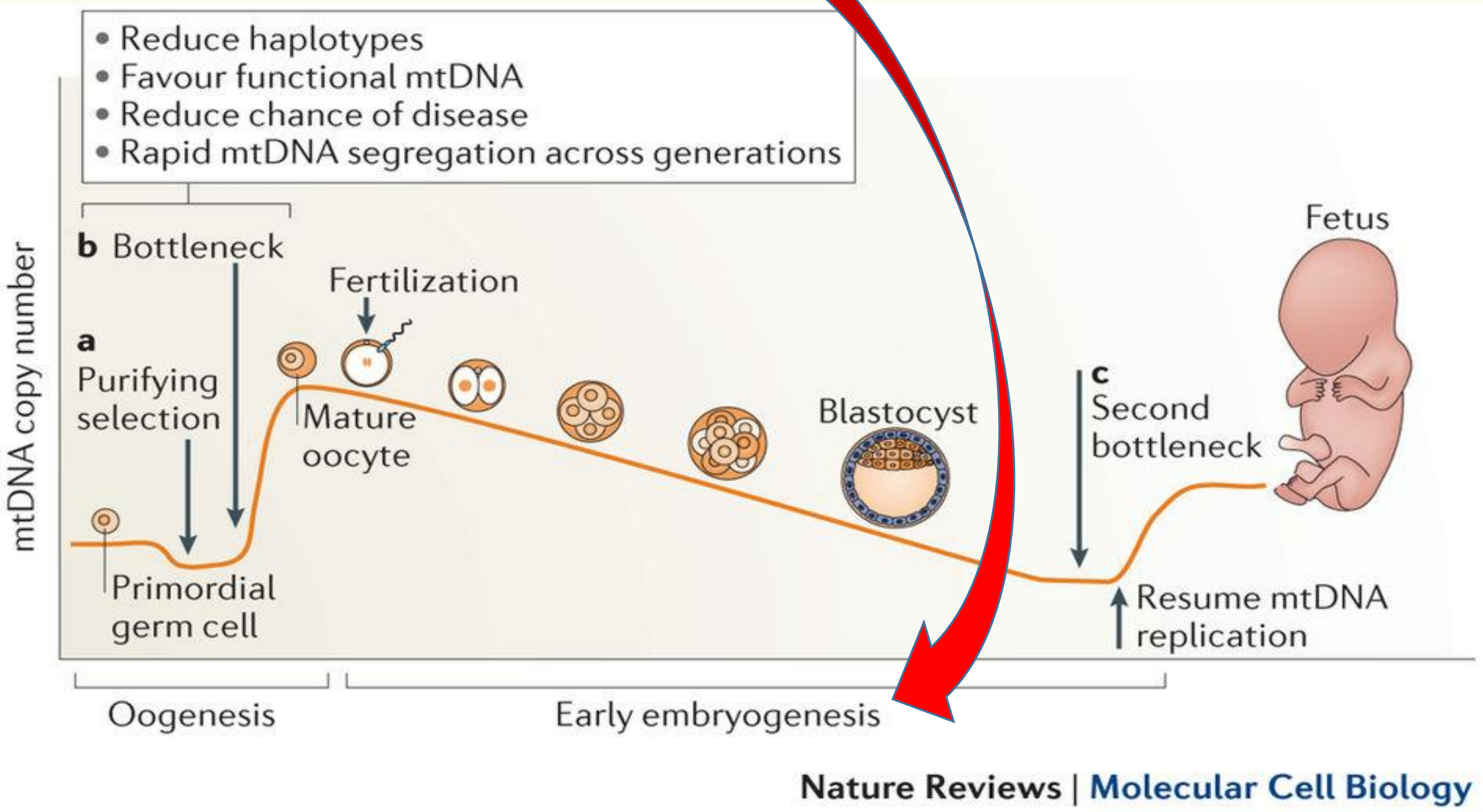
noun | em·bryo·gen·e·sis | \ˌem-brē-ō-ˈje-nə-səs\

Popularity: Bottom 10% of words

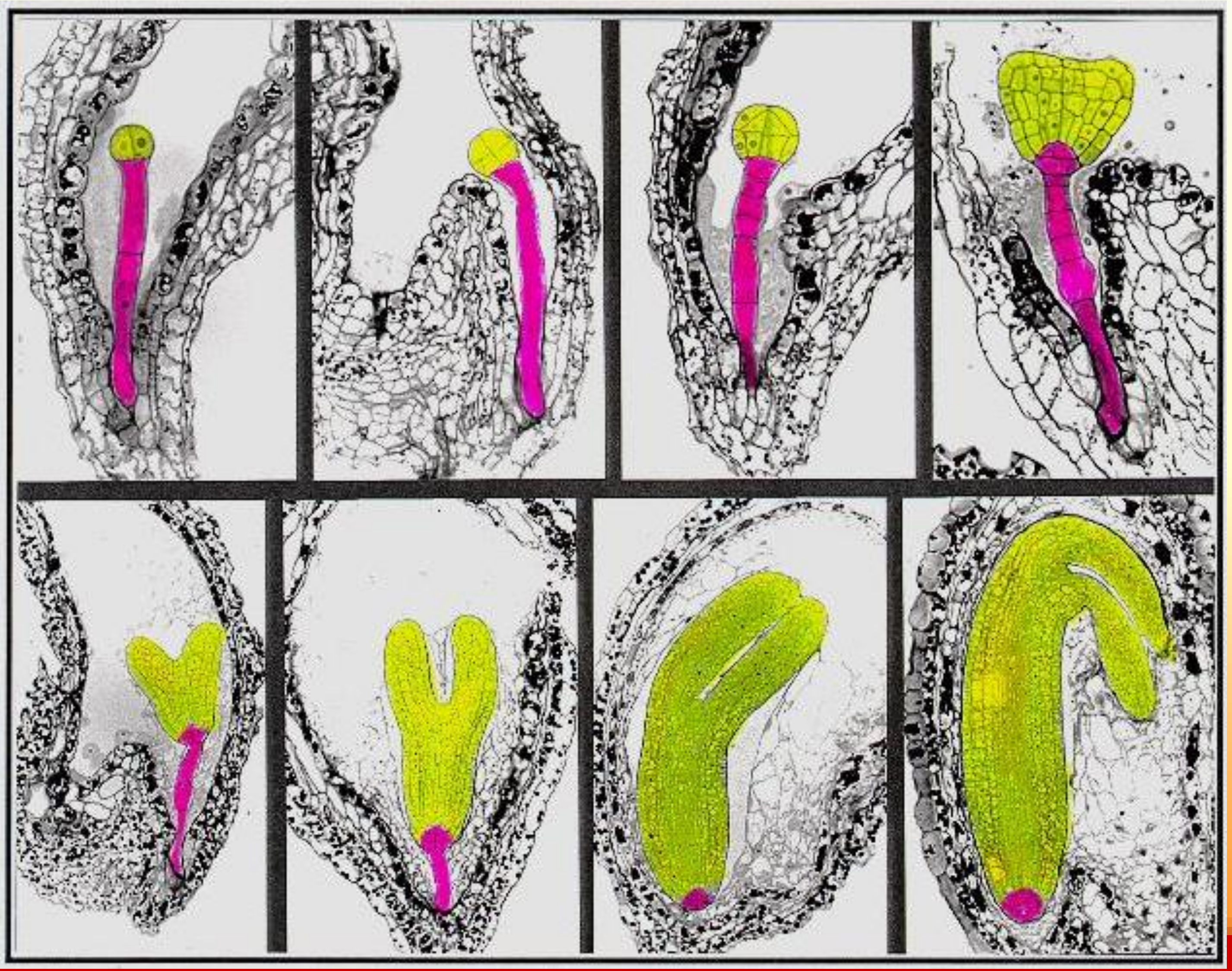
: **the formation and development of the embryo**

Merriam-Webster®

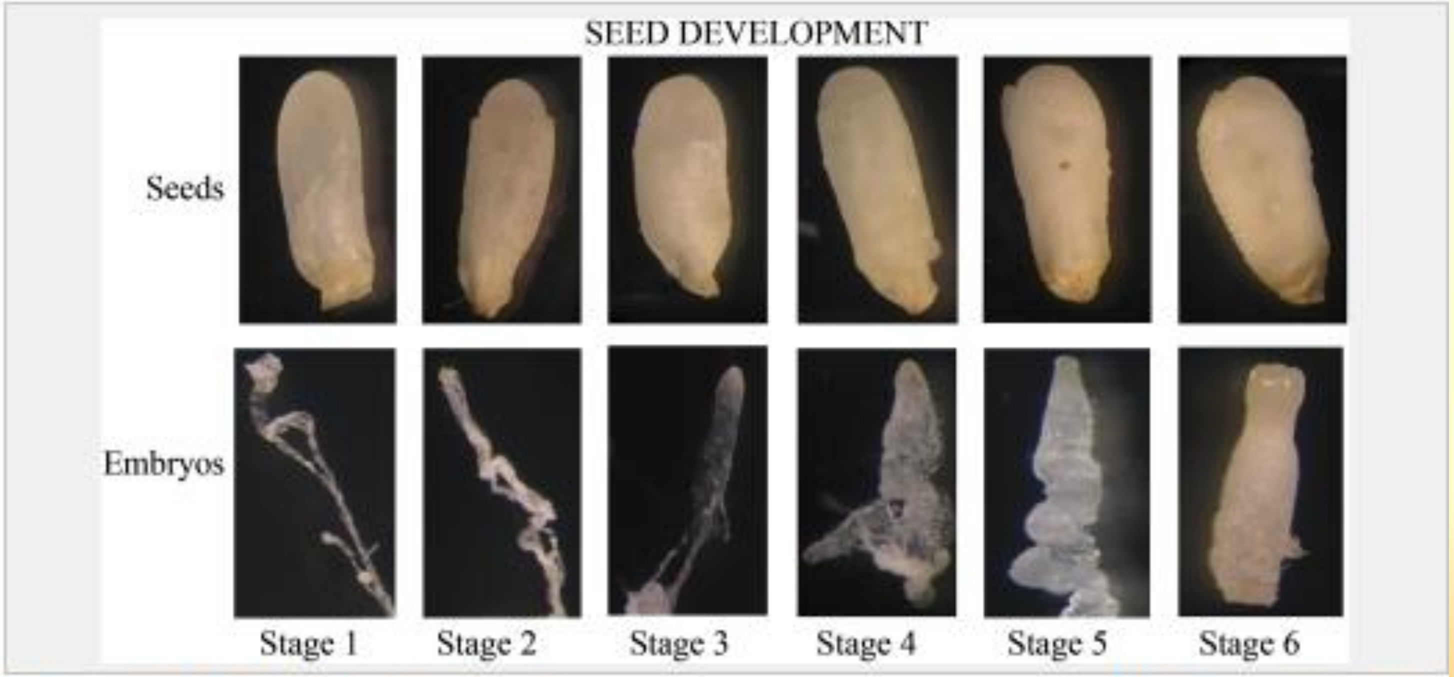
Embryogenesis



Plant Embryogenesis



Zygotic Plant Embryogenesis



Related In: Results - Collection
Show All Figures

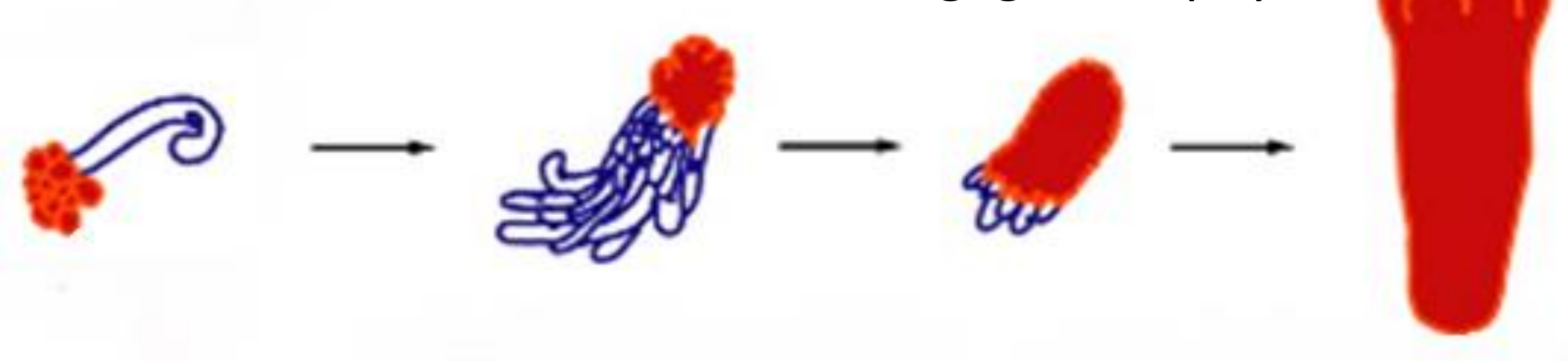
(Conifer example)

fig1: Dissected characteristic zygotic embryo stages of the Chinese fir during seed development. Stages 1, 2, and 3: cleavage polyembryony stage; stage 4: dominant embryo stage; stage 5: columnar embryo stage; stage 6: early cotyledon stage. (This figure is available in colour at JXB online.)

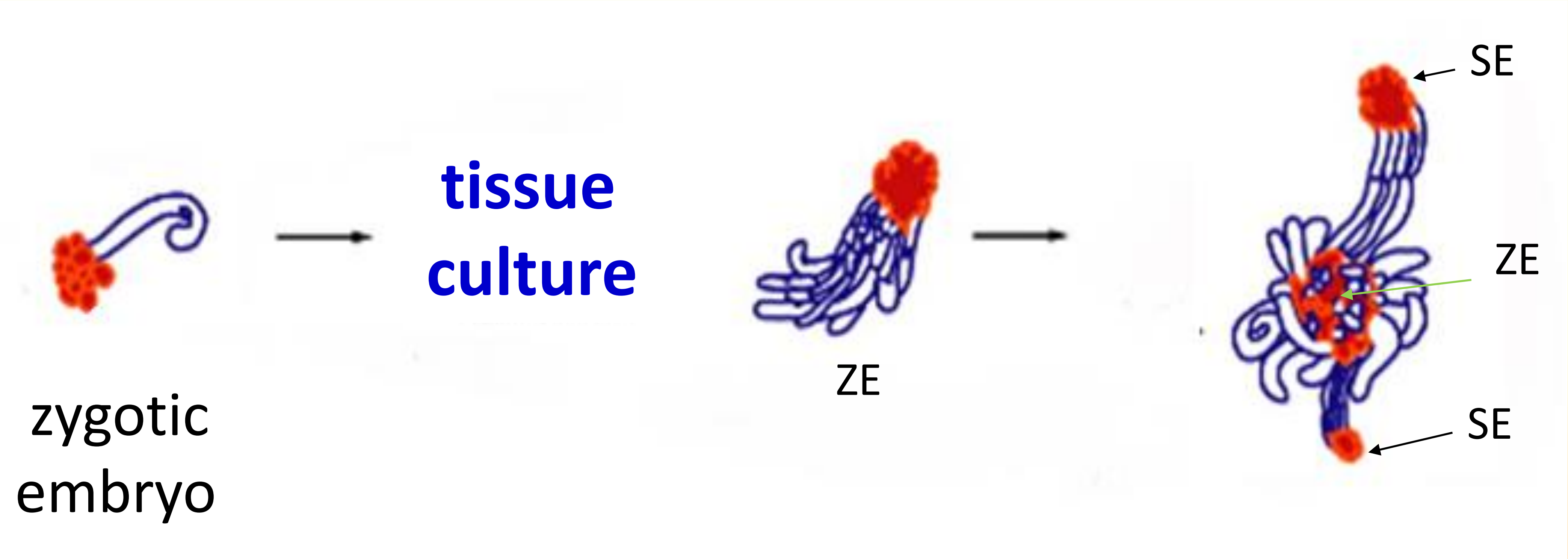
[Zygotic](#) Plant Embryogenesis

(Conifer example)

Inside a seed,.... Inside the megagametophyte



Somatic Plant Embryogenesis



Somatic Embryogenesis

SE

SE is often reported in conifers

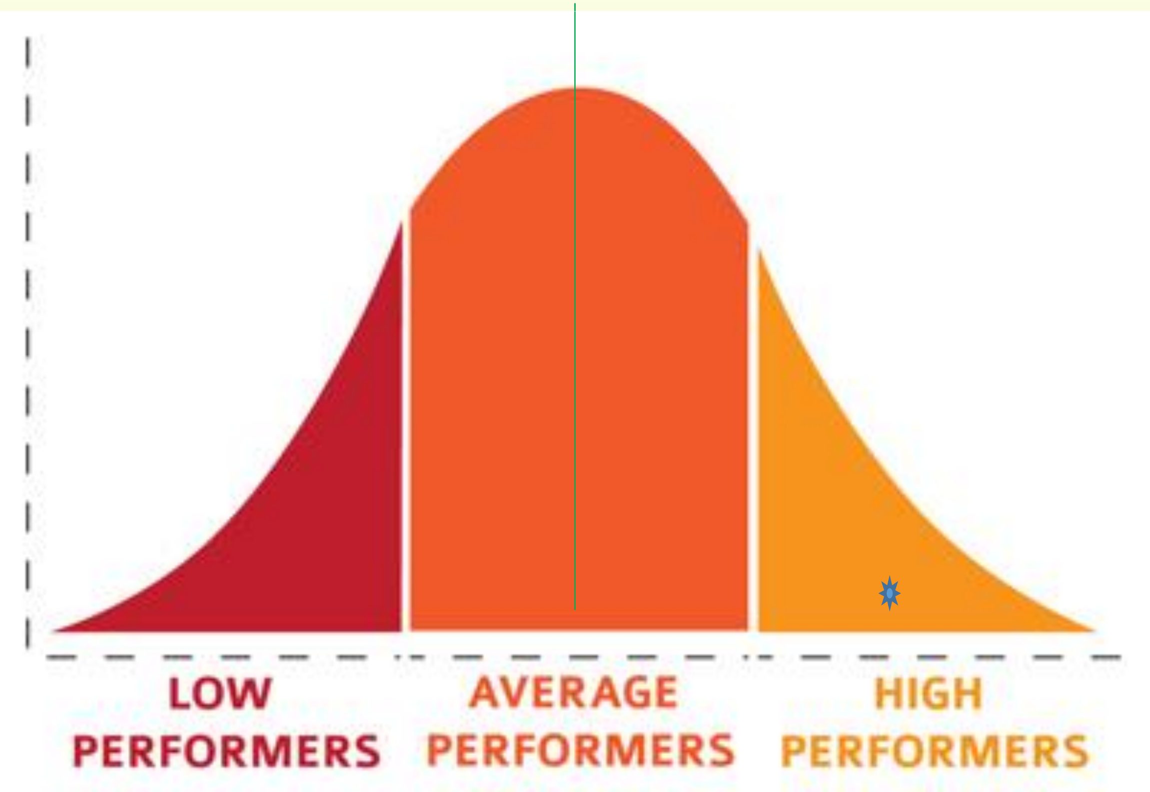
Genus	*species*
• *Abies*	6
• *Larix*	6
• *Picea*	10
• *Pinus*	15*
• other	6

* Klimaszewska et al,
Tree and Forestry Science and Biotechnology ©2007 Global Science Books

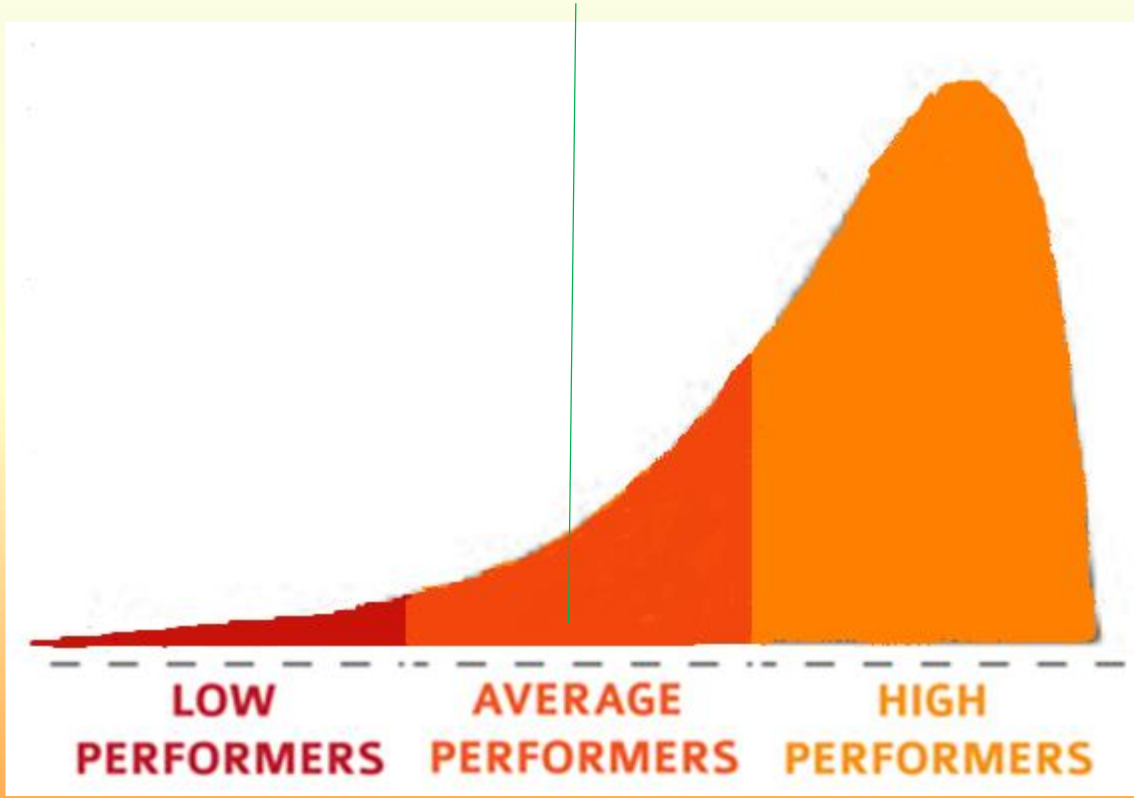
Claudio Stasolla, Lisheng Kong, Edward C. Yeung and Trevor A. Thorpe
Source: In Vitro Cellular & Developmental Biology.
Plant, Vol. 38, No. 2 (Mar. - Apr., 2002),pp. 93-105

Why SE ?

- A method of cloning - make copies of one starting plant



Plant seeds



Plant clonal SE

Why SE ?

- Crop uniformity
- Embryos can be cryo-preserved for future use
- A vehicle for genetic engineering

Steps of the conifer SE process

Sterile *in vitro* culture

• Initiation	establish cultures of immature SE
• Proliferation	make millions of immature SE
• Maturation	cause the SE to mature into plantlets
• Acclimatization	adapt the plantlets to soil and *ex vitro* conditions
• Deployment	plant in field

Steps of the conifer SE process

In vitro culture

• Initiation	establish cultures of immature SE
• Proliferation	make millions of immature SE
[cryopreservation]	
• Maturation	cause the SE to mature into plantlets
• Acclimatization	adapt the plantlets to soil and *ex vitro* conditions
• Deployment	plant in field

Molecular Tree Breeding lab

North Carolina State University

Frampton lab - *Abies fraseri* SE accomplishments

• Initiation	establish cultures of immature SE
• Proliferation [cryopreservation]	make millions of immature SE
• Maturation	cause the SE to mature into plantlets
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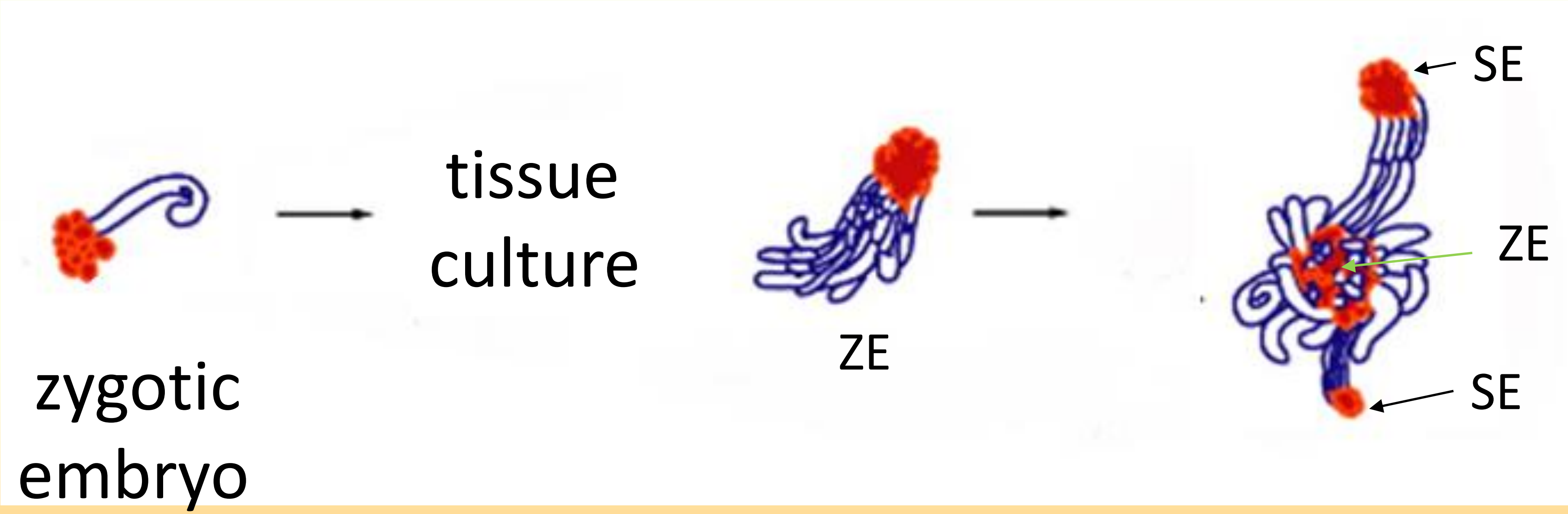
Initiation of SE cultures

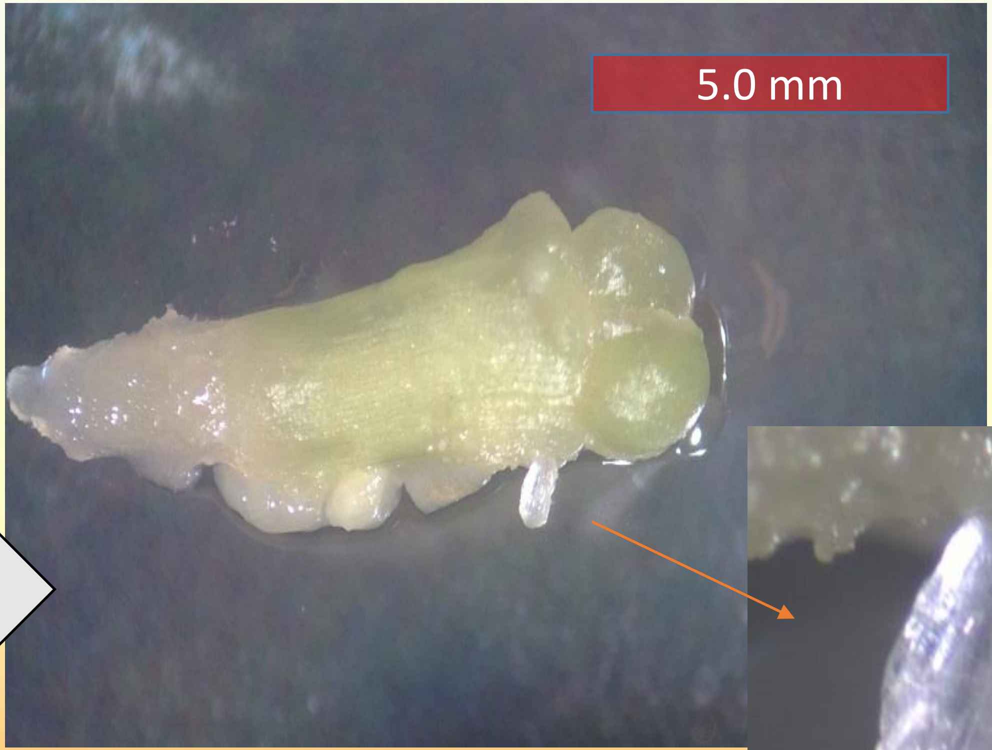
Somatic Embryogenesis



SE Where does it begin?

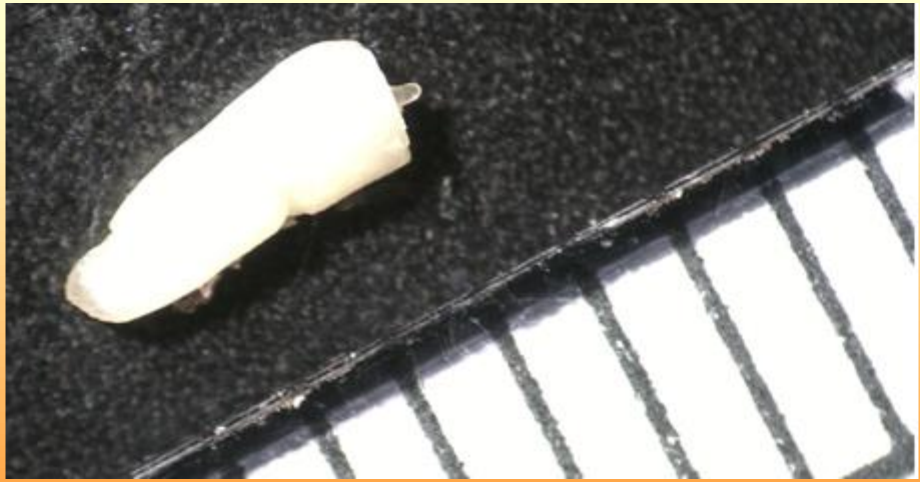
Mature zygotic embryo, stored 6 years, -20^{o}C

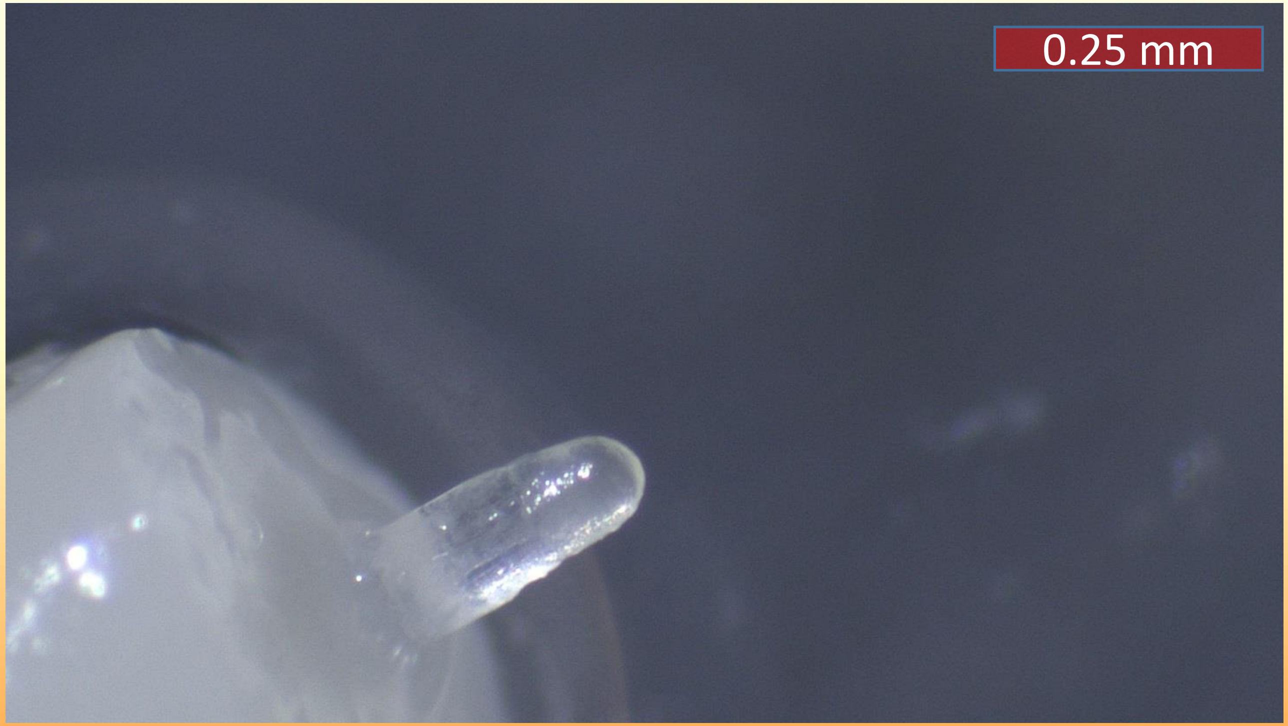
~ 3 weeks



SE   Where does it begin?

Immature zygotic embryo,
~ 30 post pollination



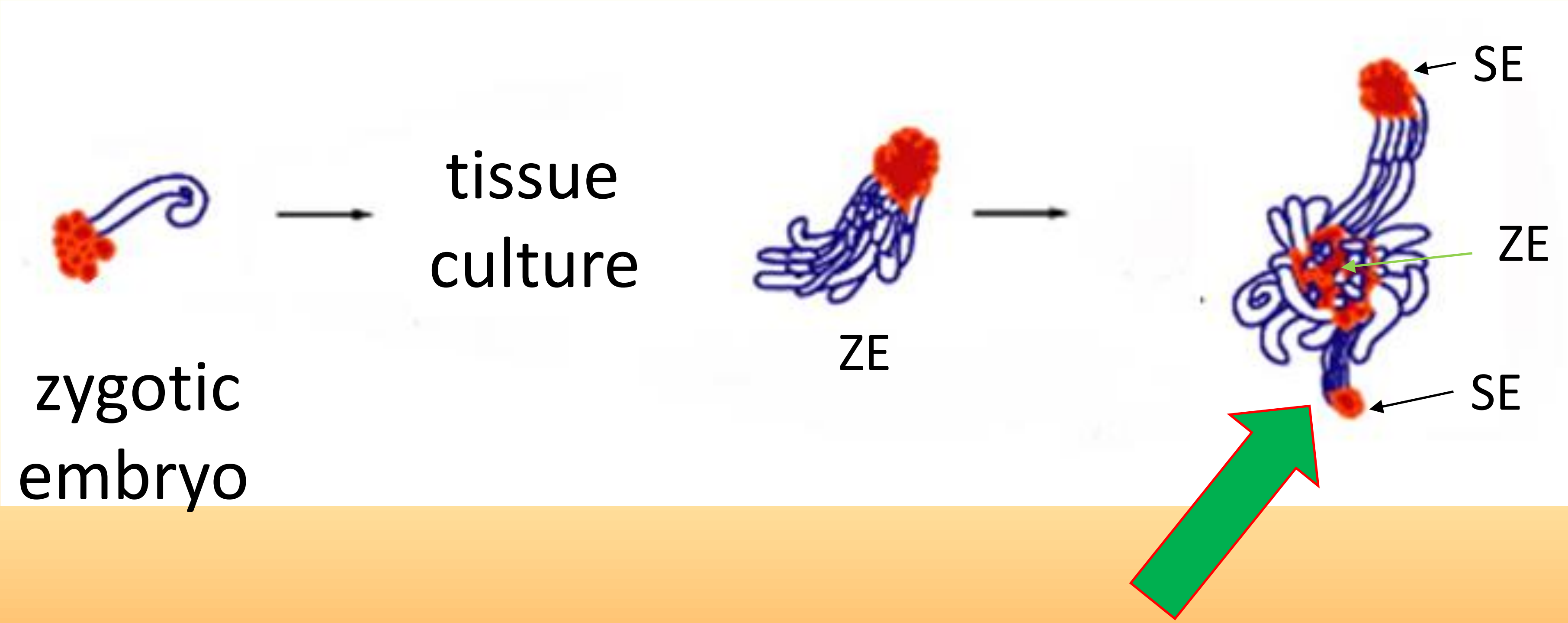


2016 SE Initiation Totals

zygotic embryo stage	# processed	# embryogenic responses	%
immature	**4177**	**323**	**7.7**
mature	**1991**	**129**	**6.5**

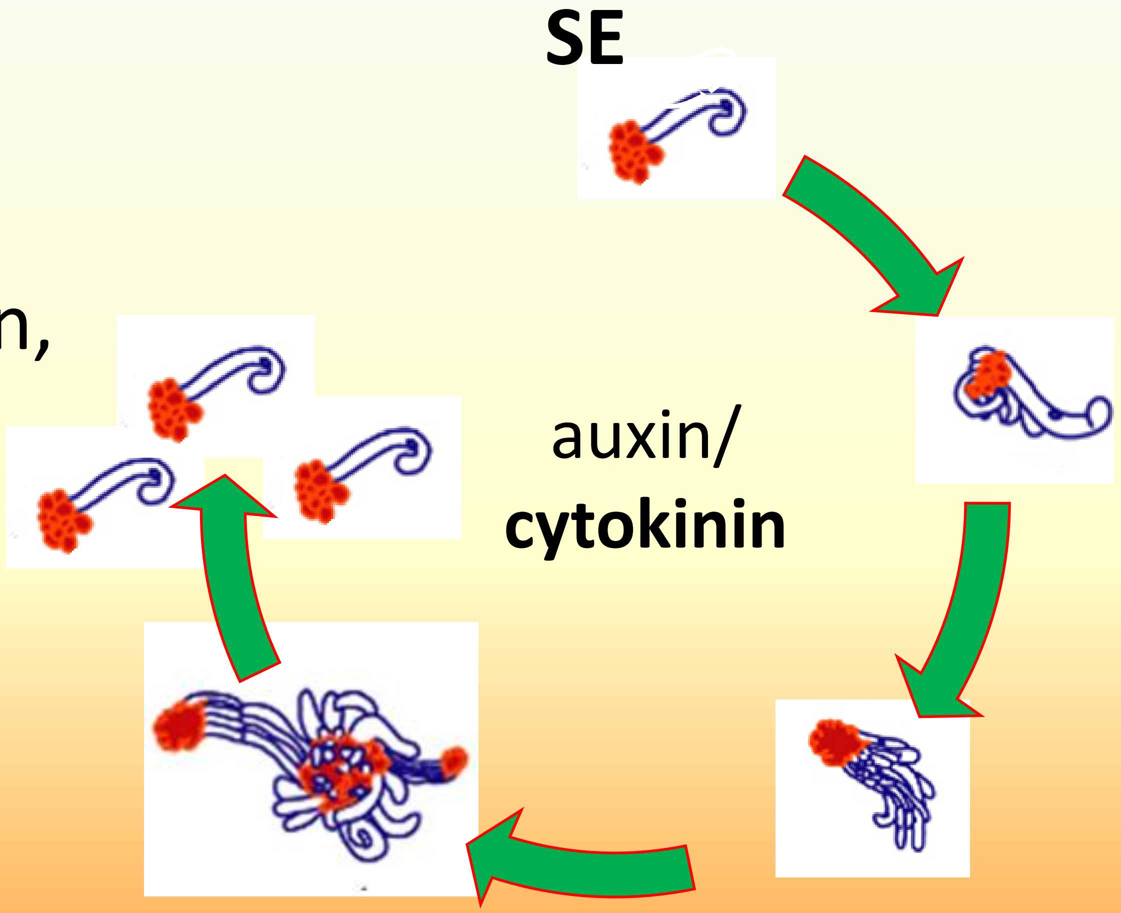
Proliferation of SE cultures

Somatic Embryogenesis



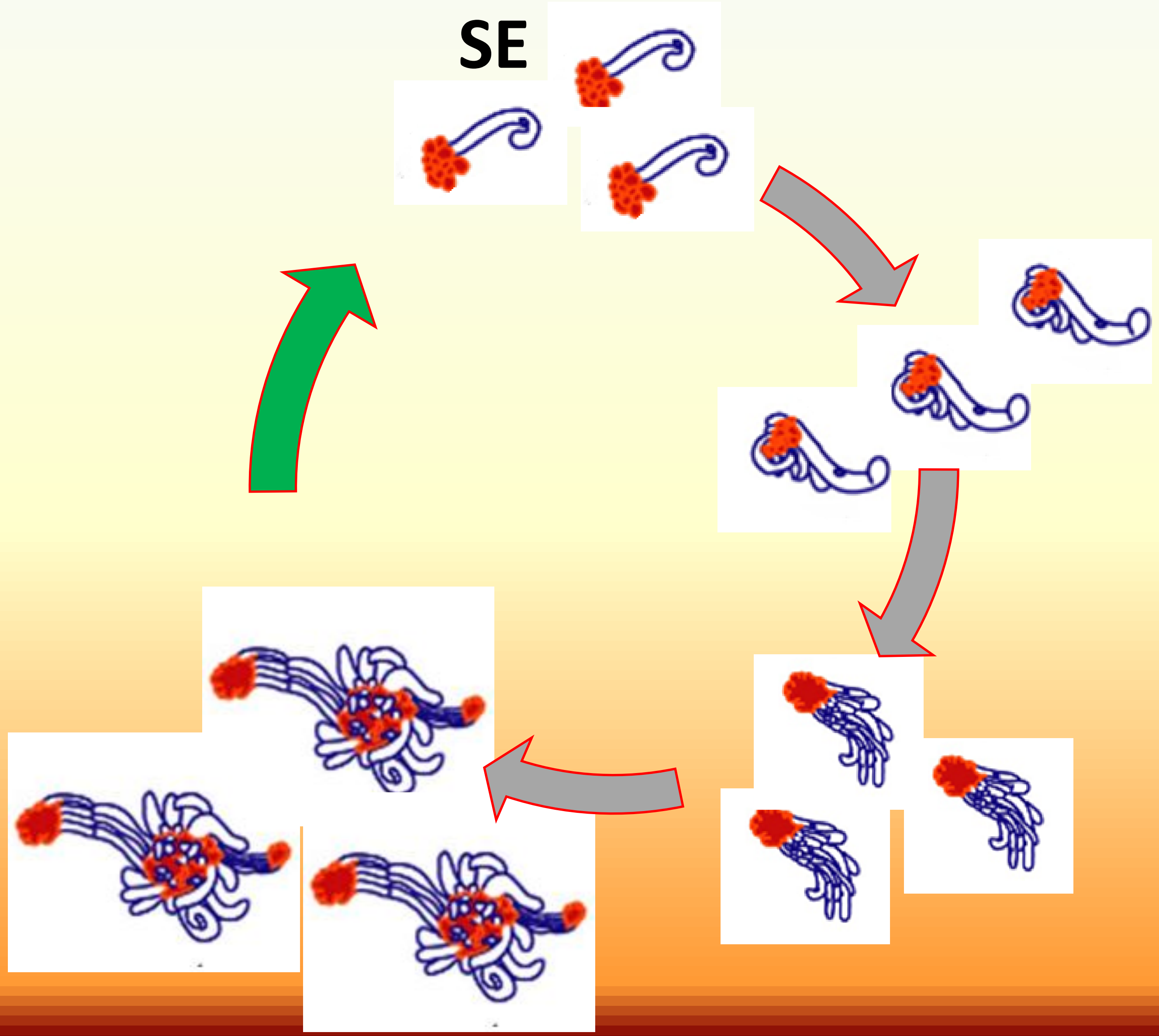
SE proliferate via cleavage of the embryo head

- Most conifer SE cultures are proliferated using auxin:cytokinin, often in a 2:1 ratio.
- *Abies* SE cultures require **only cytokinin**



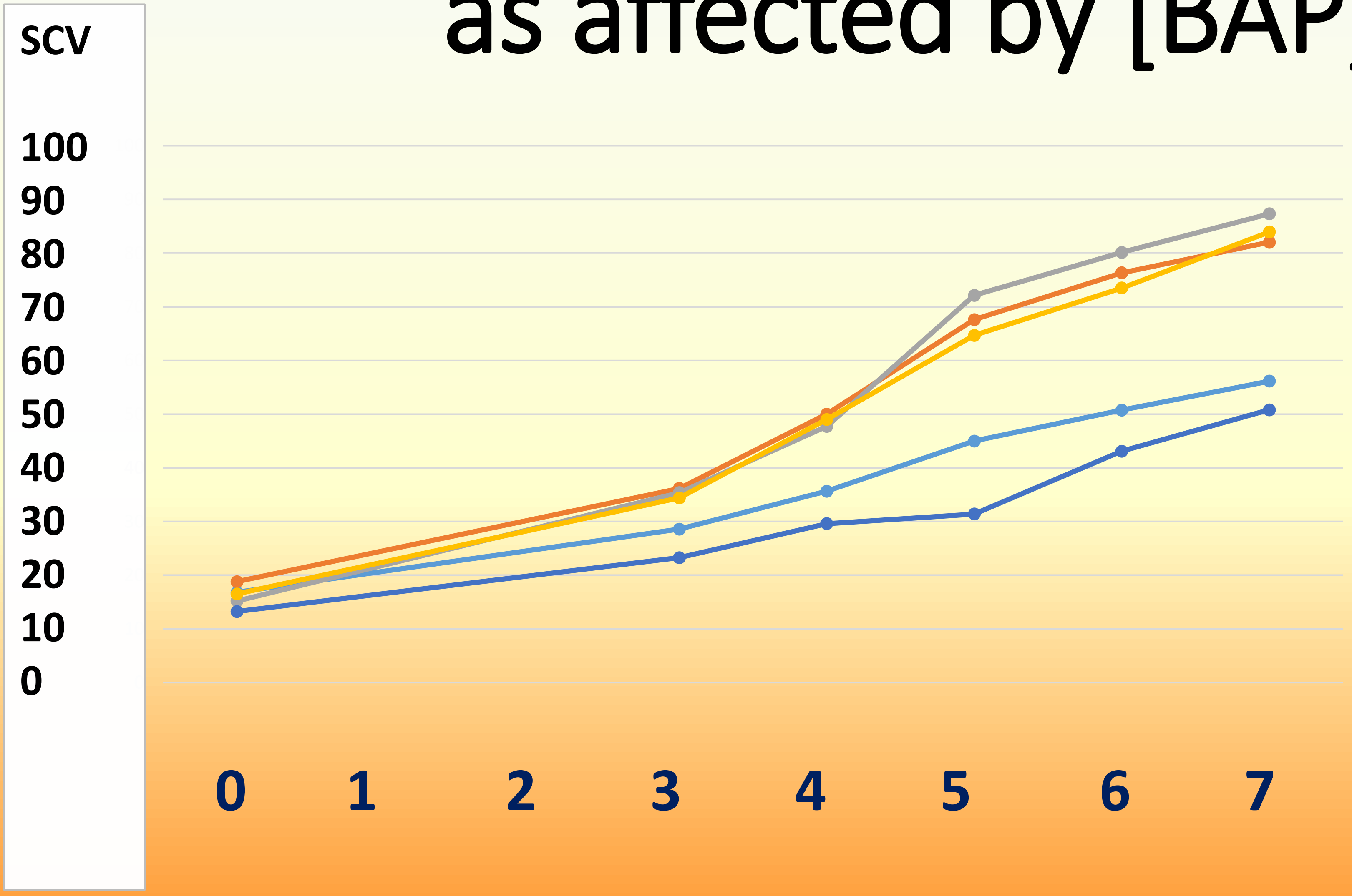
SE proliferate via cleavage of the embryo head

- **Millions of copies from 1 starting embryo**



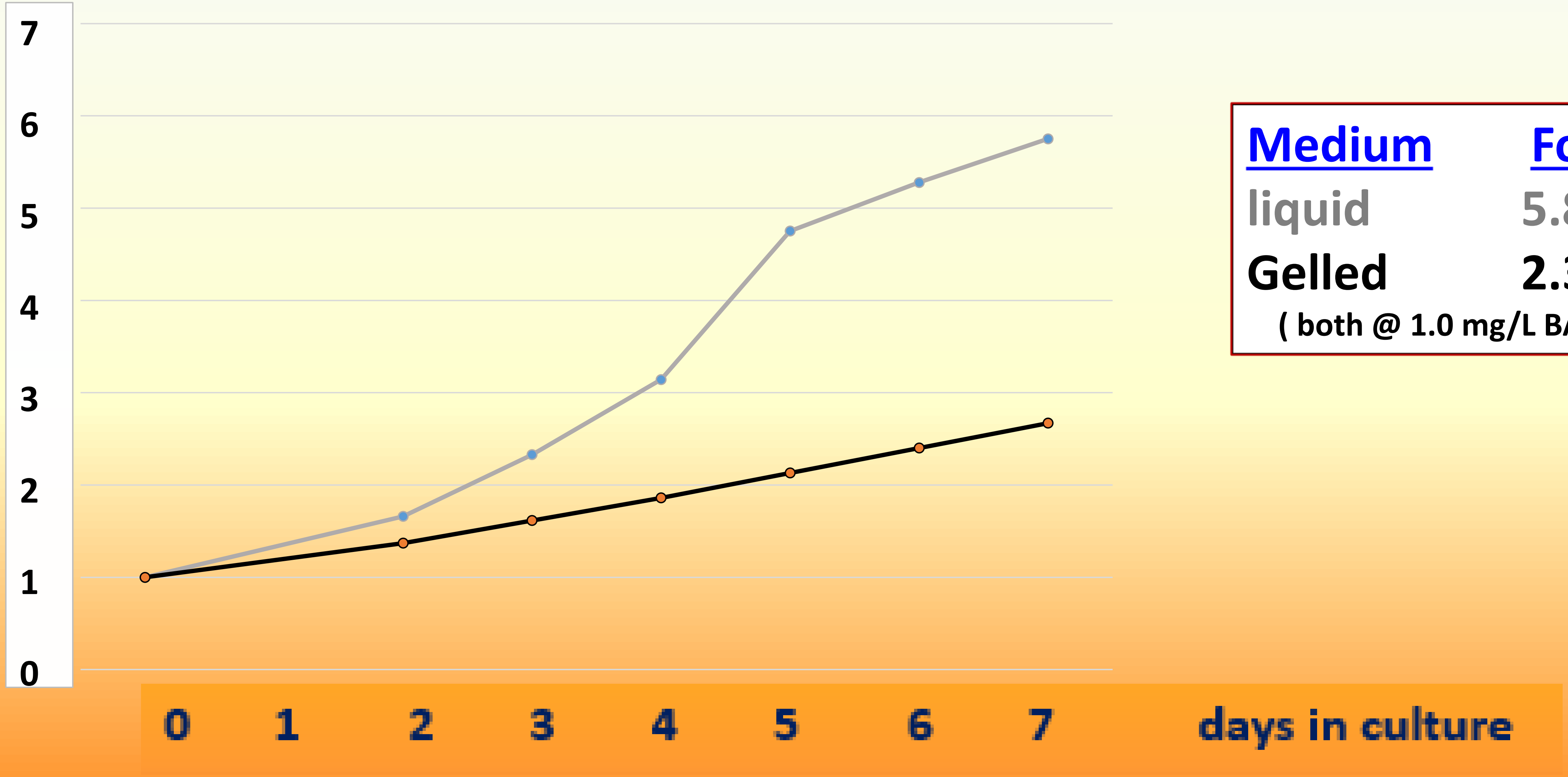
Proliferation with gelled or liquid media

Liquid proliferation rates as affected by [BAP]



mg/L BAP	fold
0	3.3x
0.3	4.4x
1.0	5.8x
3.0	5.1x
9.0	3.9x

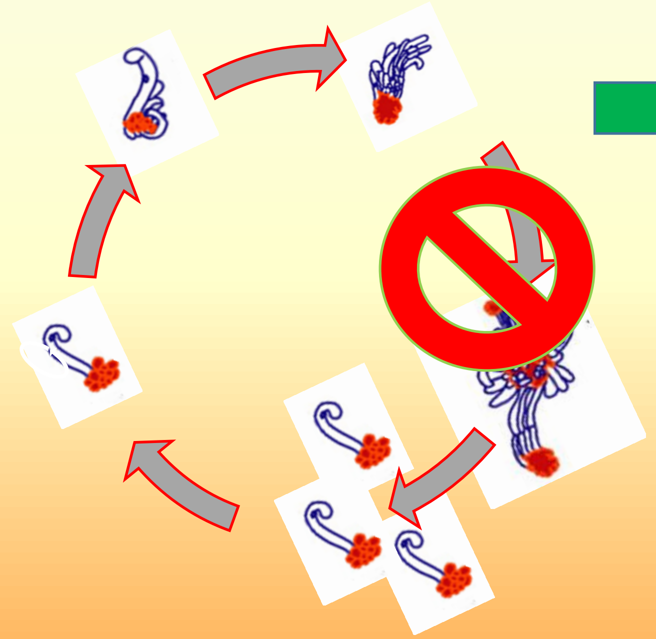
Liquid vs. gelled proliferation rates

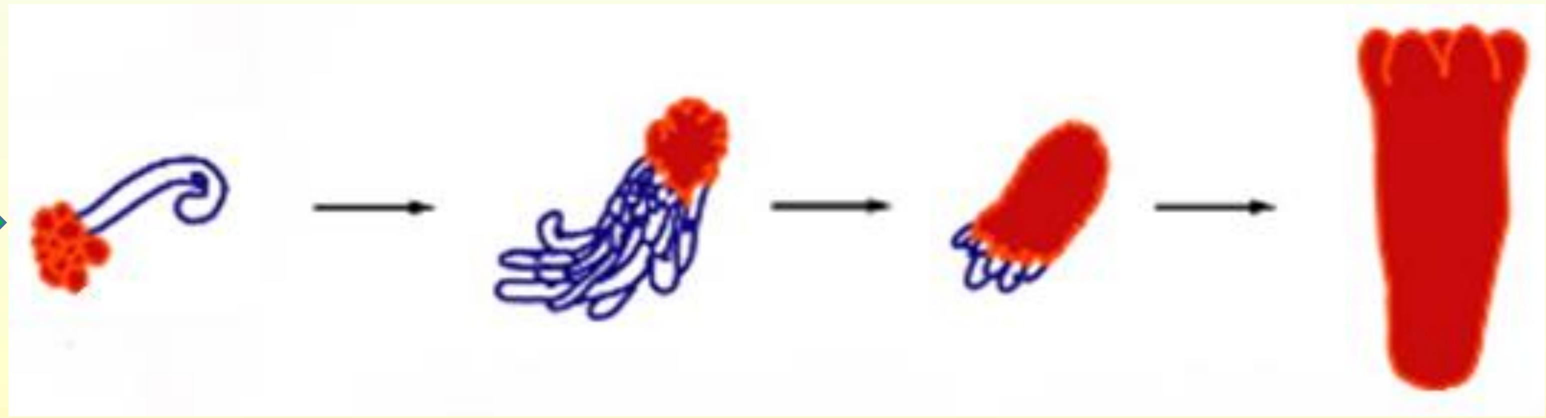


Medium	Fold
liquid	5.8x
Gelled	2.3x

(both @ 1.0 mg/L BAP)

SE Maturation





#1 - Remove auxin & cytokinin

SE Maturation #2-4….

3 main components often used to achieve conifer SE maturation

- ABA — signal to accumulate storage products, developmental paths activated
- Maltose — possible developmental signal, osmoticant
- PEG — osmoticant, possible developmental signal

Concentrations of ABA, Maltose & PEG vary greatly across conifer species

species	*Pinus caribaea*	*Pinus taeda*	*Abies cilicica × cephalonica*	*Abies fraseri*	*Abies alba*	*Abies nordmanniana*
year	2009	2003	2011	2016	2011	2002
mg/L ABA	31.7	5.3	10	5	5.3	10.6
g/L Maltose	64	20	40	40	40	45
g/L PEG	0	120	100	100	37.5	50

Design ABA, PEG & maltose “grids”

0 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG				
66 g/L PEG				
99 g/L PEG				

4 x 4 x 4 factorial = 64 combinations

0 g/L maltose

	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG				
66 g/L PEG				
99 g/L PEG				

40 g/L maltose

	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG				
66 g/L PEG				
99 g/L PEG				

20 g/L maltose

	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG				
66 g/L PEG				
99 g/L PEG				

60 g/L maltose

	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG				
66 g/L PEG				
99 g/L PEG				

Maturation test parameters

- Place ~ 200 mg tissue in a petri dish with 20 ml gelled medium
- 5 replicate plates per each of 64 treatments, and 1 control treatment
- Cultures are weighed at 0, 3, 6, 9 & 12 weeks
- Cotyledonary stage embryos are counted at weeks 6, 9 & 12.
- Tissue from 8 different genotypes tested

Cotyledonary Embryo Production

Family	Line	# cot. E produced
11	33	0
24	42	0
24	46	0
24	50	0
24	51	0
51	19	0
51	23	369
62	07	0

51-0023 average cotyledonary embryo / plate

0 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG				
66 g/L PEG				
99 g/L PEG				

40 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG	0.6	0.1	0.6	0.6
66 g/L PEG	12.9	1.9	0.1	0.8
99 g/L PEG	3.6			

20 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG			0.2	0.1
66 g/L PEG	1.7	0.1	1.0	
99 g/L PEG	3.0			

60 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				0.1
33 g/L PEG	1.8	1		4.0
66 g/L PEG	10.0	2.0		
99 g/L PEG	0.5			

51-0023 total production - trends

- 0 maltose - no embryo production
- Majority of embryos produced with 66 g/L PEG
- 40 g/L maltose $\geq$ 60 g/L maltose
- Majority of embryos produced with 0 µM ABA

0 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG				
66 g/L PEG				
99 g/L PEG				

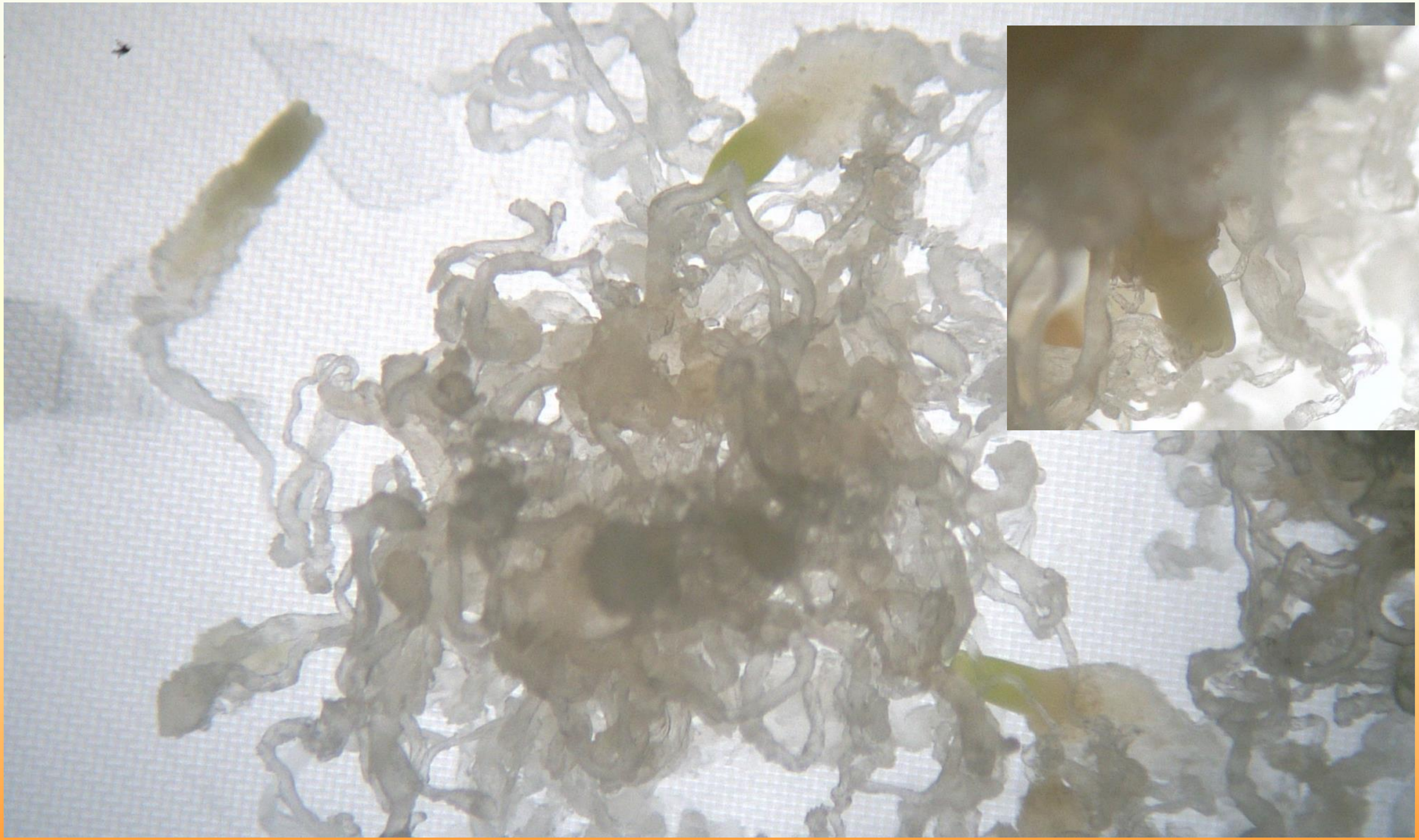
40 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG	0.6	0.1	0.6	0.6
66 g/L PEG	12.9	1.9	0.1	0.8
99 g/L PEG	3.6			

20 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				
33 g/L PEG			0.2	0.1
66 g/L PEG	1.7	0.1	1.0	
99 g/L PEG	3.0			

60 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG				0.1
33 g/L PEG	1.8	1		4.0
66 g/L PEG	10.0	2.0		
99 g/L PEG	0.5			

???

Fresh Weight Gain during Maturation



Fresh Weight Gain during Maturation

in culture for 12 weeks, number shown = total fold growth

0 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	1.2	0.7	0.6	1.0
33 g/L PEG	.7	0.7	0.8	0.7
66 g/L PEG	.7	0.7	0.7	0.8
99 g/L PEG	.6	0.6	0.3	0.6

40 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	31.9	25	8.9	29.3
33 g/L PEG	29.9	28.2	30.1	25.2
66 g/L PEG	18.2	19.9	22.3	21.5
99 g/L PEG	42.3	6.8	9.4	6.5

20 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	14.9	11.7	10	8
33 g/L PEG	15.5	10.8	8.5	8.8
66 g/L PEG	13.4	15.3	9.9	9.2
99 g/L PEG	11.8	9.7	23.6	8.8

60 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	45.9	51.8	44.9	36.5
33 g/L PEG	49.8	28.2	27.7	21.1
66 g/L PEG	13.1	15.3	6.9	6.3
99 g/L PEG	12.9	5.6	2.7	2.7

control: 4.4 uM BAP | 10 sucrose | 0 uM ABA | 0 g/L PEG

5.6 x @ 12 weeks

(9.3 x @ 6 weeks)

Next Steps……

Yue Ken Liao Æ Cherng-Kang Liao Æ
Ya Ling Ho

Plant Cell Tiss Organ Cult (2008) 93:257–268

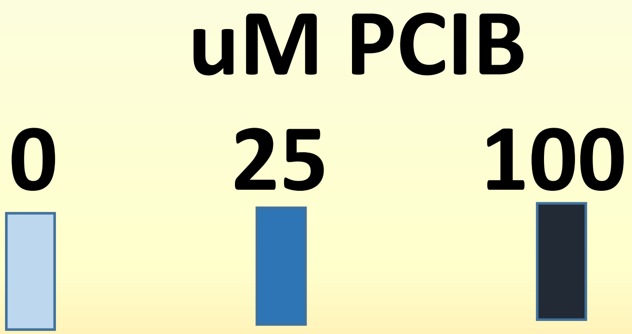
species of *L. decidua* Mill., the embryogenic cultures will keep proliferating, rather than switching to a developmental phase, if the cultures contain a high amount of IAA (Korlach and Zoglauer 1995). It is

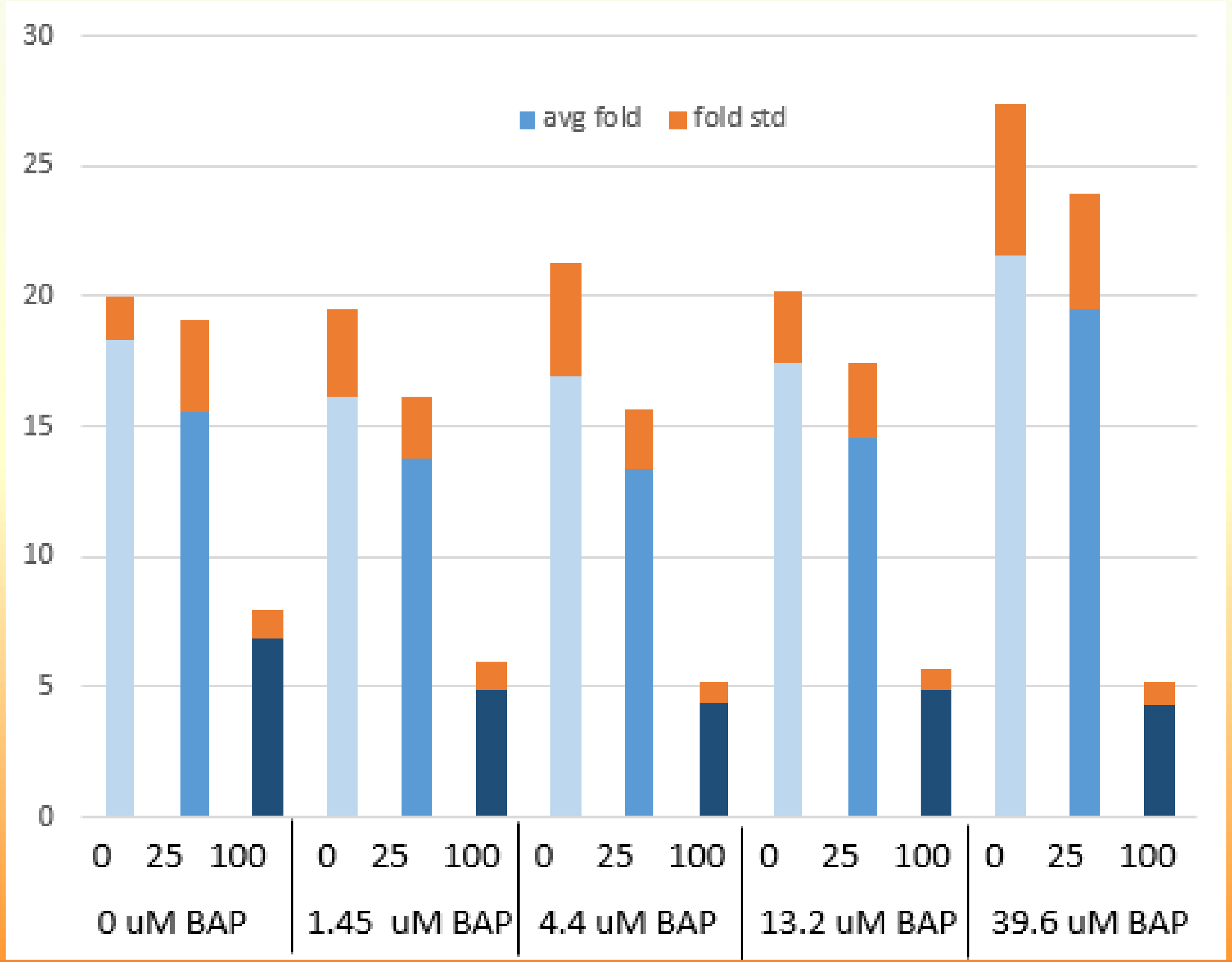
PHYSIOLOGIA PLANTARUM 116: 231–237. 2002 ISSN 0031-9317

Effect of anti-auxins on maturation of embryogenic tissue cultures of Nordmanns fir (*Abies nordmanniana*)

Inclusion of PCIB into the maturation medium had two general effects: (1) proliferation was reduced, and (2) the number of embryos that converted from proliferation to maturation was significantly increased. However, both

PCIB slows *Abies fraseri* proliferation





USDA

NC Christmas Tree Association

Molecular Tree Breeding Lab

DEPARTMENT of FORESTRY and ENVIRONMENTAL RESOURCES
NC STATE UNIVERSITY

Specialty Crop Block Grant Program
Administered by the North Carolina Department of Agriculture & Consumer Services

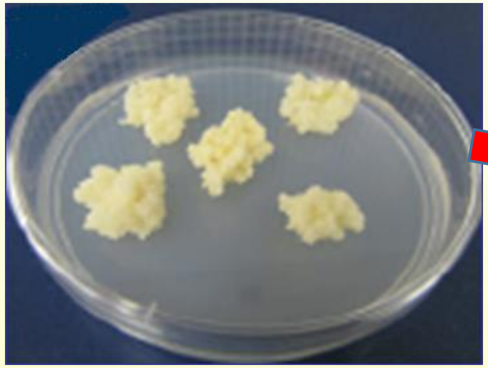
People in Denmark is currently working in SE in Normand fir!!!!!!

They have 400 clones total in 6 trials – 8000 ramets in total planted autumn 2014 and 2015

Ideal Fraser fir SE Process

1) **Pick your favorite tree**

2) **Establish tissue cultures**



3) **Cryopreserve sample**

4 & 5) **Convert embryos into seedlings**

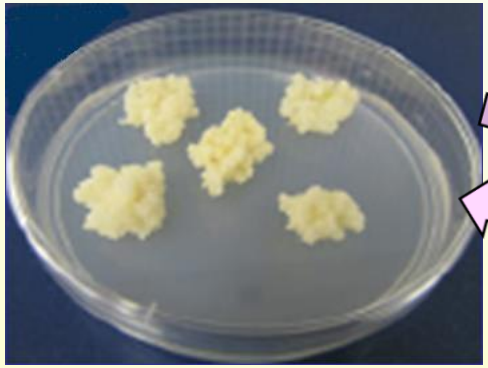
6) **Fill greenhouse with 1000's of clones, <u>exact copies</u> of your favorite tree**

Ideal Fraser fir SE Process

1) **Select an elite tree**

2) **Initiate embryogenic tissue culture**

3) **Multiple embryos**



Cryopreserve sample

4) **Mature embryos into seedlings**

5) **Acclimatize in greenhouse**

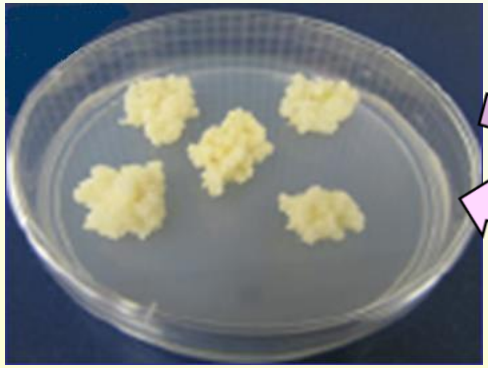
6) **Plant 1000's of copies of your favorite tree**

Ideal Fraser fir SE Process

1) **Select** your favorite tree

2) **Initiate** embryogenic tissue culture

3) **Multiple** embryos



Cryopreserve sample

4) **Mature** embryos into seedlings

5) **Acclimatize** in greenhouse

6) **Plant** 1000's of copies of your favorite tree

1) Select your favorite trees

Maturation weight trends

Increasing maltose increases F.W.T. gain

Increasing PEG decreases F.W.T. gain

0 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	1.2	0.7	0.6	1.0
33 g/L PEG	.7	0.7	0.8	0.7
66 g/L PEG	.7	0.7	0.7	0.8
99 g/L PEG	.6	0.6	0.3	0.6

40 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	31.9	25	8.9	29.3
33 g/L PEG	29.9	28.2	30.1	25.2
66 g/L PEG	18.2	19.9	22.3	21.5
99 g/L PEG	42.3	6.8	9.4	6.5

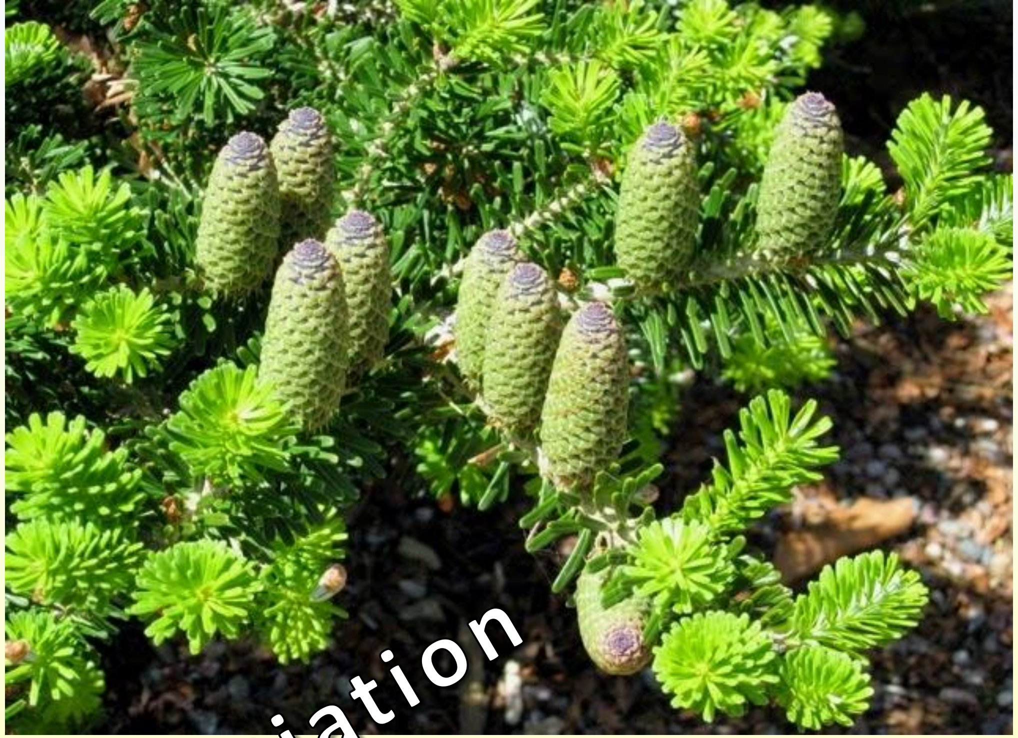
20 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	14.9	11.7	10	8
33 g/L PEG	15.5	10.8	8.5	8.8
66 g/L PEG	13.4	15.3	9.9	9.2
99 g/L PEG	11.8	9.7	23.6	8.8

60 Maltose	0 mg/L ABA	5 mg/L ABA	10 mg/L ABA	15 mg/L ABA
0 g/L PEG	45.9	51.8	44.9	36.5
33 g/L PEG	49.8	28.2	27.7	21.1
66 g/L PEG	13.1	15.3	6.9	6.3
99 g/L PEG	12.9	5.6	2.7	2.7

control:	4.4 uM BAP	10 sucrose	0 uM ABA	0 g/L PEG

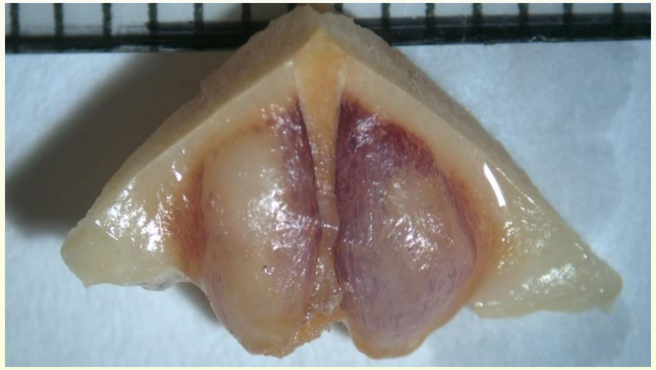
5.6 x @ 12 weeks

(9.3 x @ 6 weeks)



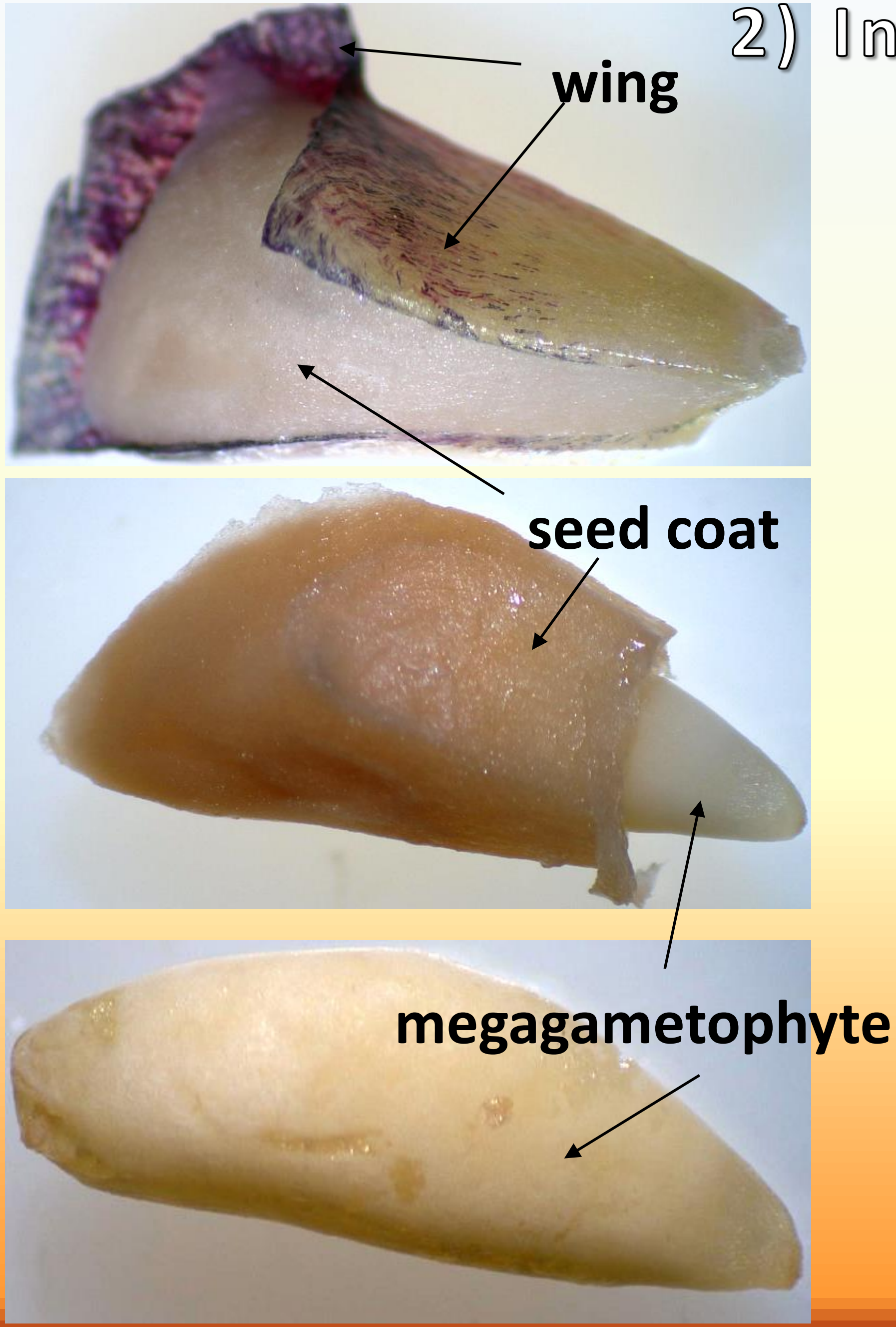
2) Initiation

Immature embryos, 2-4 weeks/year



Mature embryos year round

2) Initiation



Immature embryo

Mature embryo

2) Initiation +

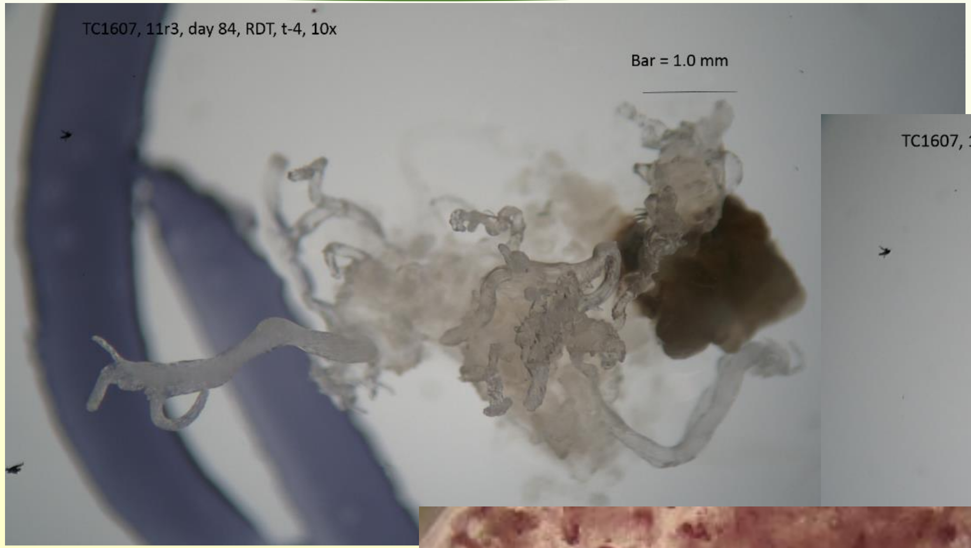
Mature embryo

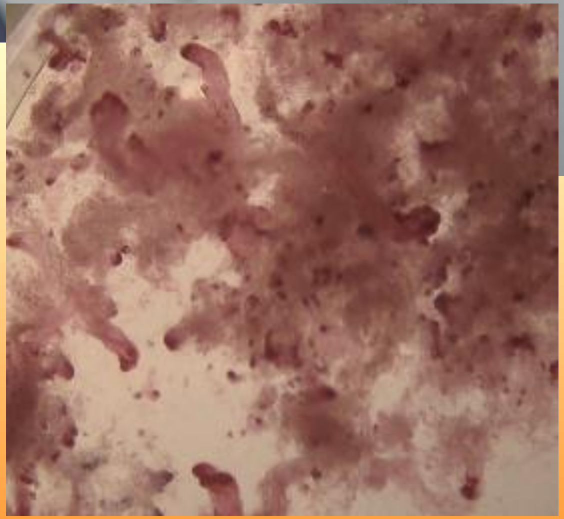
~ 3 weeks

Immature embryo

~ 10 weeks

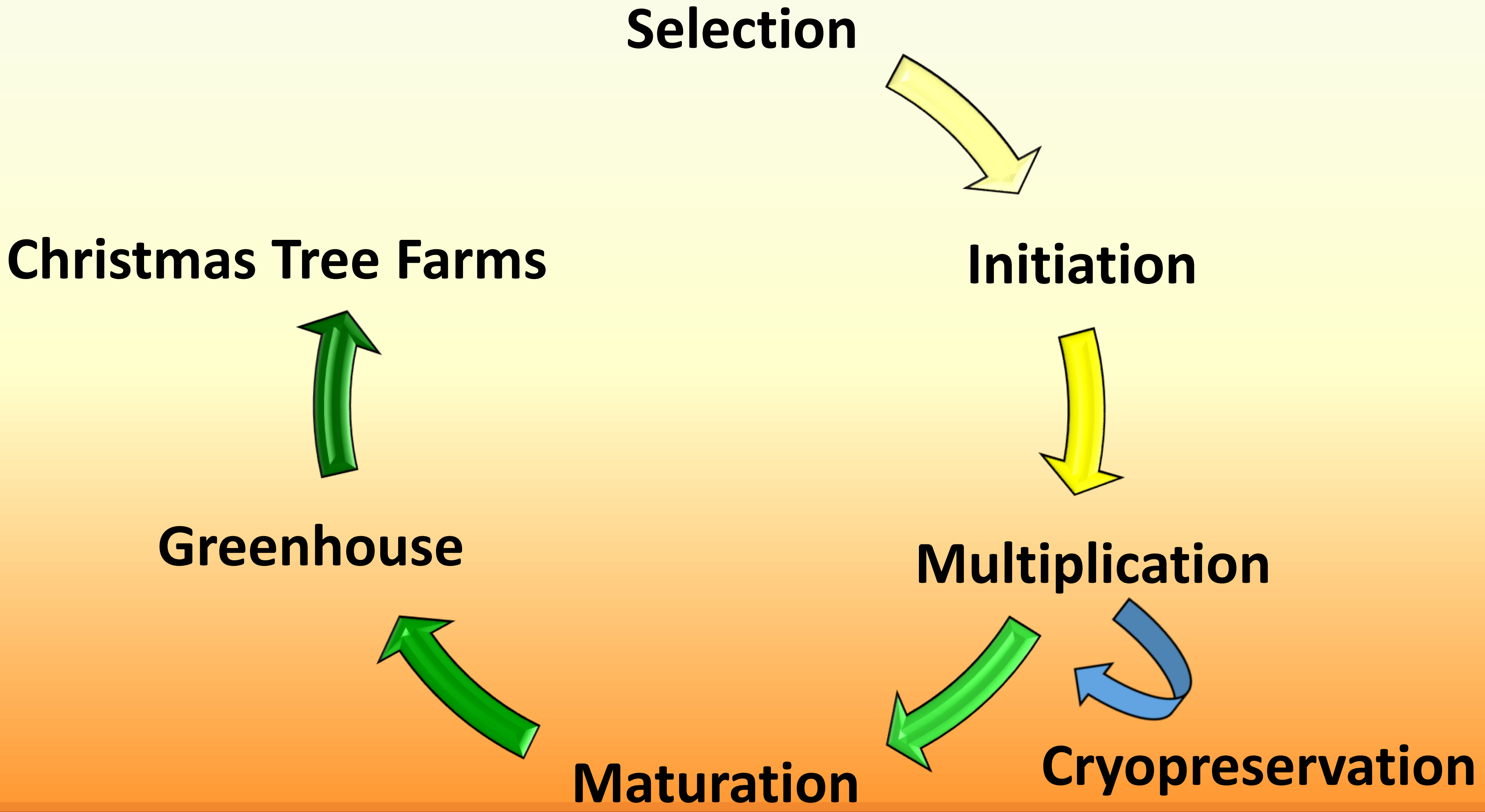
3) Multiplication



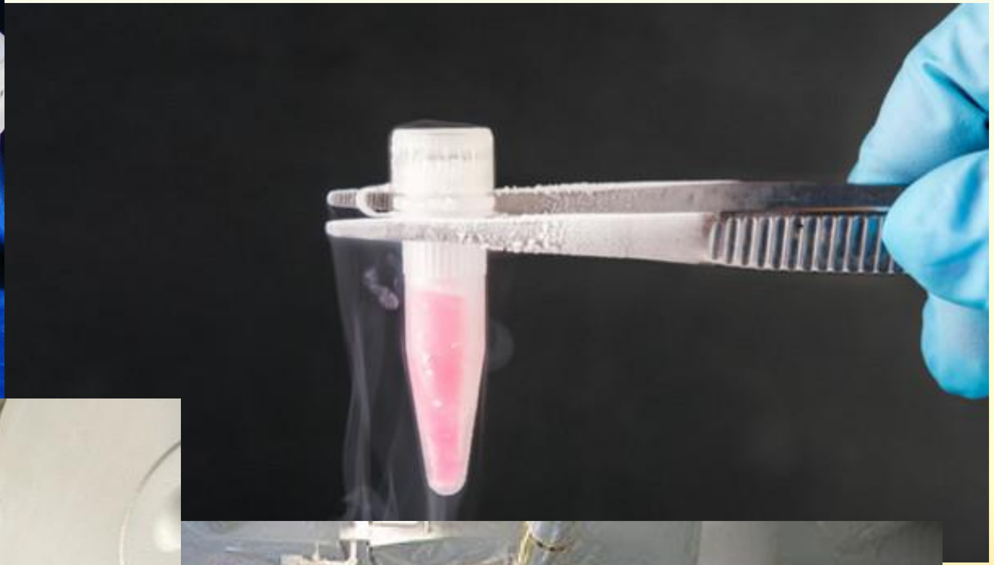


3) Multiplication

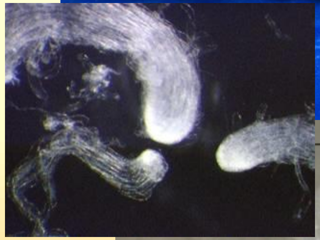
Somatic Embryogenesis Process Flow

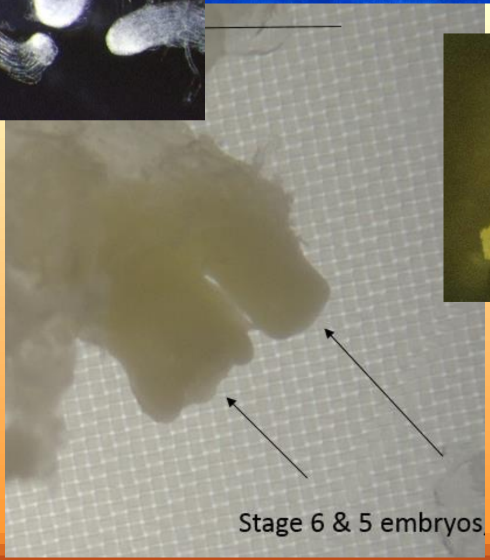


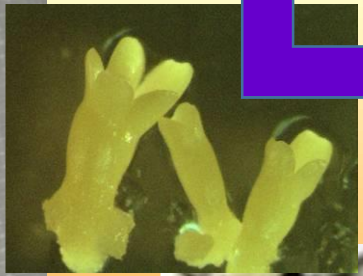
Cryopreservation



4) Maturation





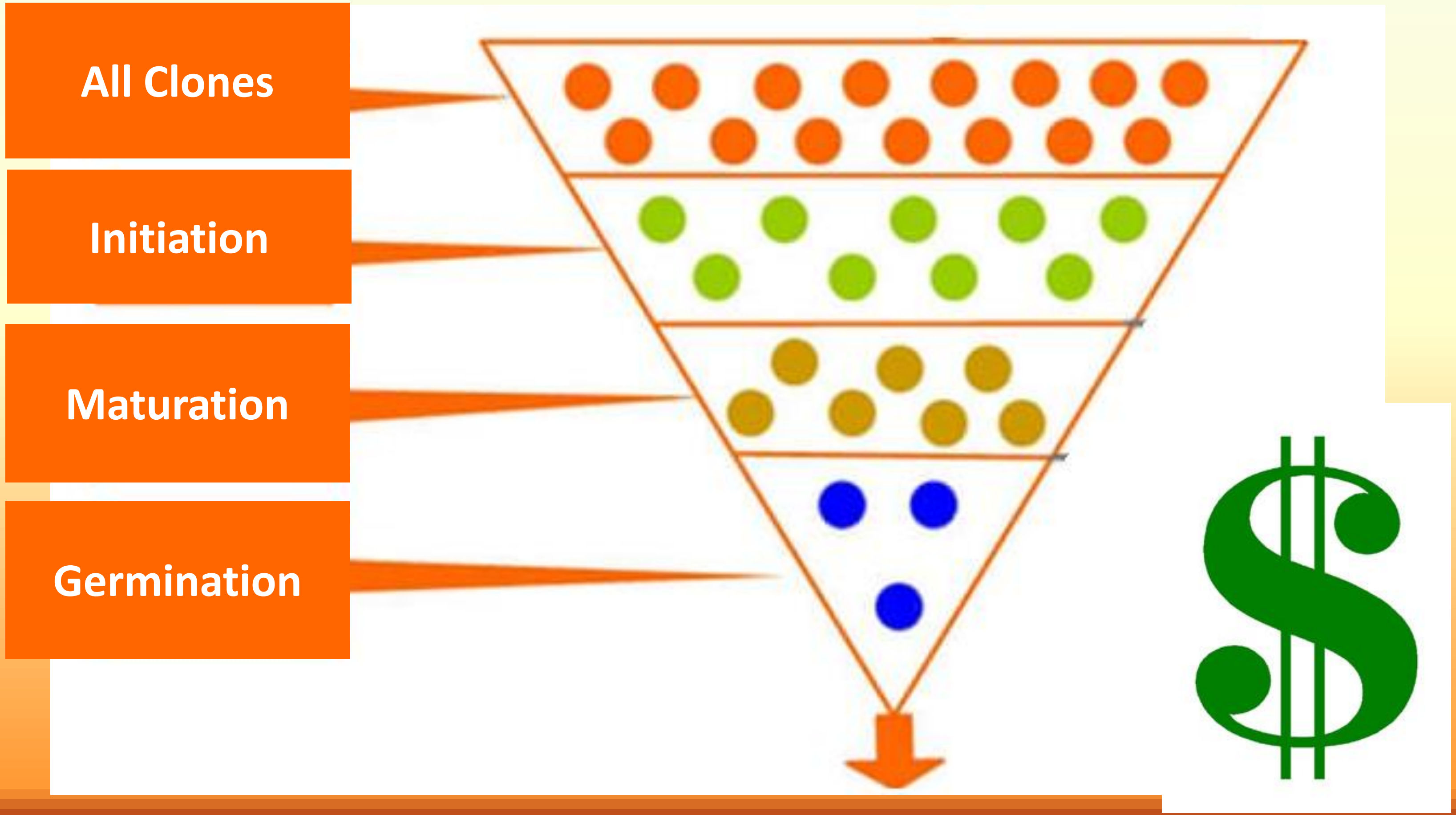


5) Acclimatization

Plant a Christmas Tree Farm

Current Challenges

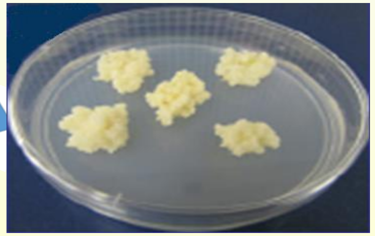
Techniques work on only some clones



Controlled Pollination

1) **Clonal Selection**

2) **Initiation**



Embryo Rescue

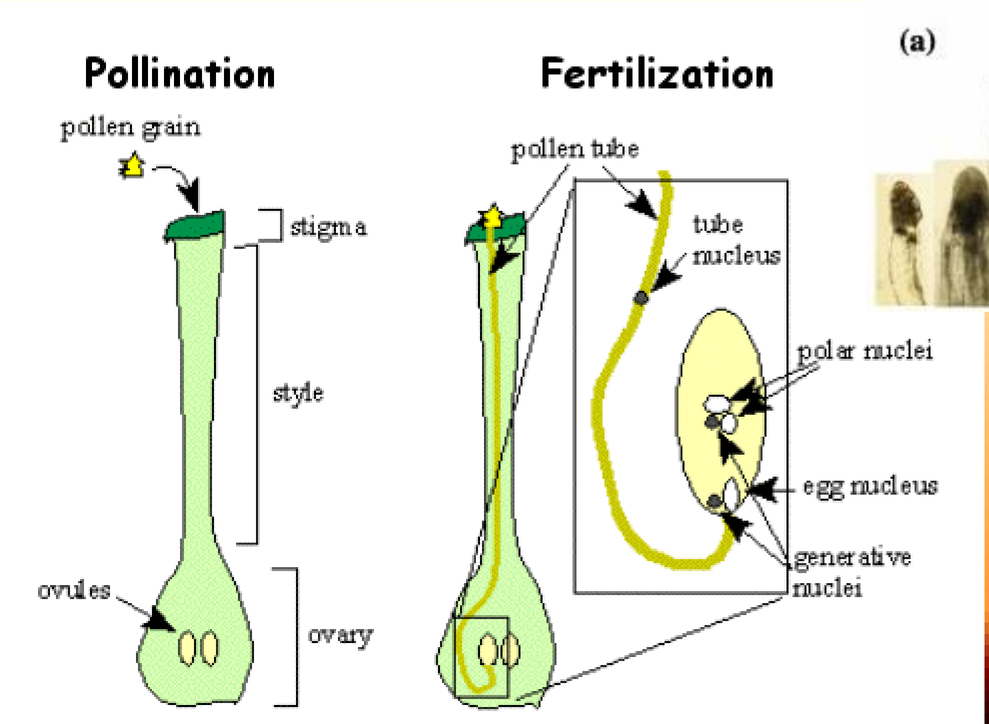
Abies fraseri

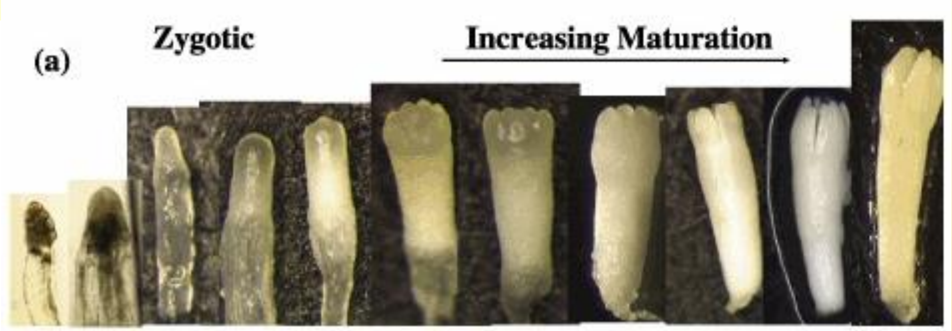
Abies firma

Cross 2 different species & get ~no viable seed

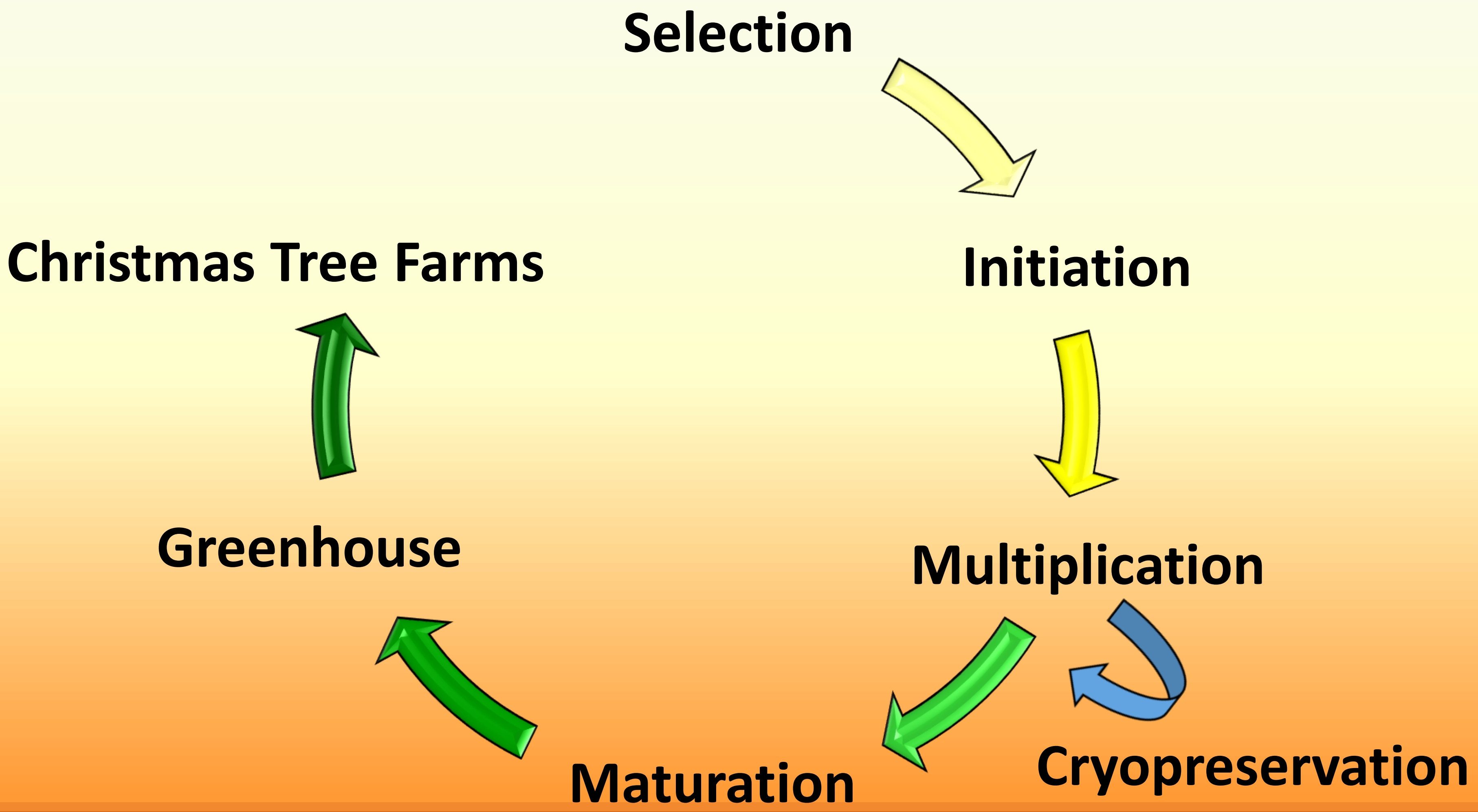
Interspecific hybrids

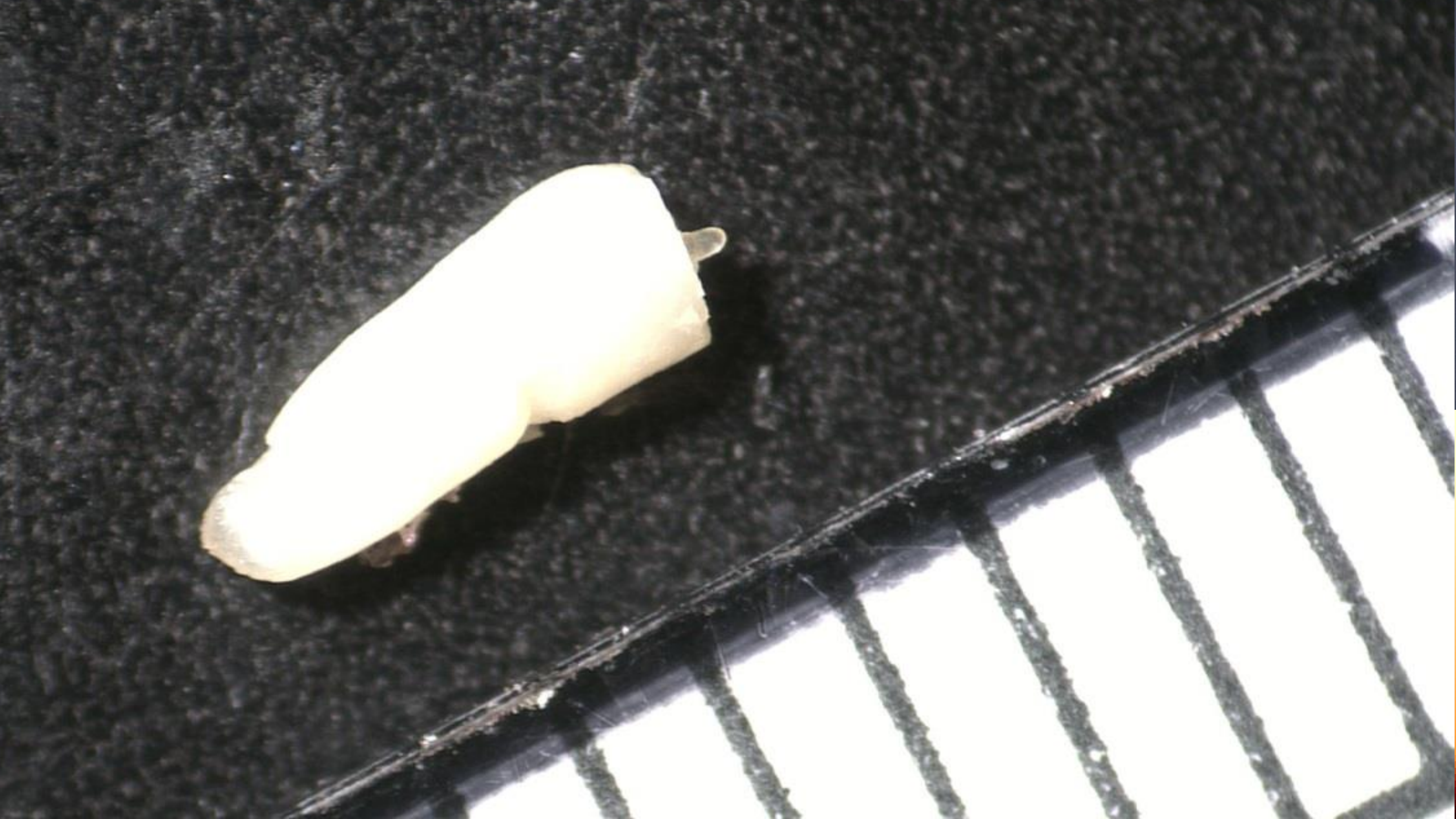
Interspecific hybrids are bred by <u>mating two species</u>, normally from within the same genus. The <u>offspring display traits of **both** parents</u>, but <u>are often sterile</u> .

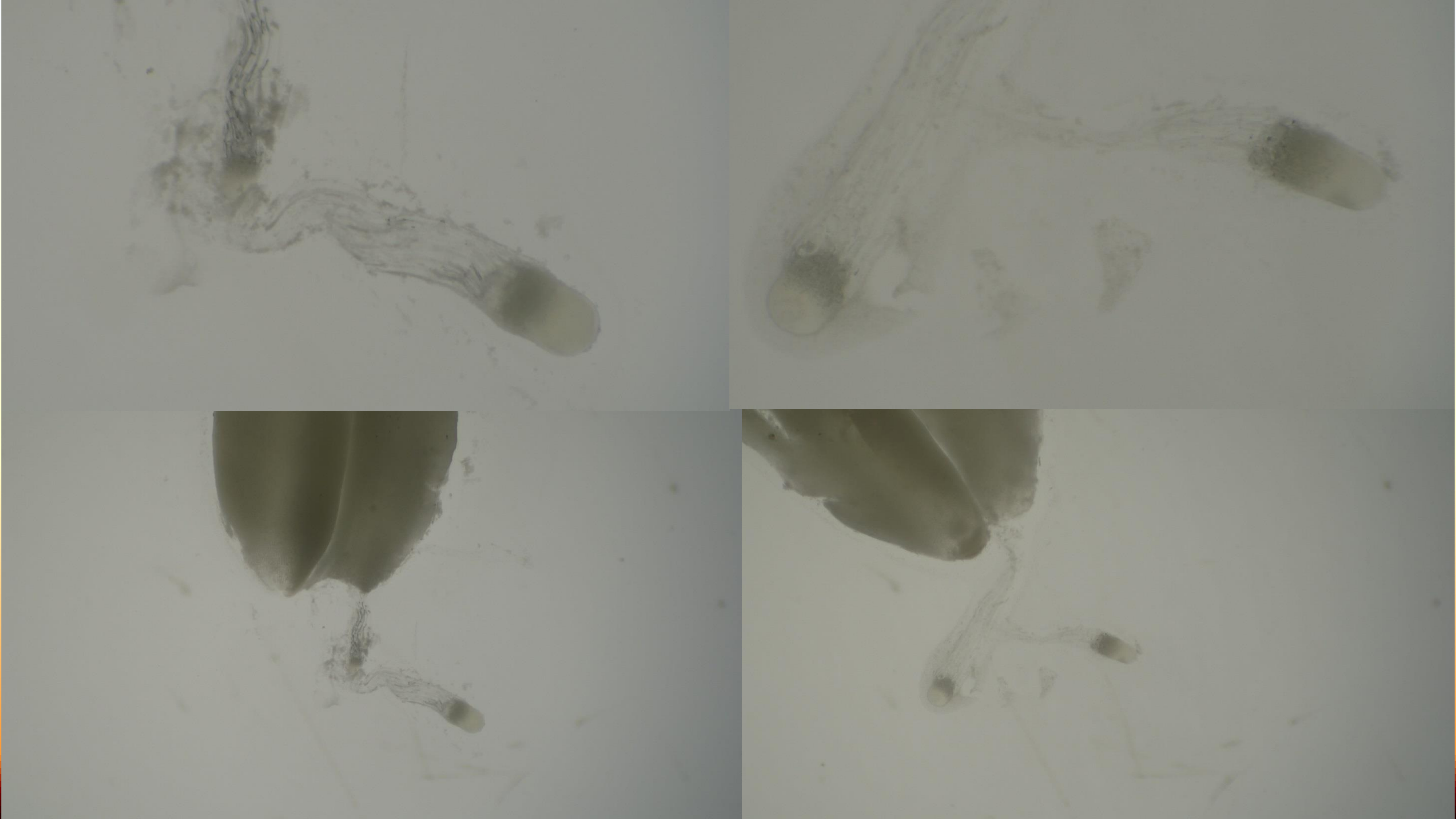




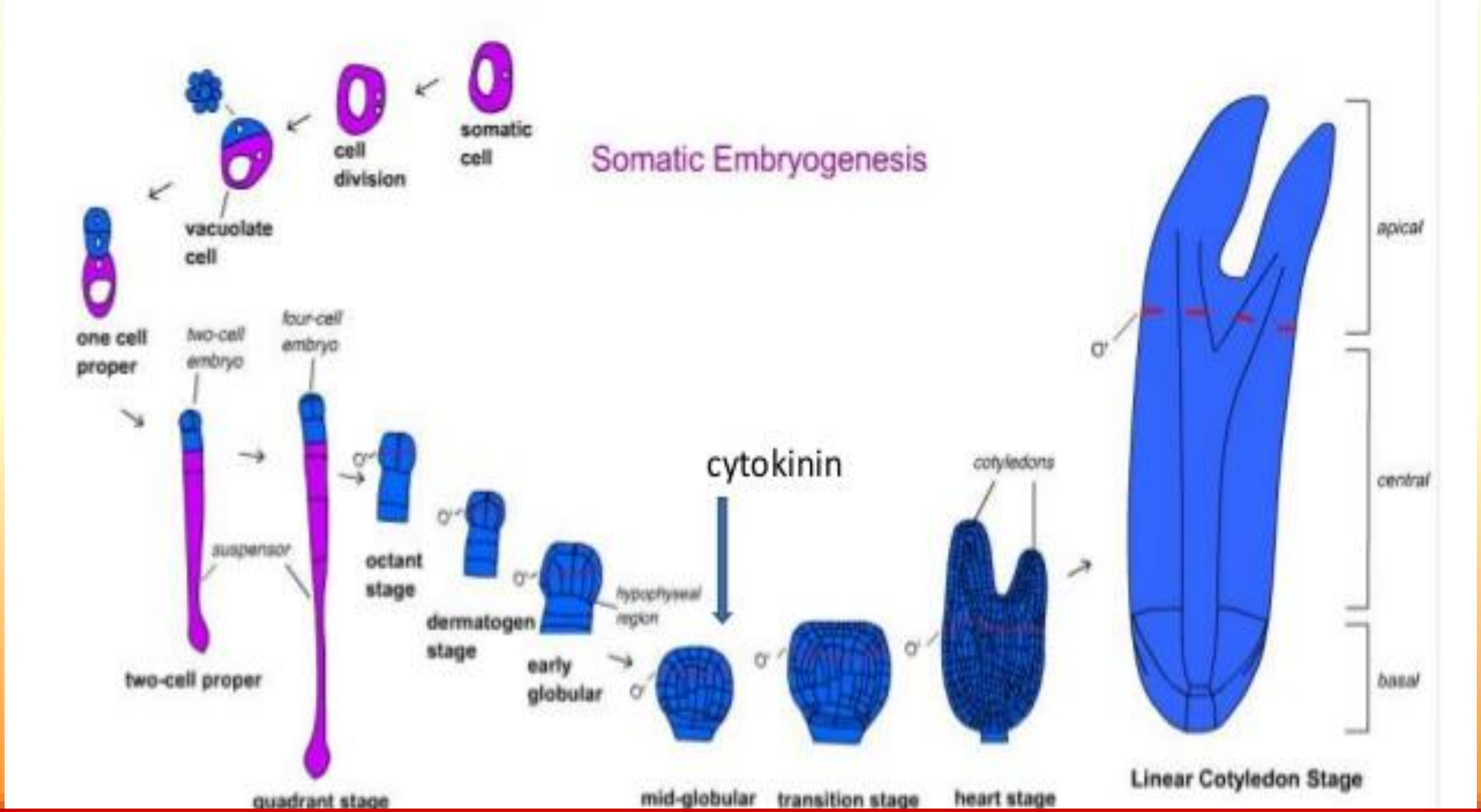
Somatic Embryogenesis Process Flow







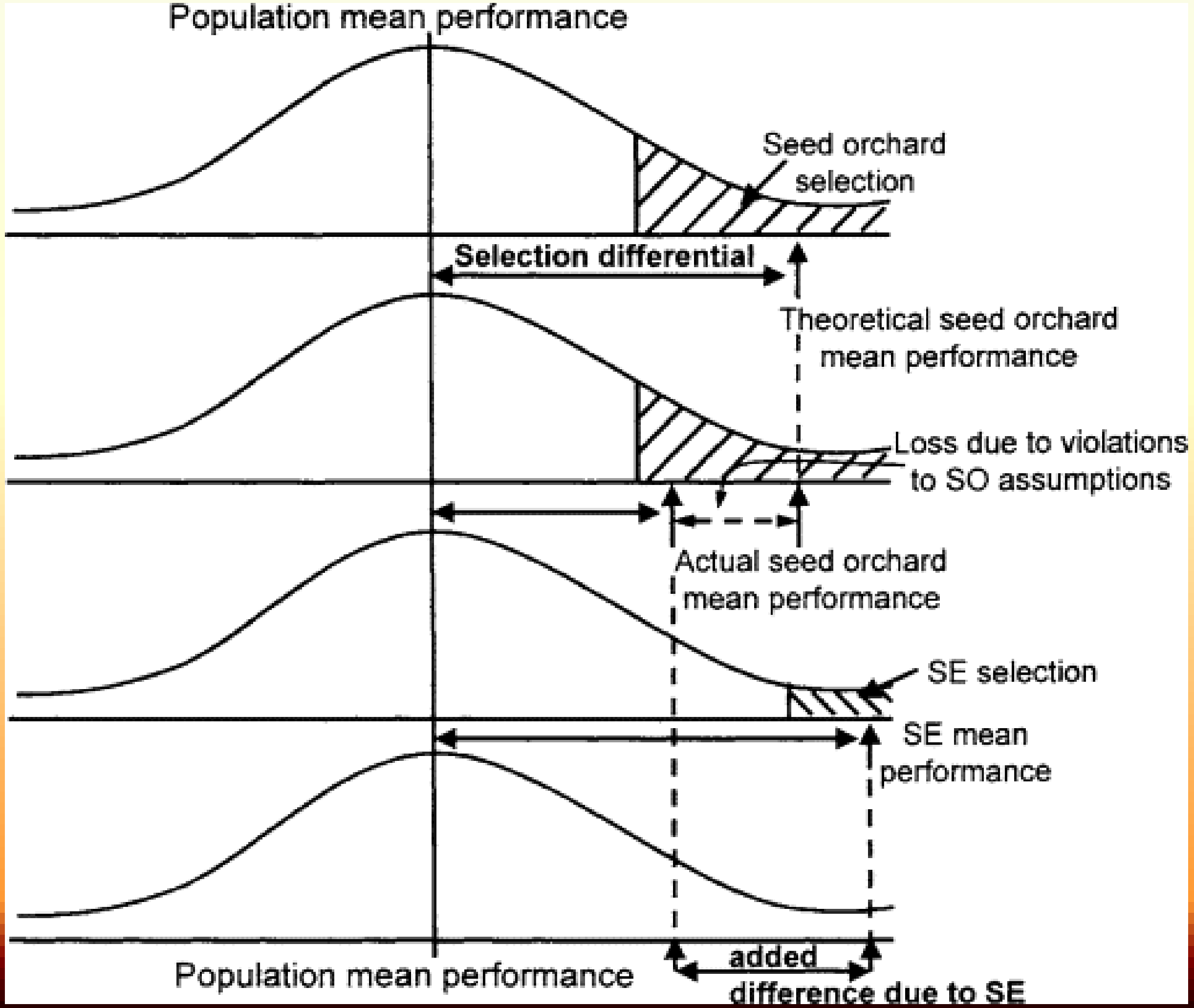
Stages of Somatic Embryogenesis





Why SE ?

- A method of cloning - make copies of a starting plant



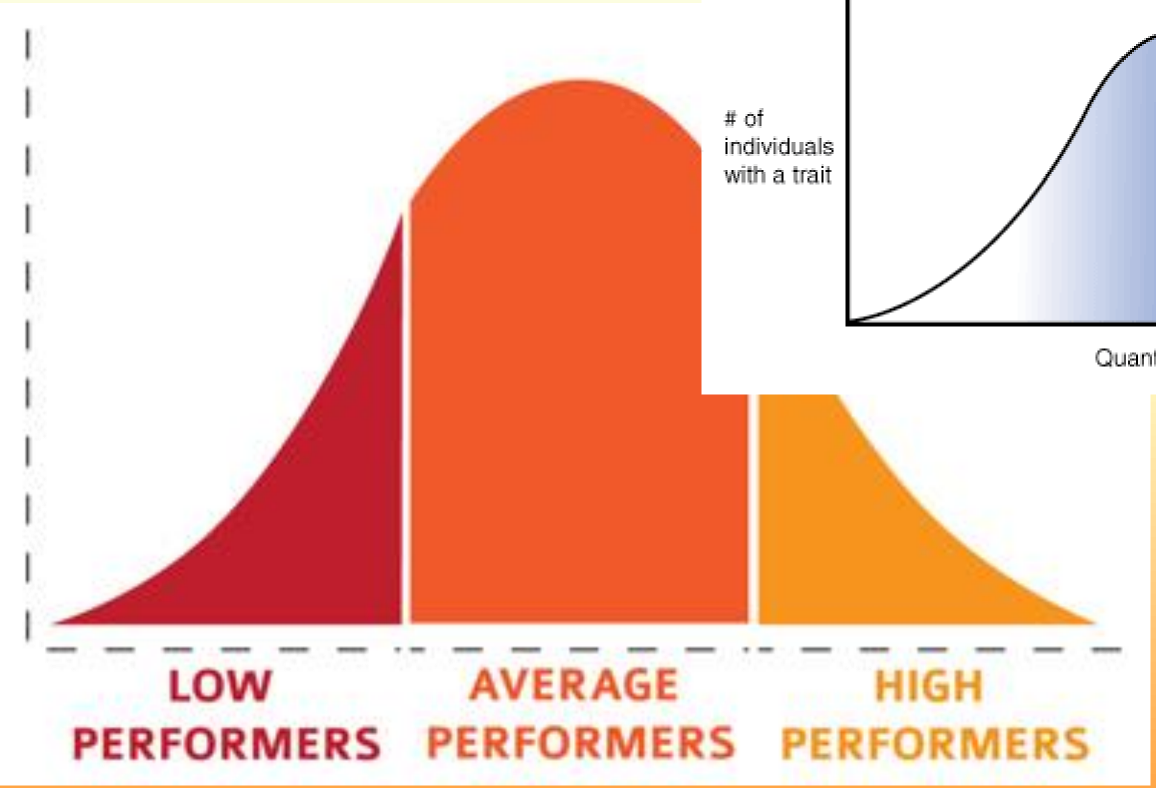
Increased Productivity

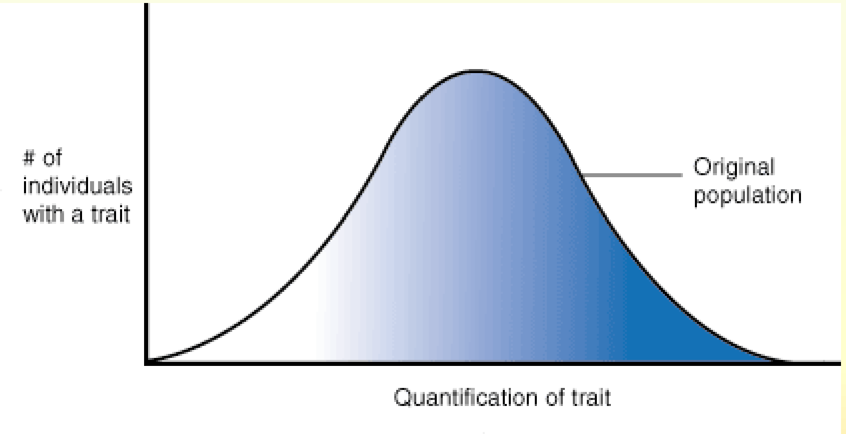
SE Process Flow

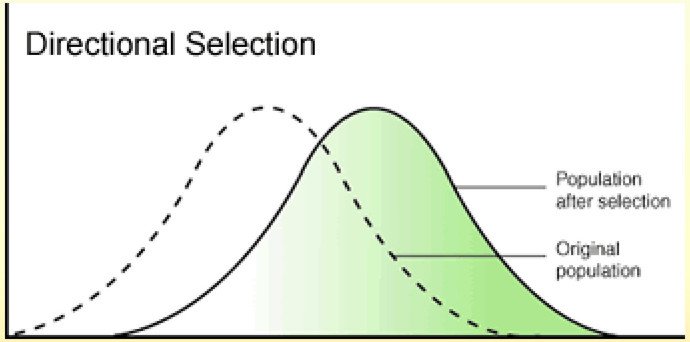
• Initiate	SE from zygotic embryos
• Proliferate	the few immature SE up to millions/line
• Mature the	SE into cotyledonary embryos with roots
• Acclimate	the SE to soil/greenhouse coniditons
• Deploy	to field

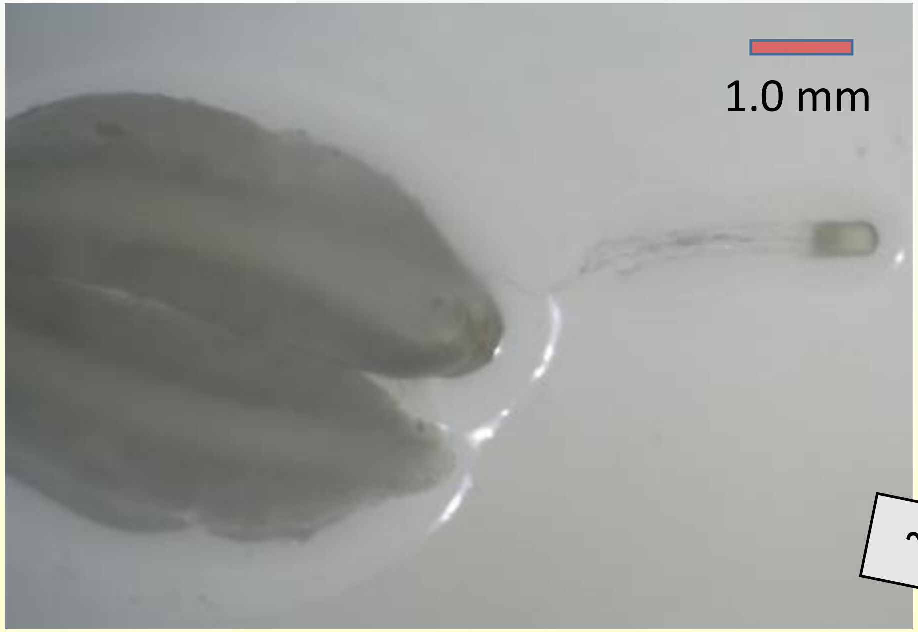
Why SE ?

- A method of cloning - make copies of one starting plant







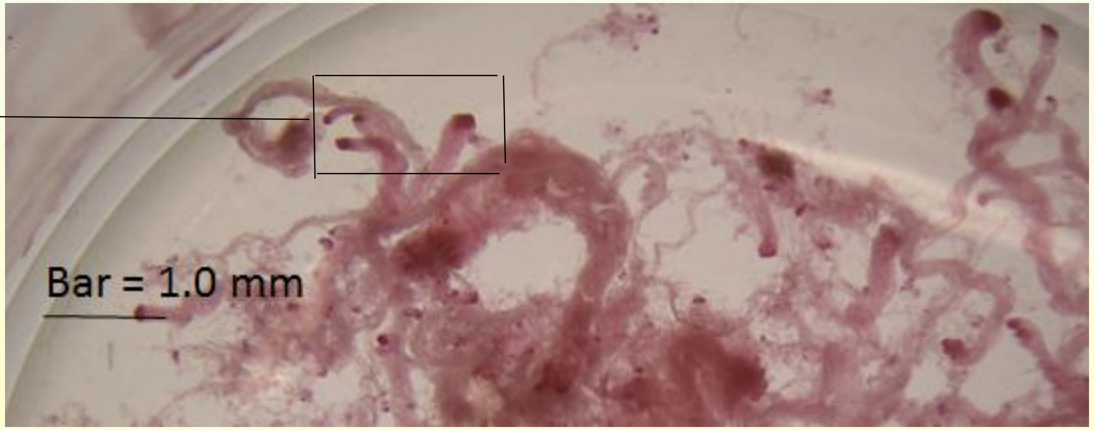


zygotic embryo

~ 10 weeks

proliferating
somatic embryos

SE stained with acetocarmine



Somatic Embryos

S Embryo head

S Embryo suspensor

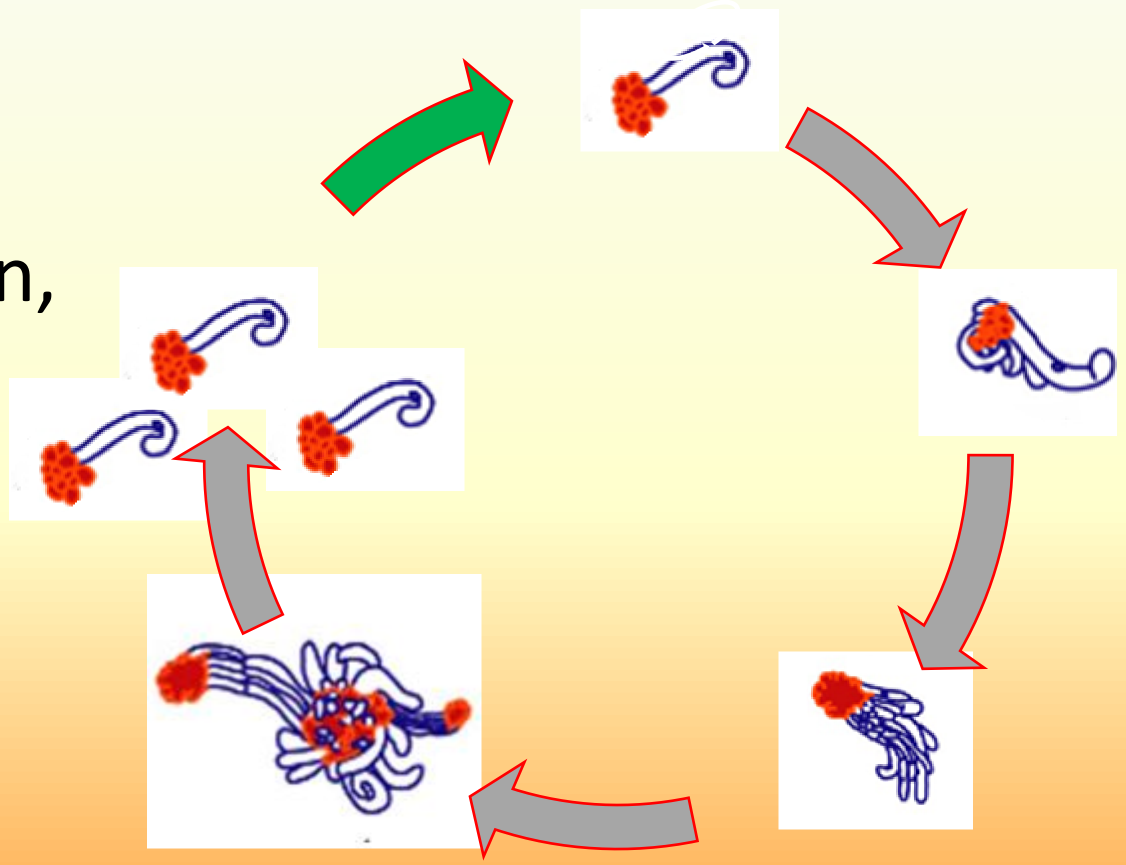
Zygotic Embryo

Additional advantages of suspension culturing

- uniform exposure of tissue to any chemical variable being tested
- uniform tissue inoculum when aliquoting tissue to experiments
- general ease of handling
- reduces plastic waste – petri dishes

SE proliferate via cleavage of the embryo head

- Most conifer SE cultures are proliferated using auxin:cytokinin, often in a 2:1 ratio.
- *Abies* SE cultures require **only cytokinin**



SE proliferate via cleavage of the embryo head