



45^o Congresso Brasileiro de Olericultura
15^o Congresso Brasileiro de Floricultura e Plantas Ornamentais
2^o Congresso Brasileiro de Cultura de Tecidos de Plantas

Protoplastos vegetais: de Kerkler até nossos dias

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Departamento de Biologia Vegetal
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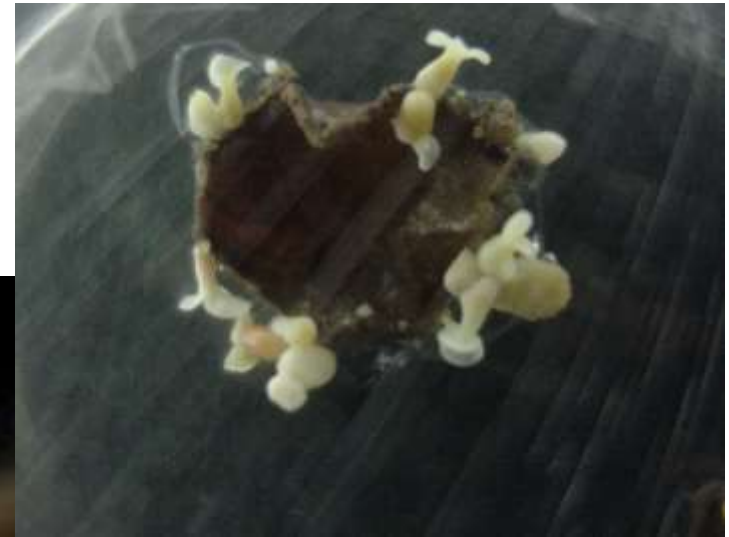


Gottlieb Haberlandt

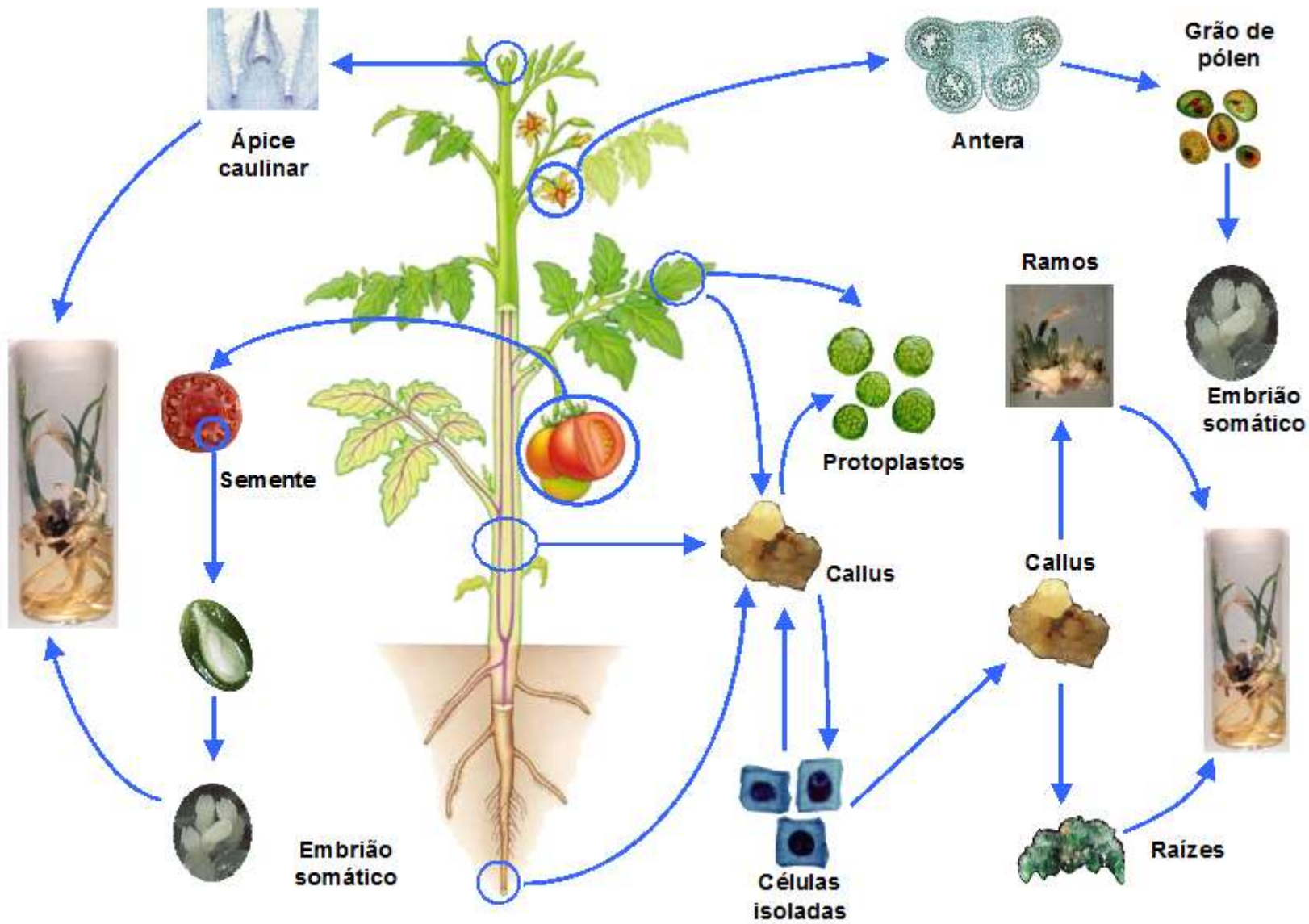


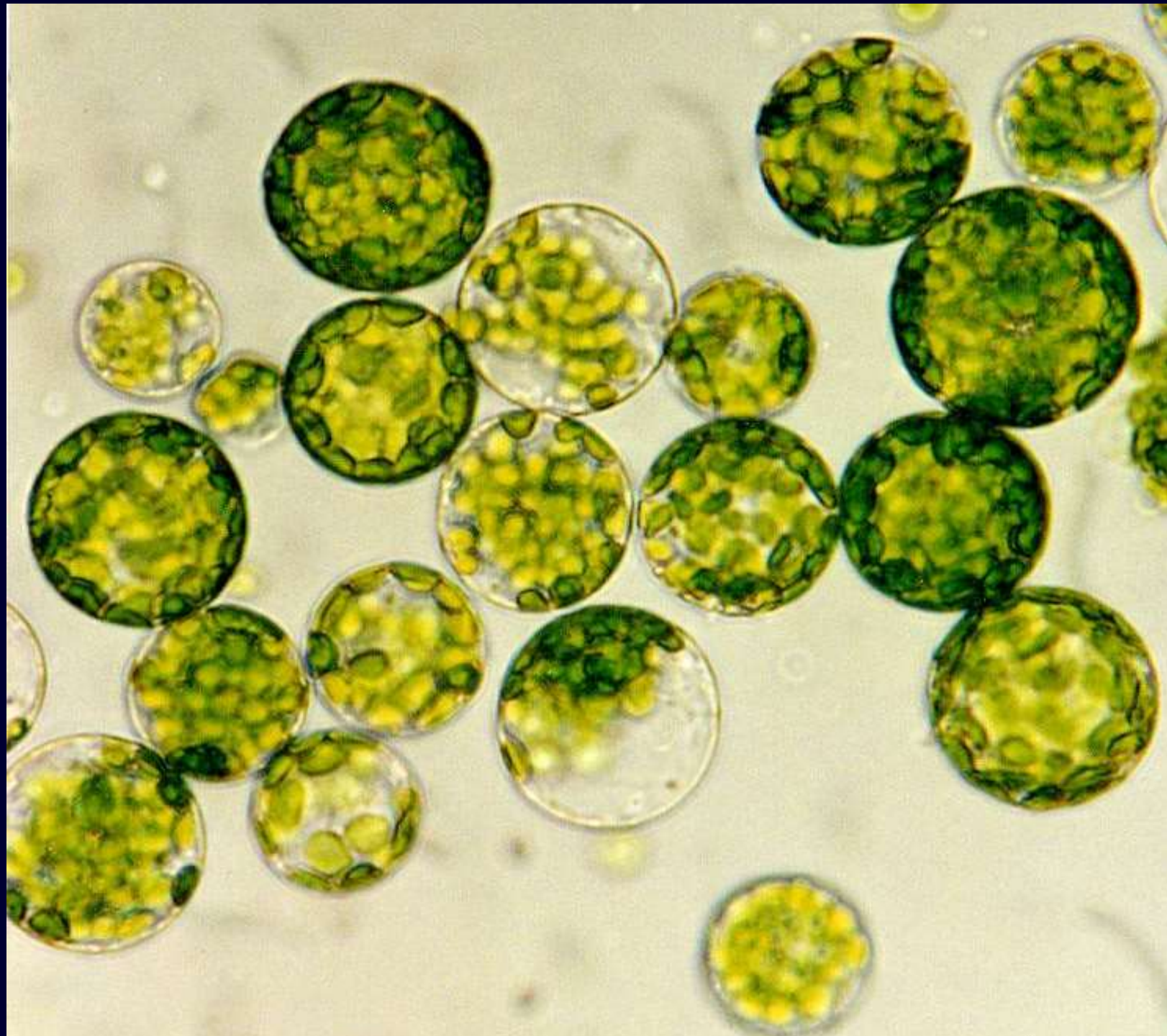
“Theoretically all plant cells are able to give rise to a complete plant”

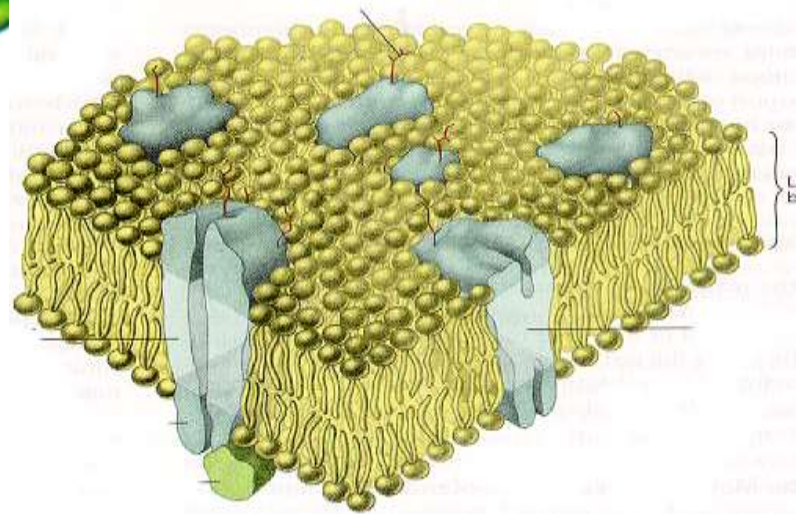
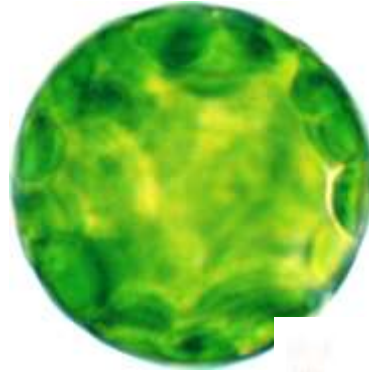
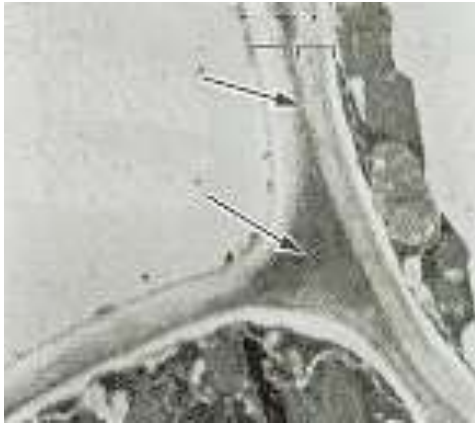
Se a totipotência existe, ela pode ser explorada...

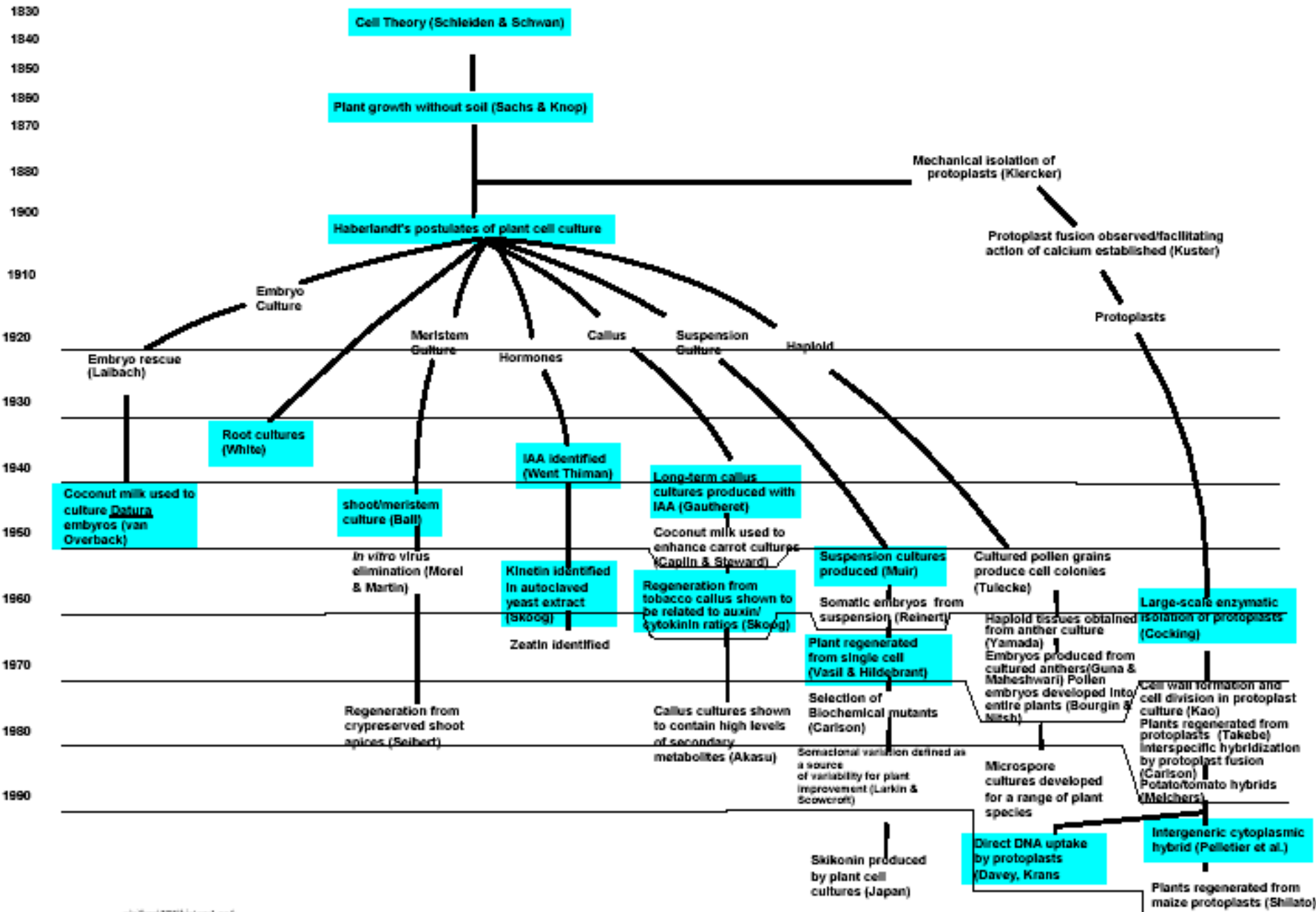


Embriogênese somática direta





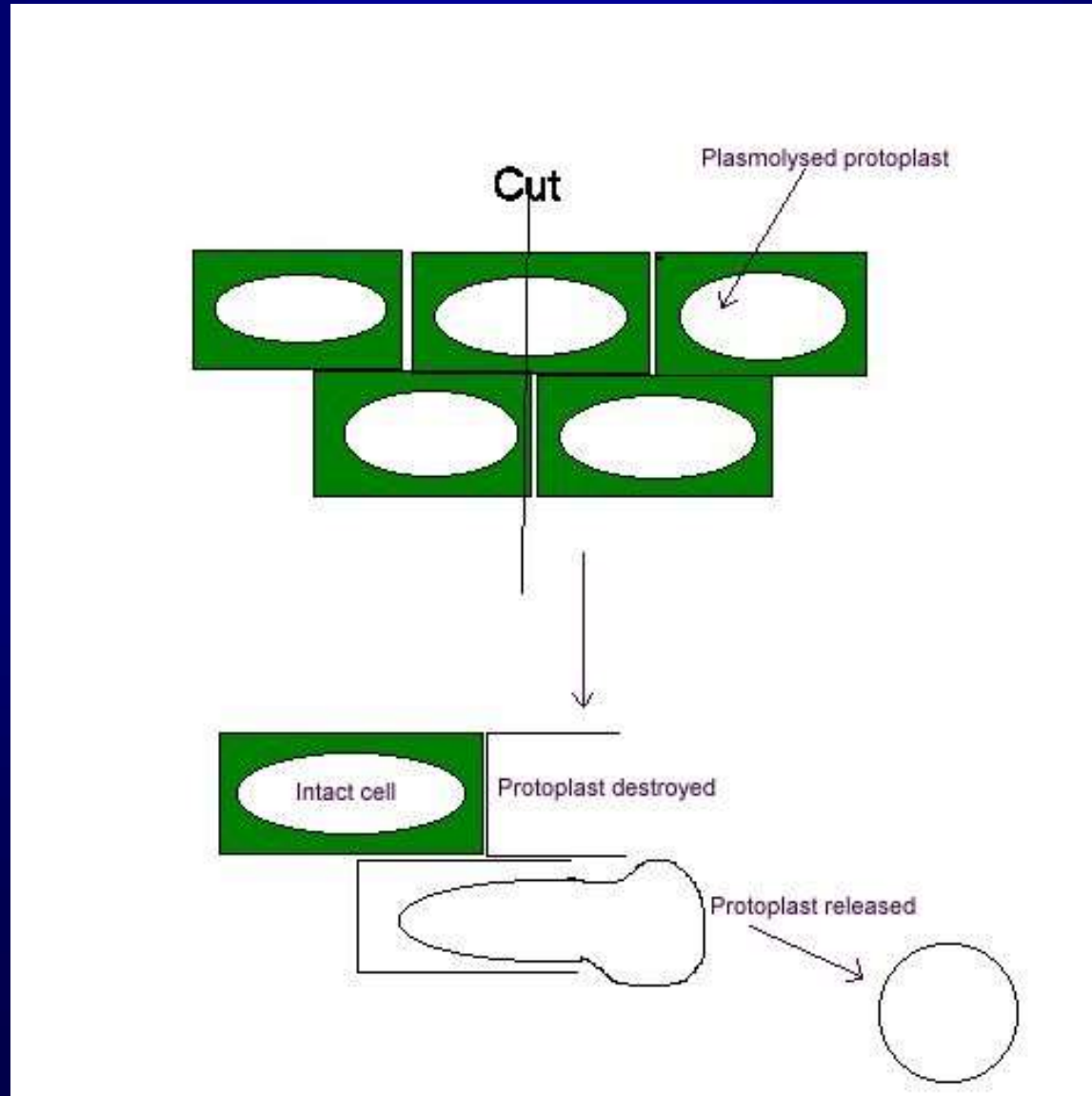




Histórico

- 1882 – Klercker obteve protoplastos isolados via mecânica
- 1960 – Isolamento de protoplastos usando enzimas oriundas de fungo (*Myrotecium verrucaria*) (Cocking, 1960)
- 1962-1975 – Os meios básicos de cultura de tecidos e células foram estabelecidos (MS, SH, **KM**) (**Kao & Michayluk, 1975**)
- 1971 – Primeira planta regenerada de protoplastos (Takebe et al., 1971)
- 1972- Primeiro híbrido somático (Carlson et al. 1972)
- 1978- Primeiro híbrido somático intergenérico (Melchers et al., 1978)

ISOLAMENTO MECÂNICO



In Vitro Cell. Dev. Biol.—Plant 38:84–92, March–April 2002
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1054-5476/02 \$10.00+0.00

PLANT TISSUE CULTURE. BIOTECHNOLOGY. MILESTONES

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(Received 1 November 2001; accepted 28 November 2001; editor T. A. Thorpe)

PLANT TISSUE CULTURE AND BIOTECHNOLOGY

ROCKEFELLER FOUNDATION CONFERENCE ON

"CROP IMPROVEMENT THROUGH TECHNIQUES OF PLANT CELL AND TISSUE CULTURE"

Villa Serbelloni, Bellagio (Como), Italy

May 13-17, 1969



Standing	GAMBORG (Canada)	COCKING (England)	YAMADA (Japan)	IFAMATO (Italy)	ERIKSSON (Sweden)	RILEY (Japan)/(U.S. Rockefeller)	ROBERTS (U.S.)
Seated	HILDEBRANDT (U.S.)	NICKELL (U.S.)	REINERT (Germany)	TORREY (U.S.)	BRAUN (U.S.)	MOREL (France)	CIRLSON (U.S. NSF)

In Vitro Cell. Dev. Biol.—Plant 36:77–82, March–April 2000
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1054-5476/99 \$10.00+0.00

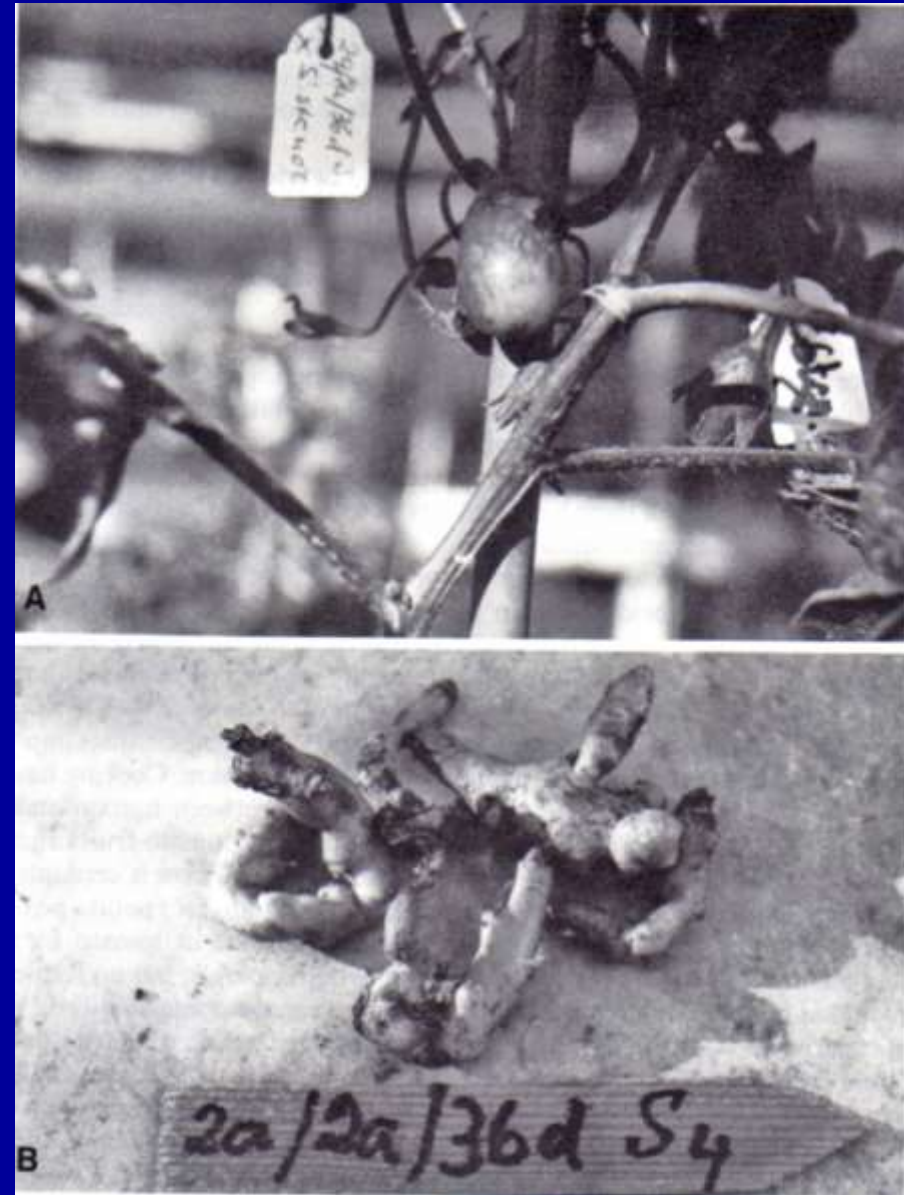
TURNING POINT ARTICLE
PLANT PROTOPLASTS

EDWARD C. COCKING*

Plant Science Division, University of Nottingham, Nottingham NG7 2RD, United Kingdom

(Accepted 24 June 1999; editor I. K. Vasil)





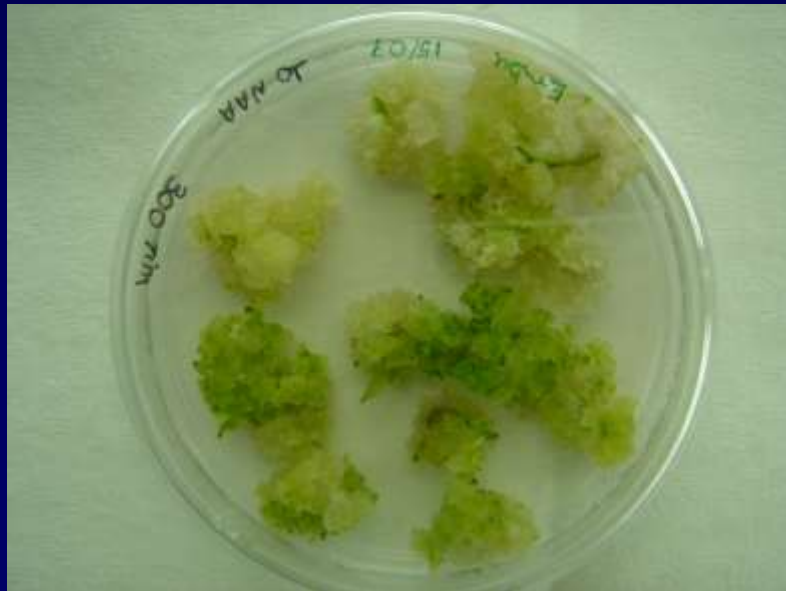
Gleba & Sytnik, 1984

ISOLAMENTO E CULTIVO

- Obtenção material estéril (ou partir de material estabelecido *in vitro*)
- Utilização de um osmótico adequado
- Facilitação da atividade enzimática
- Tratamento enzimático seqüencial ou mistura enzimática
- Purificação dos protoplastos isolados (remoção de enzimas e restos celulares, quantificação e ajuste de densidades)
- Transferência para meio de cultivo adequado

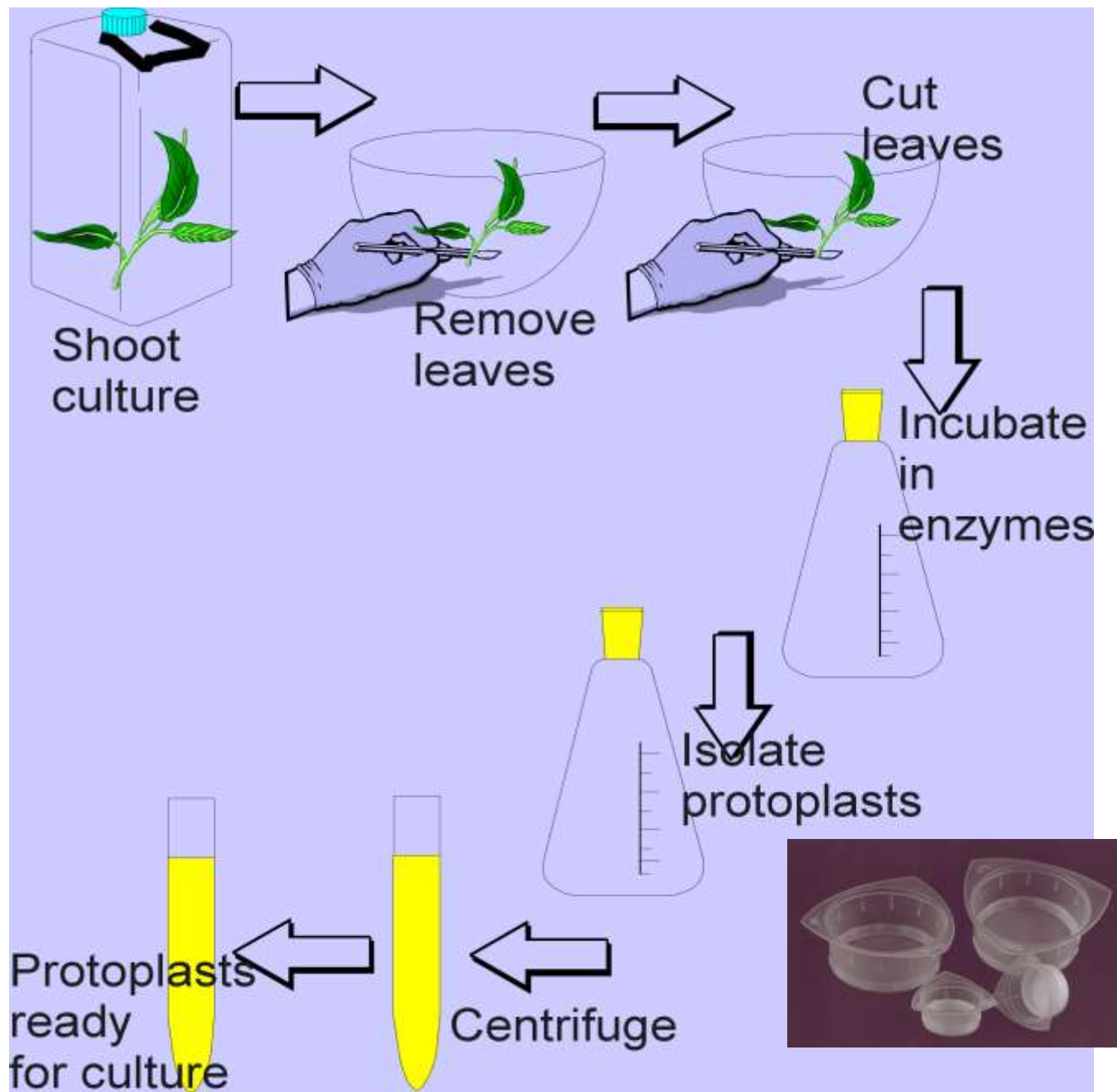
ISOLAMENTO

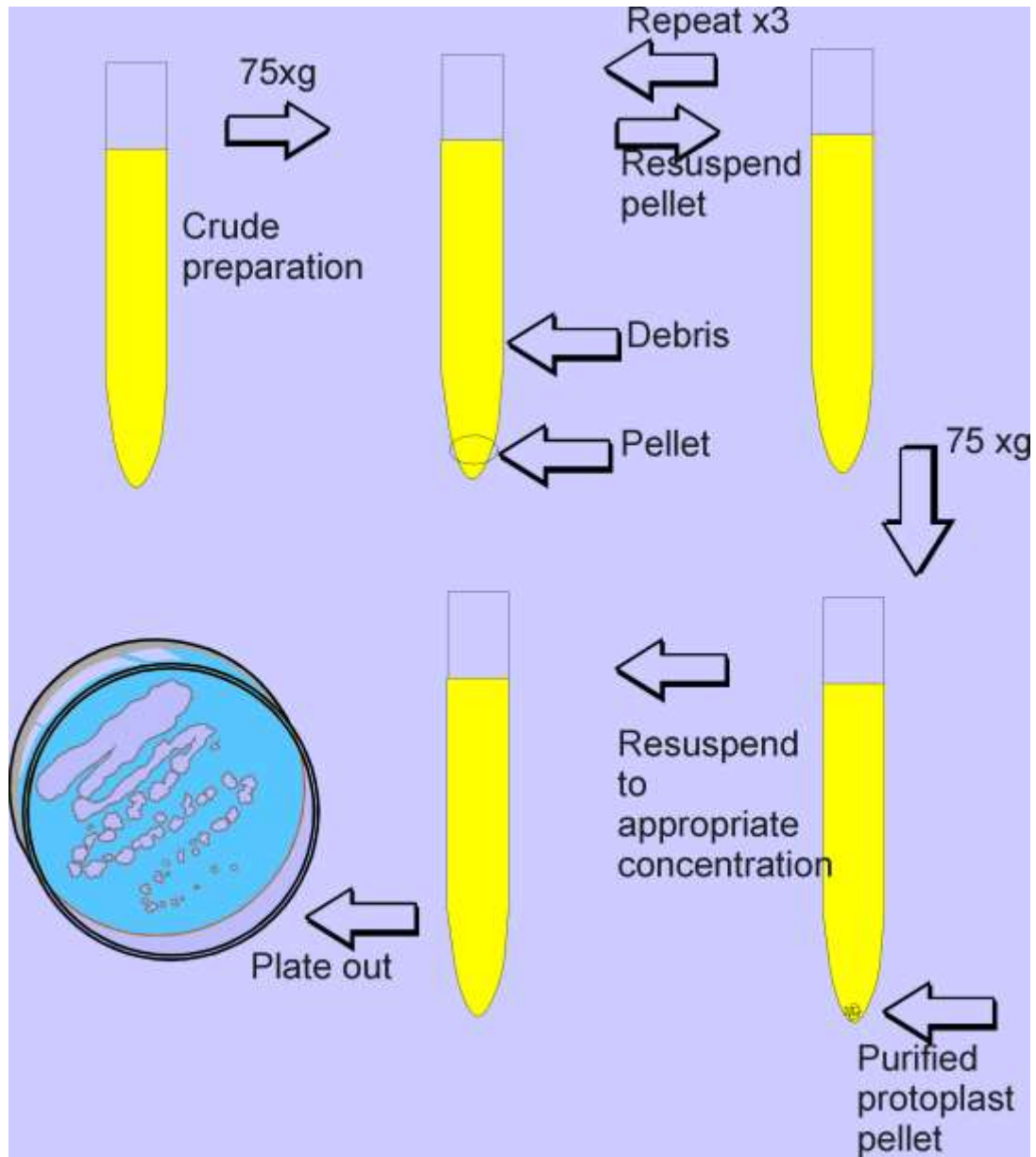
- Folhas
- Suspensão celular
- Raízes
- Pétalas
- Anteras
- Grãos de pólen
- Cotilédones
- Frutos



Enzimas Pectocelulolíticas

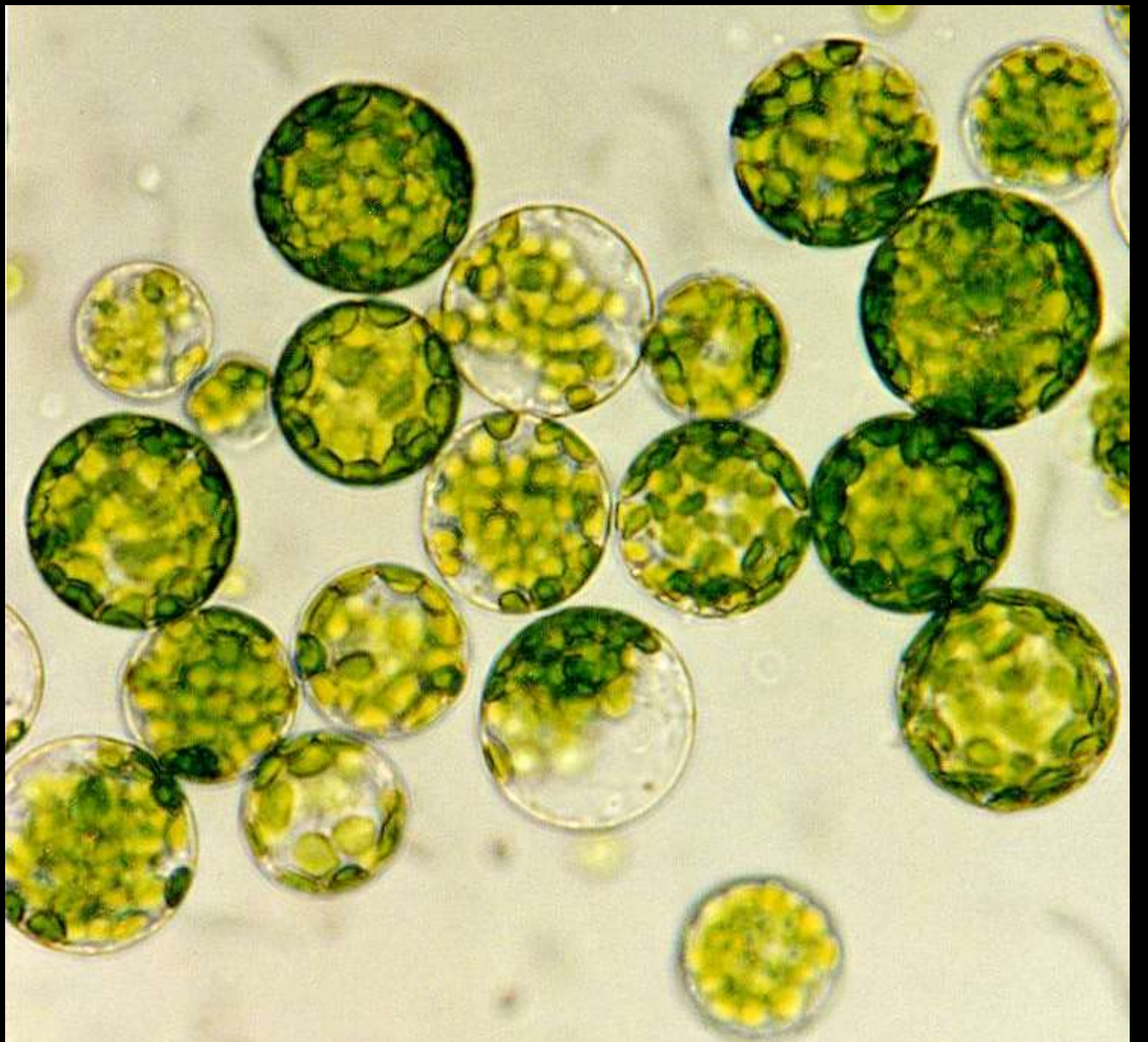






Purificação





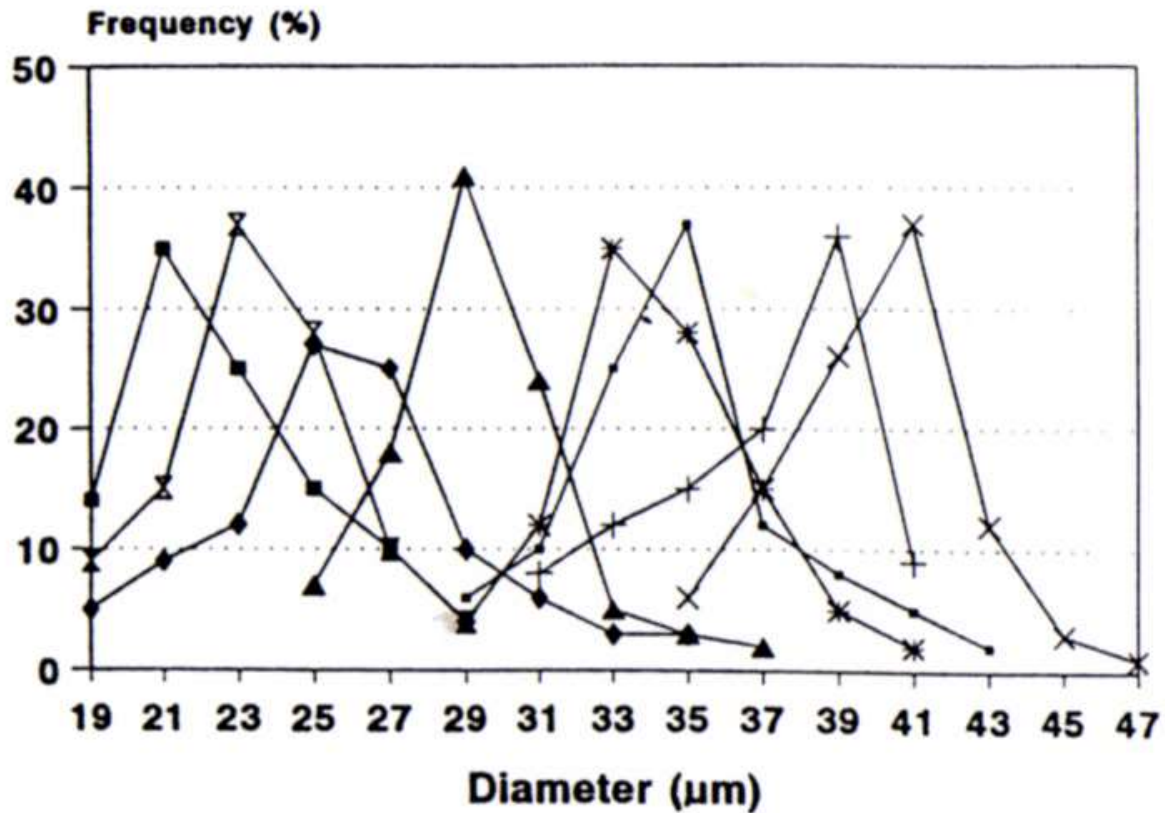
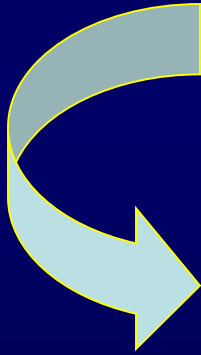
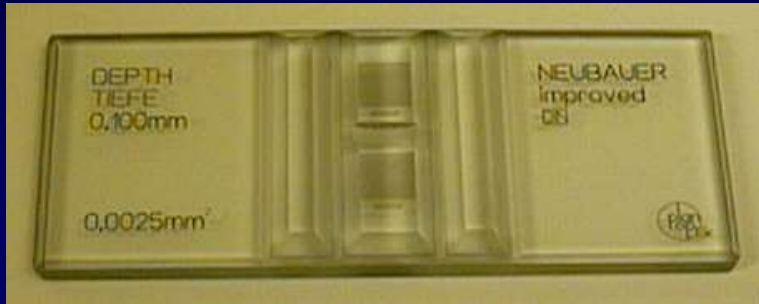


Fig. 2. *Passiflora* spp. protoplast diameters

RENDIMENTO



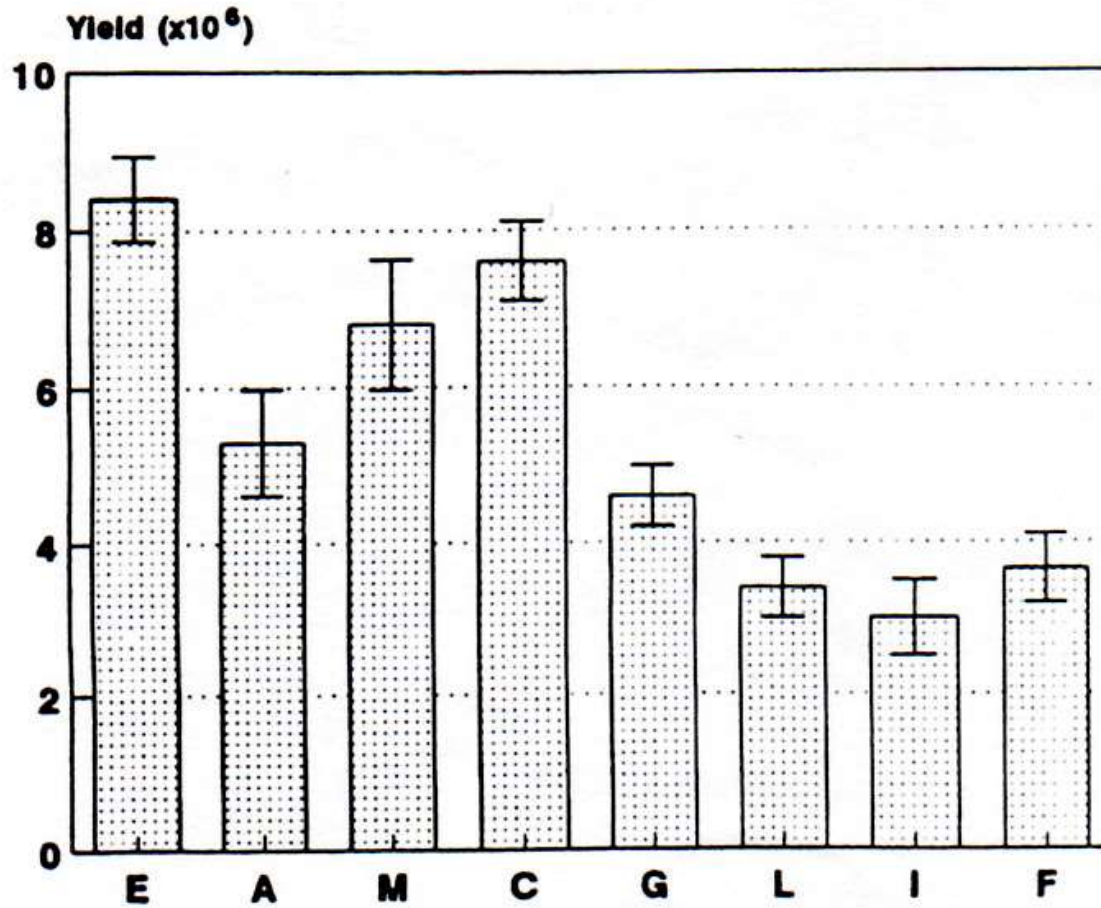
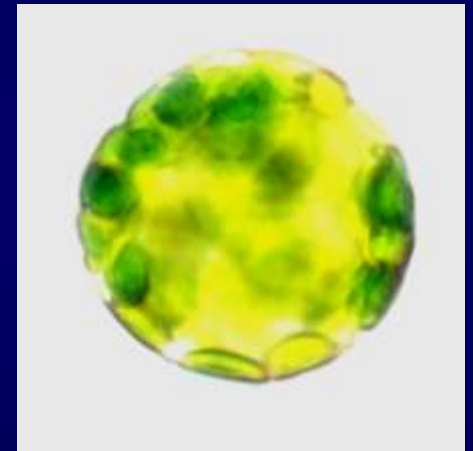
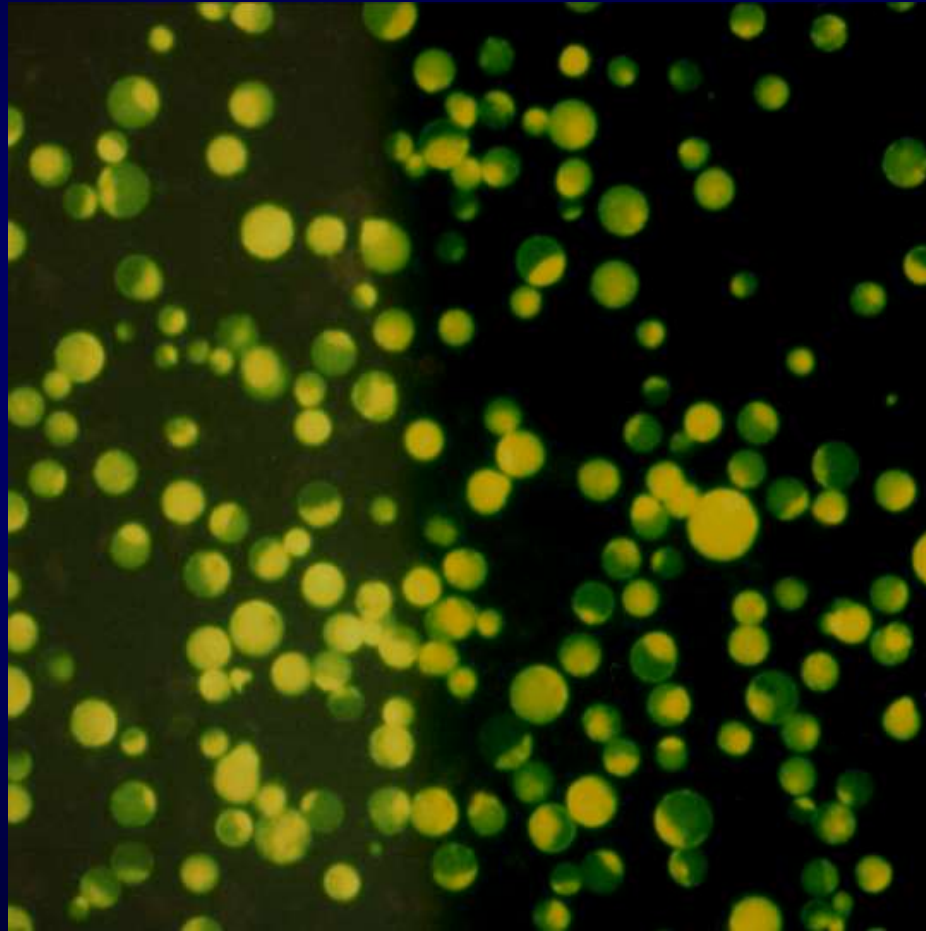


Fig. 1. Yield of protoplasts per 500 mg of leaf tissue of *P. edulis* var. *flavicarpa* (E), *P. alata* (A), *P. amethystina* (M), *P. cincinnata* (C), *P. giberti* (G), *P. mollissima* (L), *P. maliformis* (I) and *P. foetida* (F)

VIABILIDADE



AJUSTES DE DENSIDADE

Eficiência de Plaqueamento (Plating Efficiency)

EIP (IPE) = Eficiência Inicial de Plaqueamento

EFP (FPE) = Eficiência Final de Plaqueamento

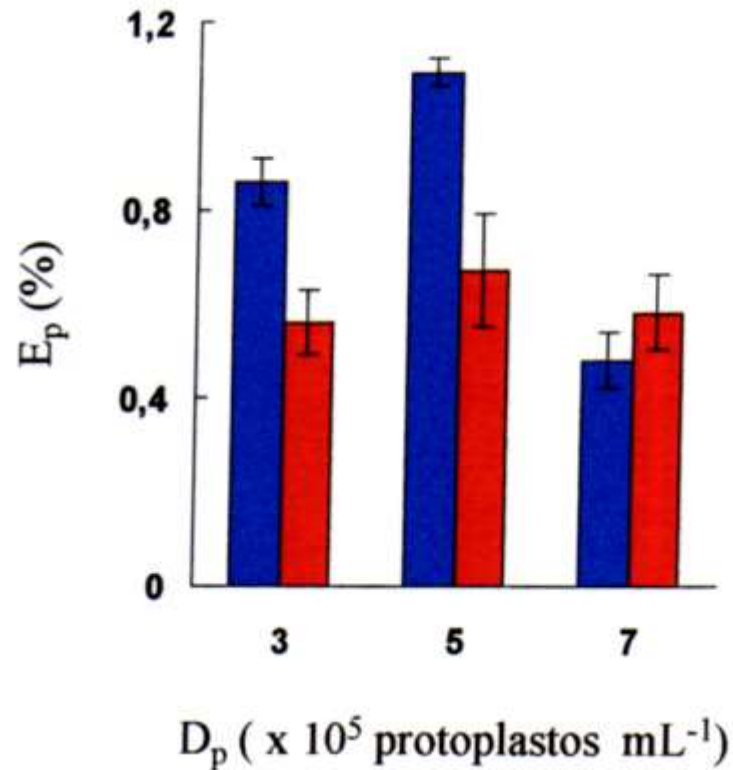
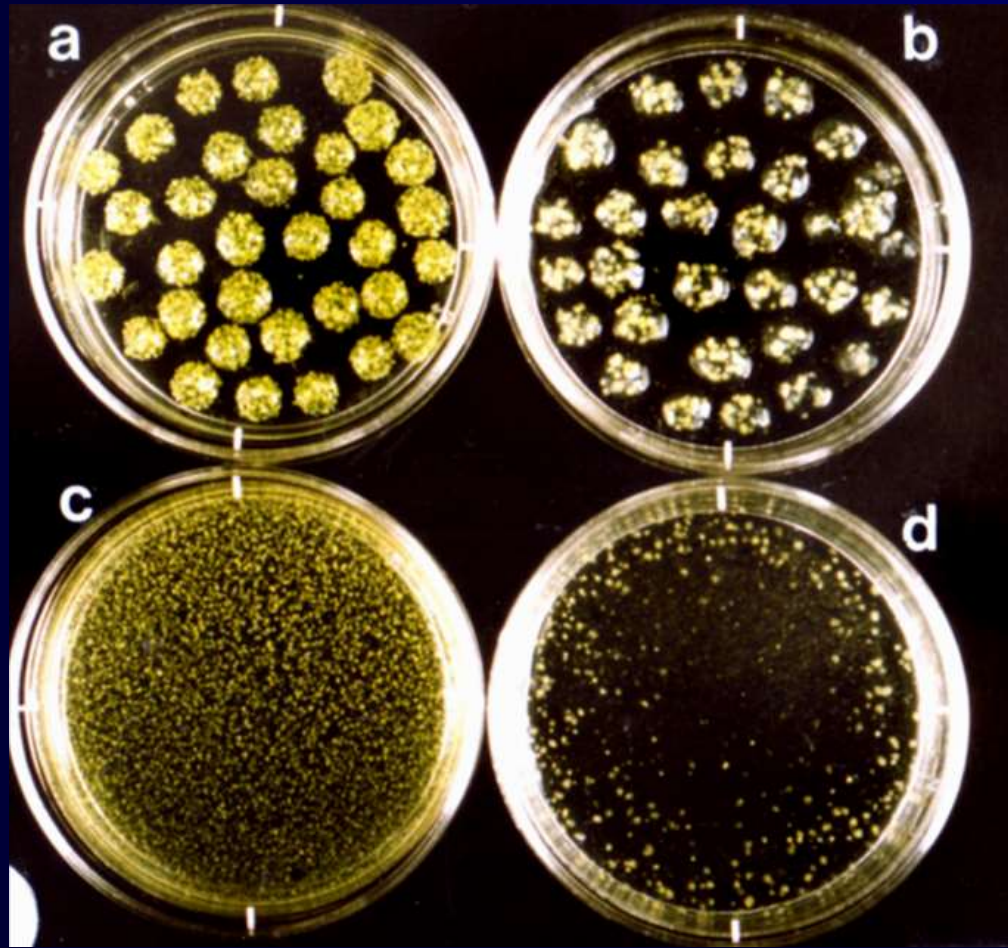


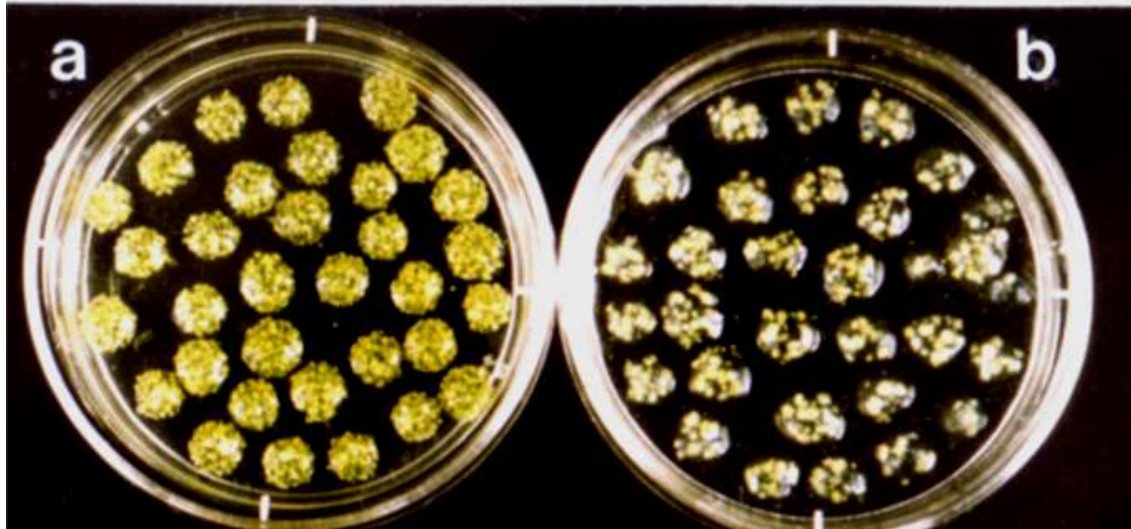
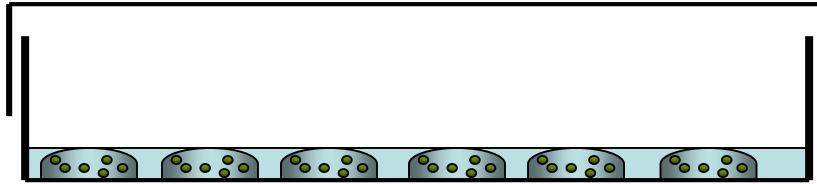
Figura 7 - Eficiência de plaqueamento (E_p) de protoplastos dos genótipos arábica diplóide DH₃ (■) e canéfora Apoatã (■) após três meses de cultivo, em função da densidade de plaqueamento (D_p). As barras indicam o erro padrão da média obtida com 7, 2 e 9 repetições para as respectivas D_p 's em ordem crescente.

Cordeiro (1999)

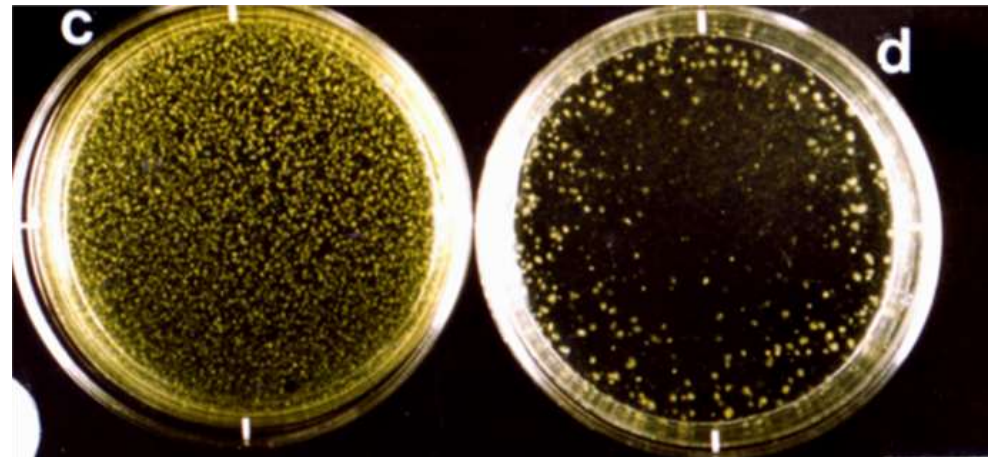
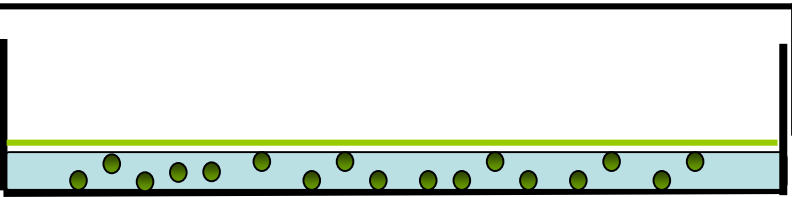
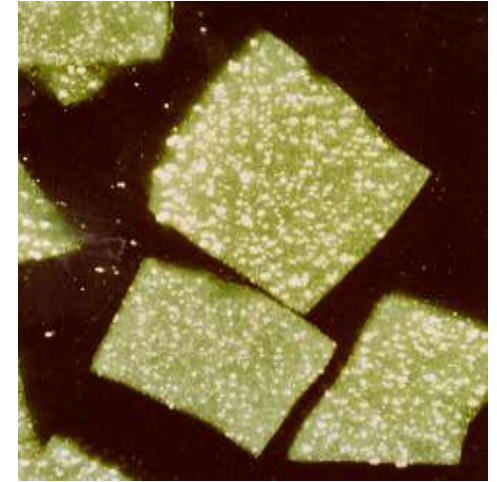
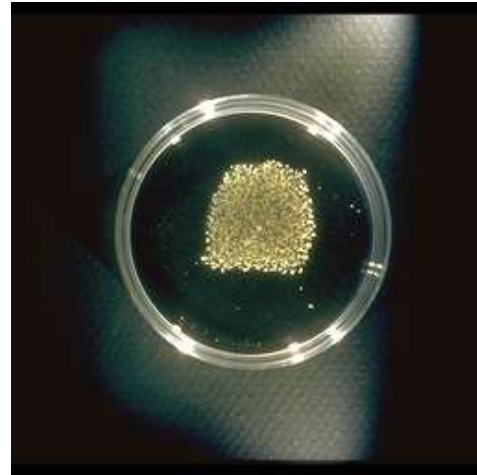
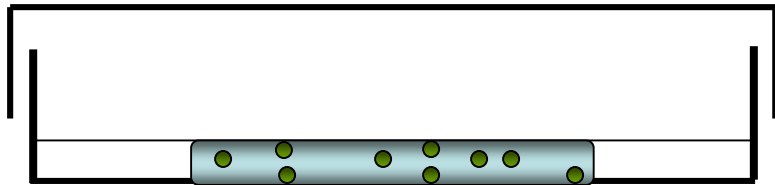
MODALIDADES DE CULTIVO



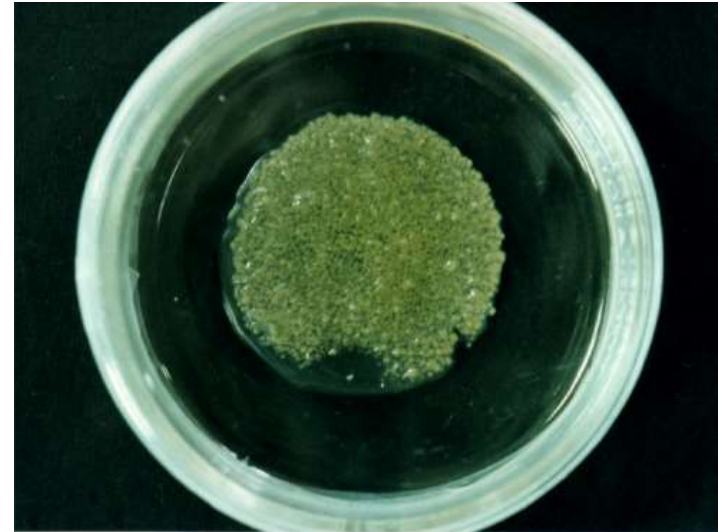
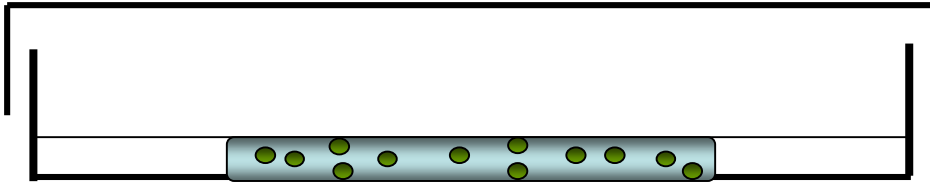
Gotas de agarose (Agarose droplets)



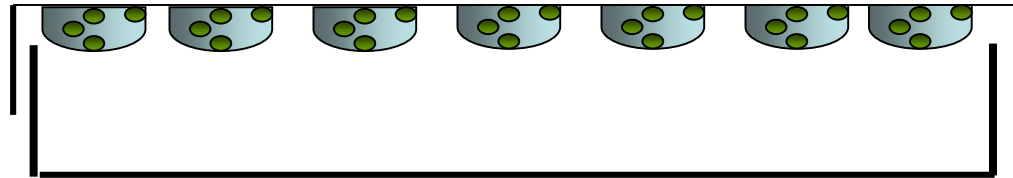
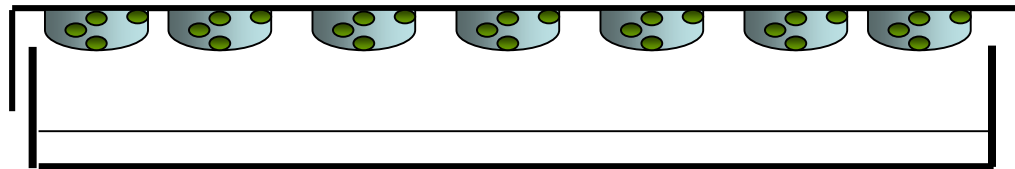
Camada de Agarose (Agarose-embedded Layer)



Alginato (“Alginate-embedded”)

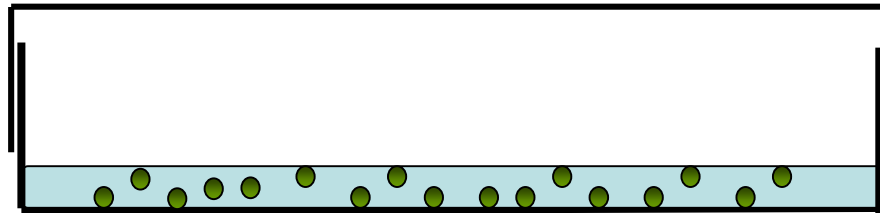


Gotas suspensas (“Hanging-droplets”)

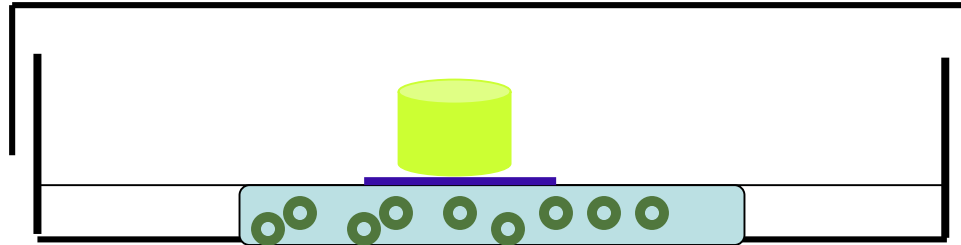


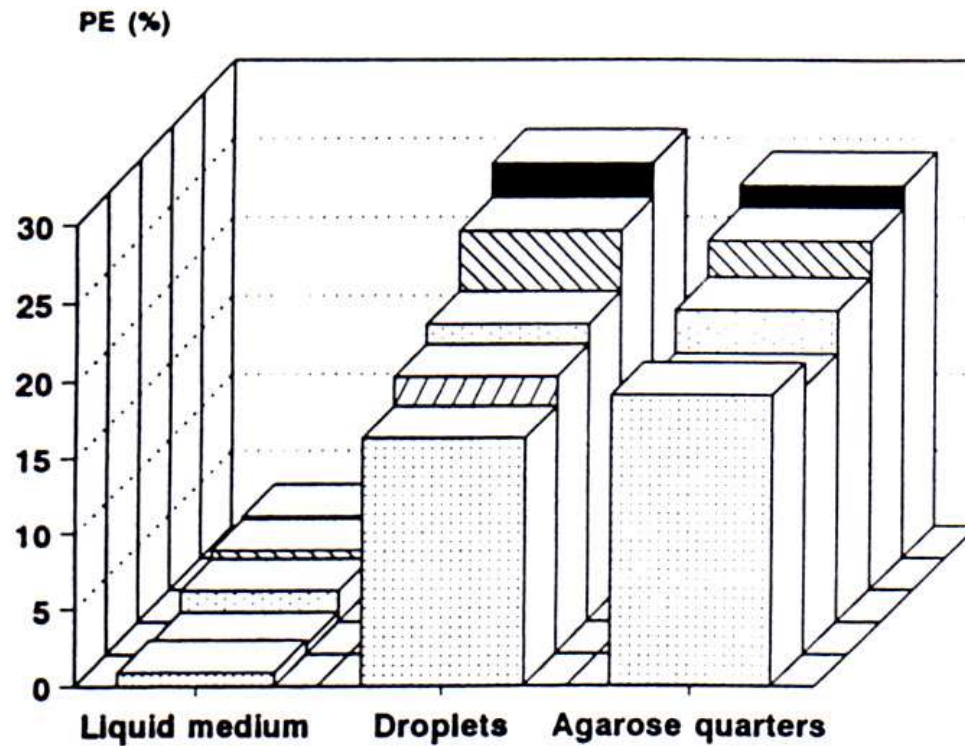
(Kao et al. 1970, Potrykus et al. 1977)

Meio Líquido



Cultura nutridora (Nurse-culture)

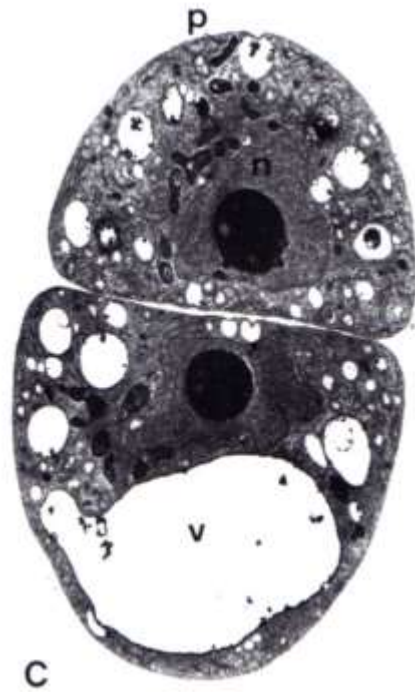
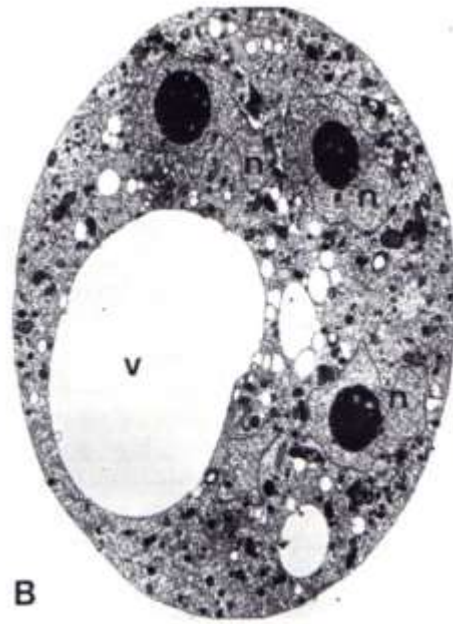
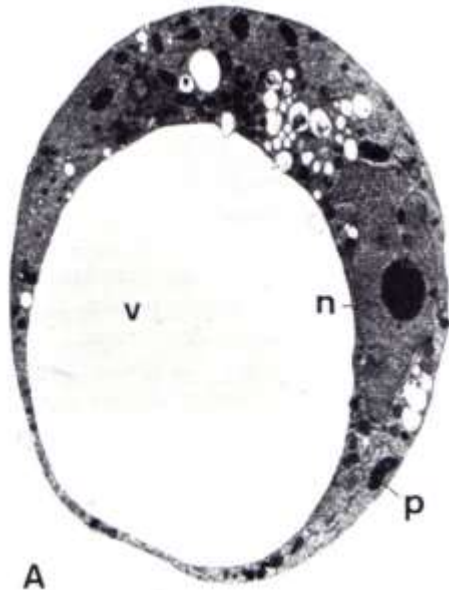




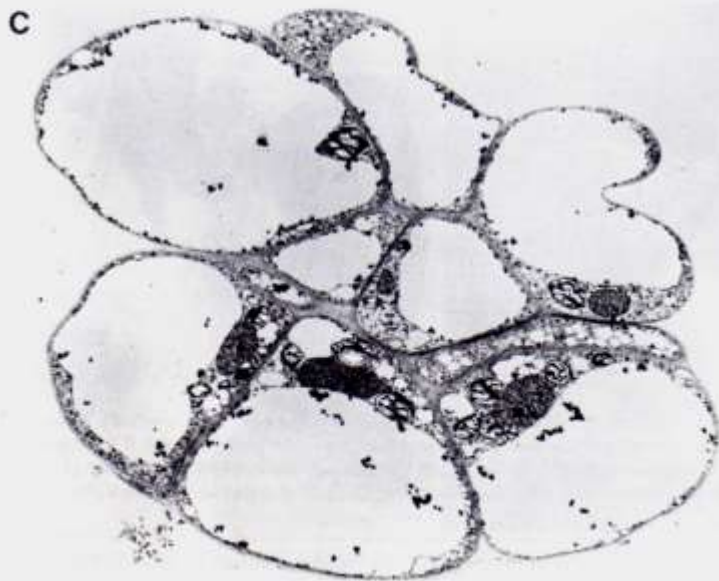
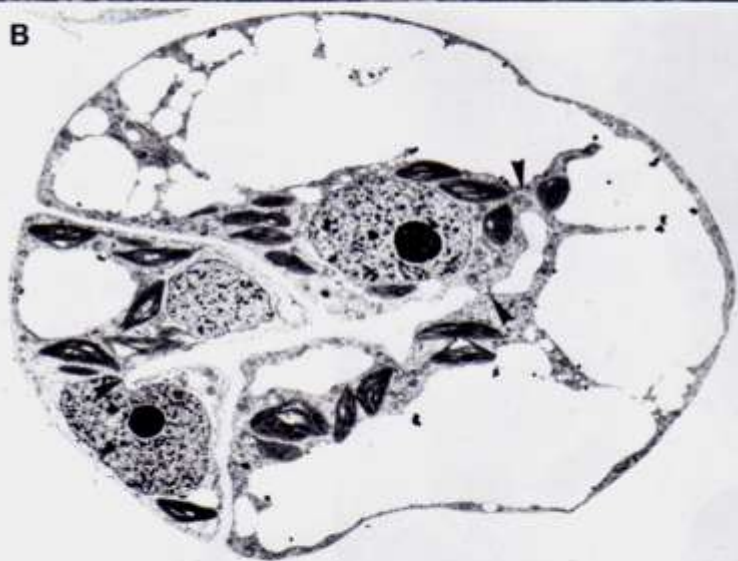
P. amethystina
 P. cincinnata
 P. alata
 P. giberti
 P. edulis

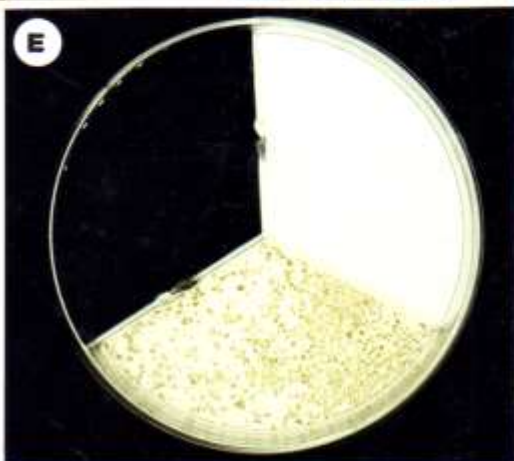
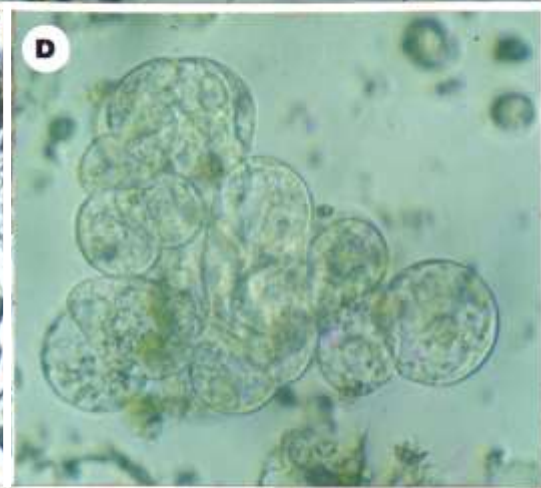
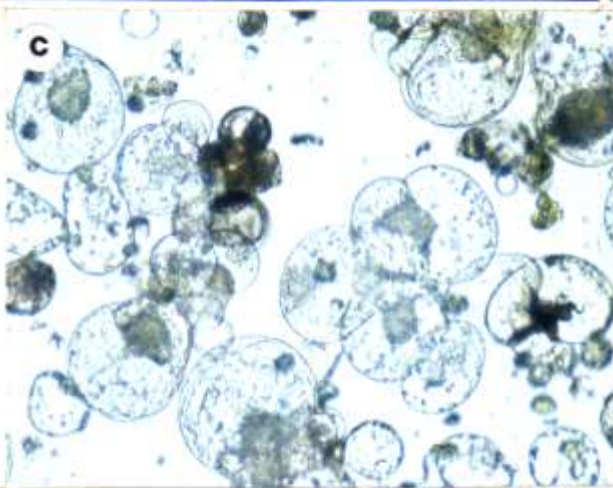
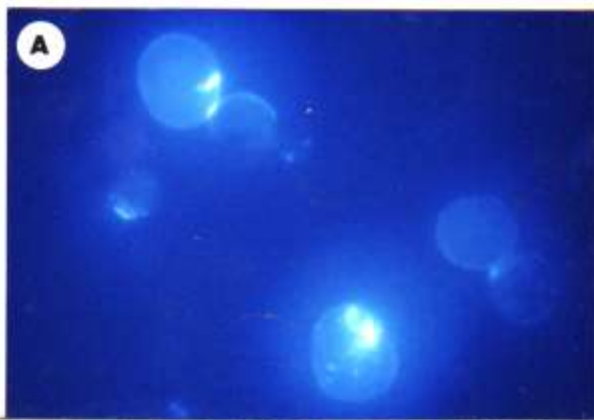
Fig. 4. Plating efficiencies of *Passiflora* spp. protoplast cultures according to species and culture system

Vieira & Dornelas (1996)



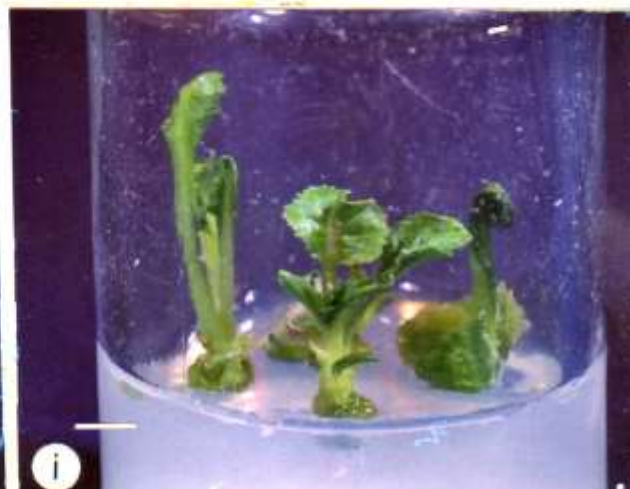
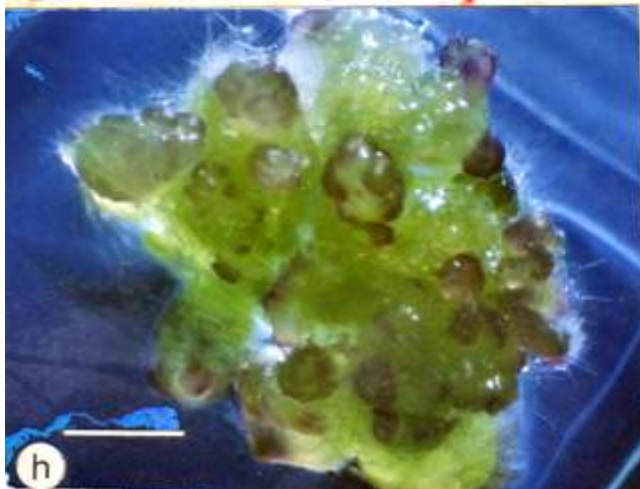
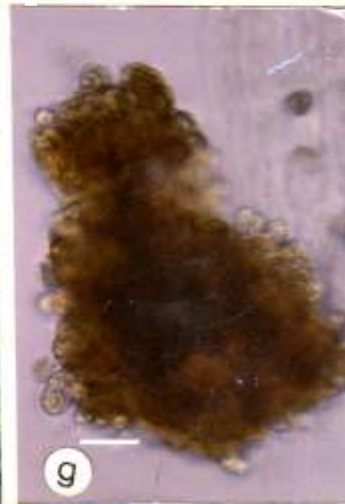
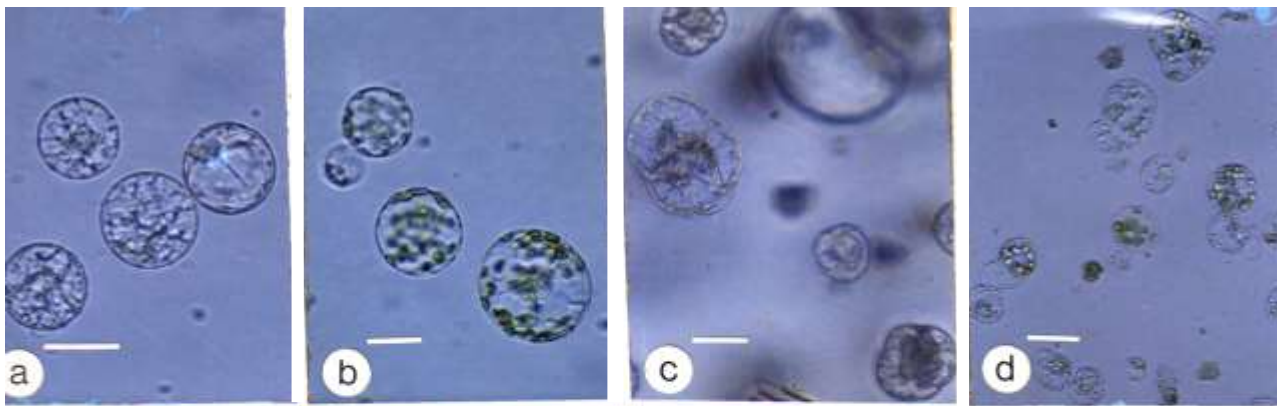
Gleba & Sytnik (1984)

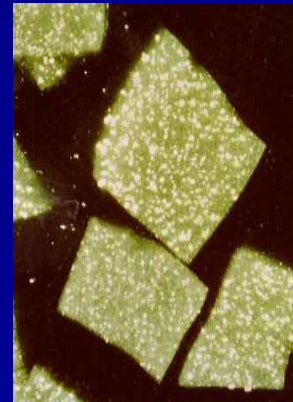
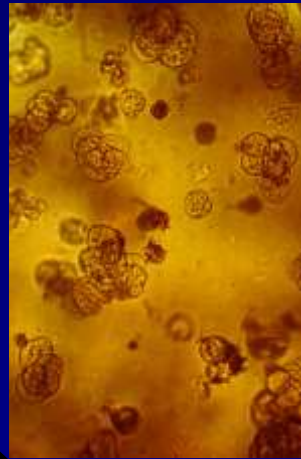
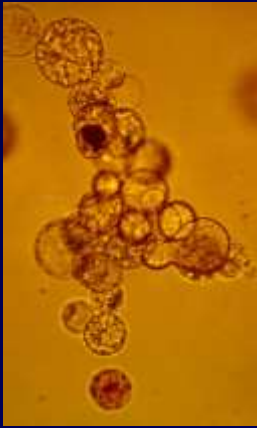




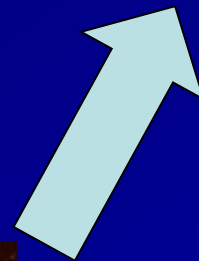
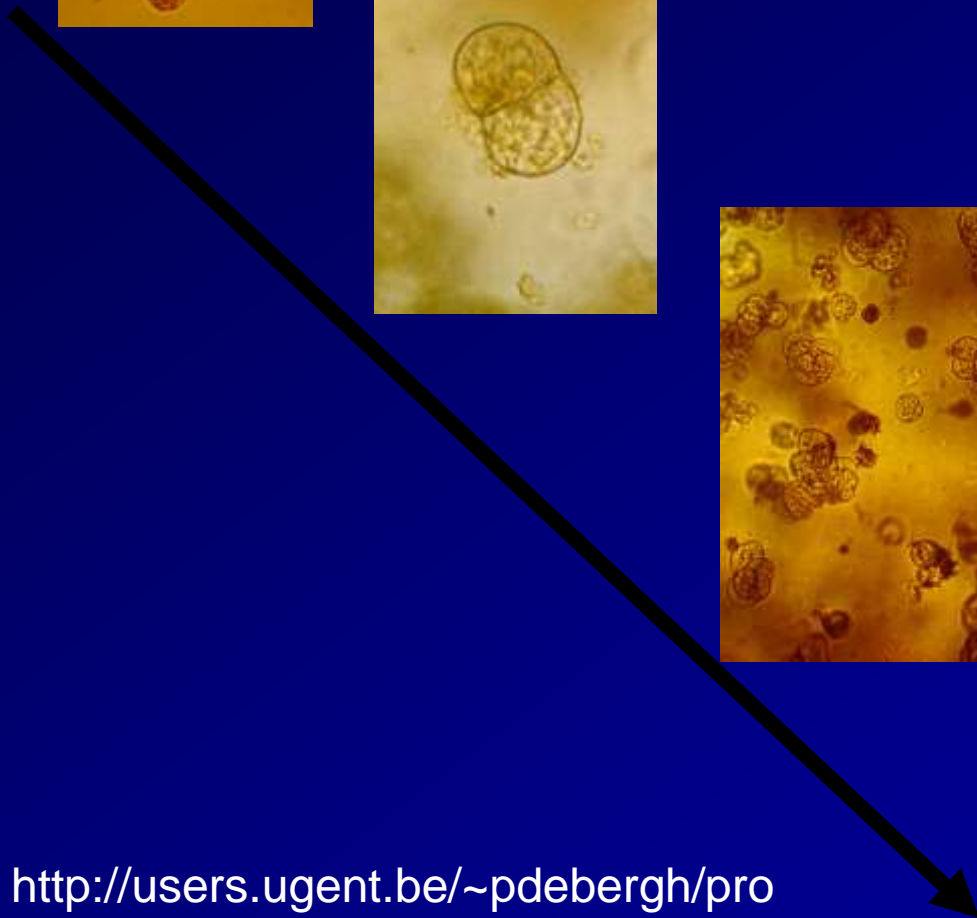
REGENERAÇÃO





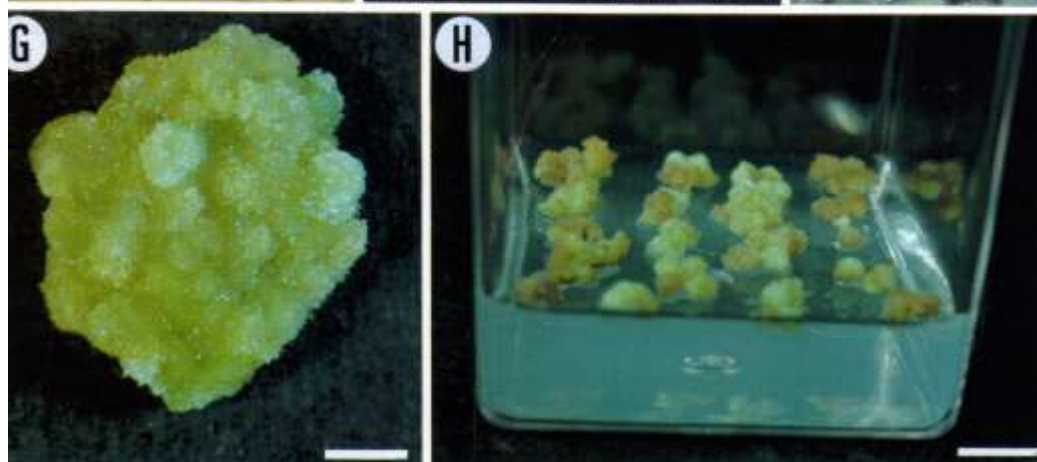
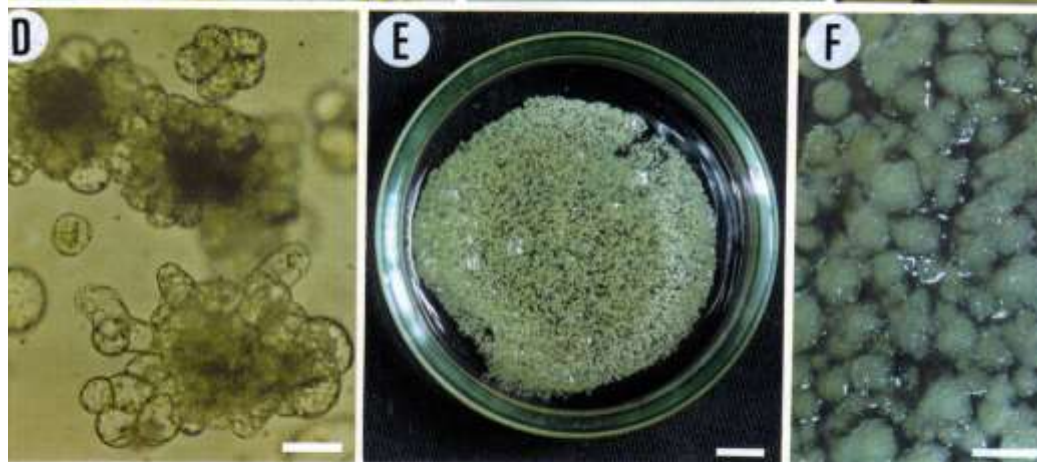
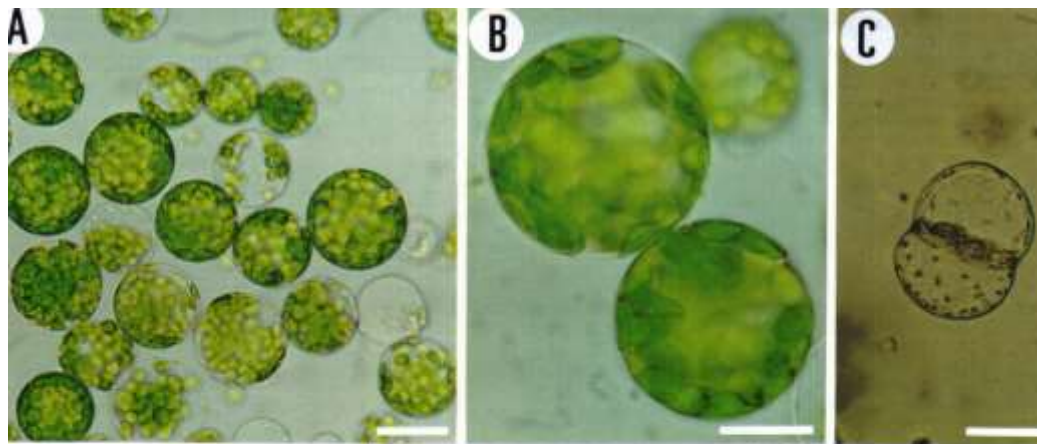


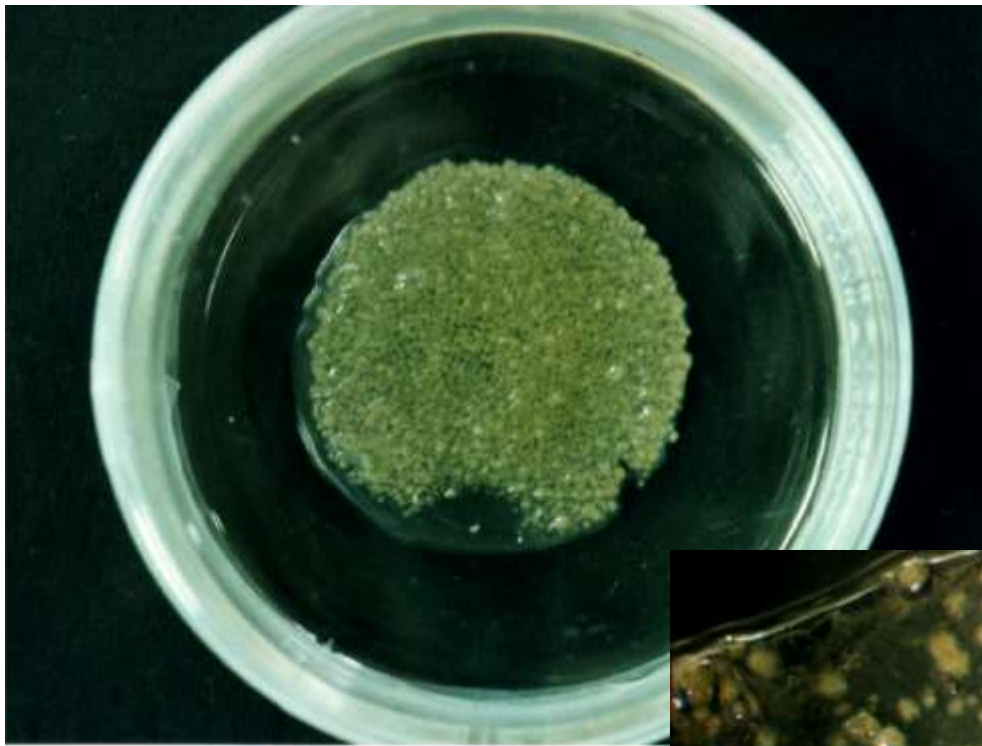
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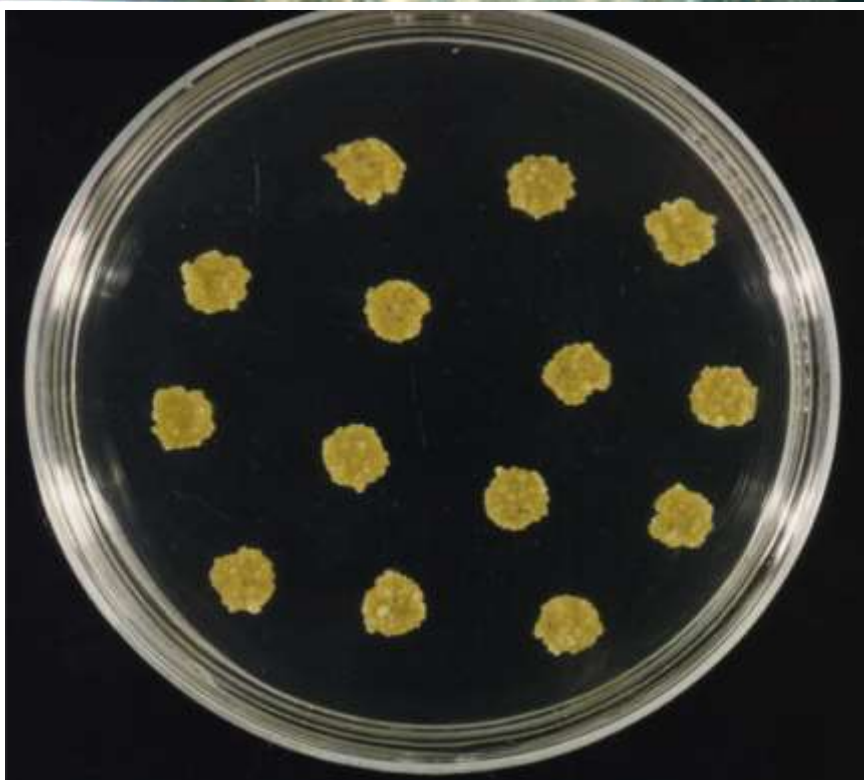
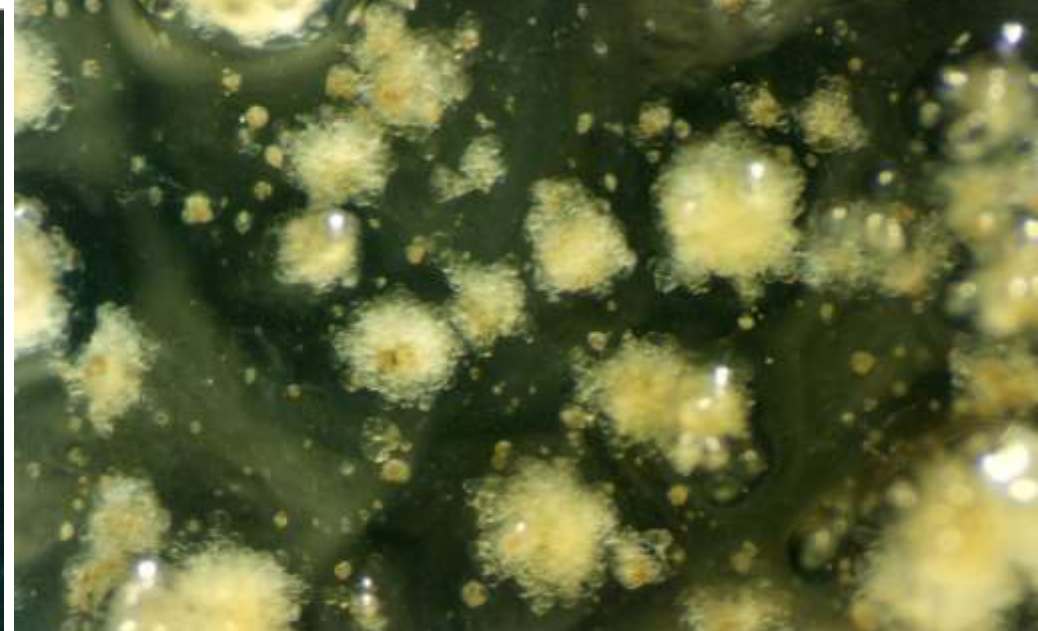
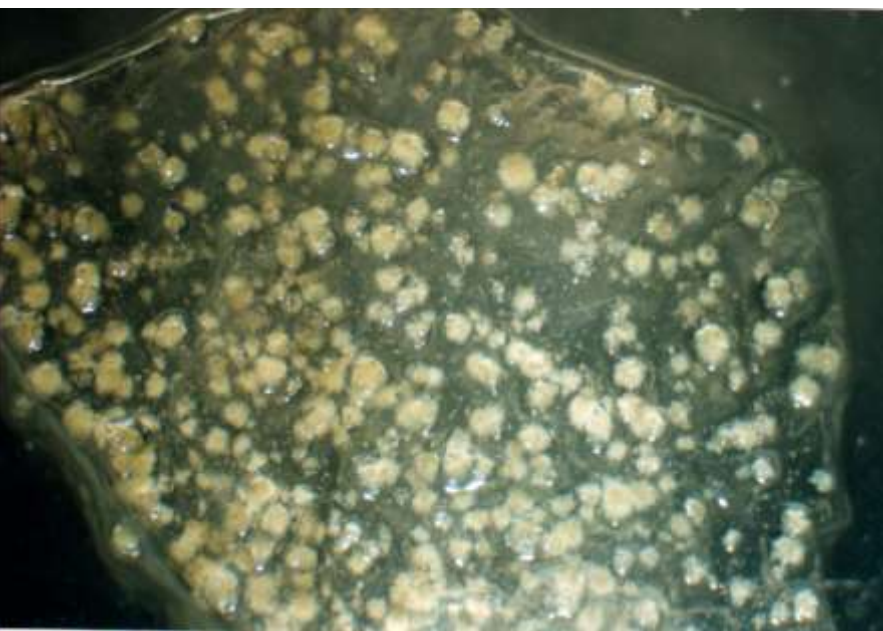


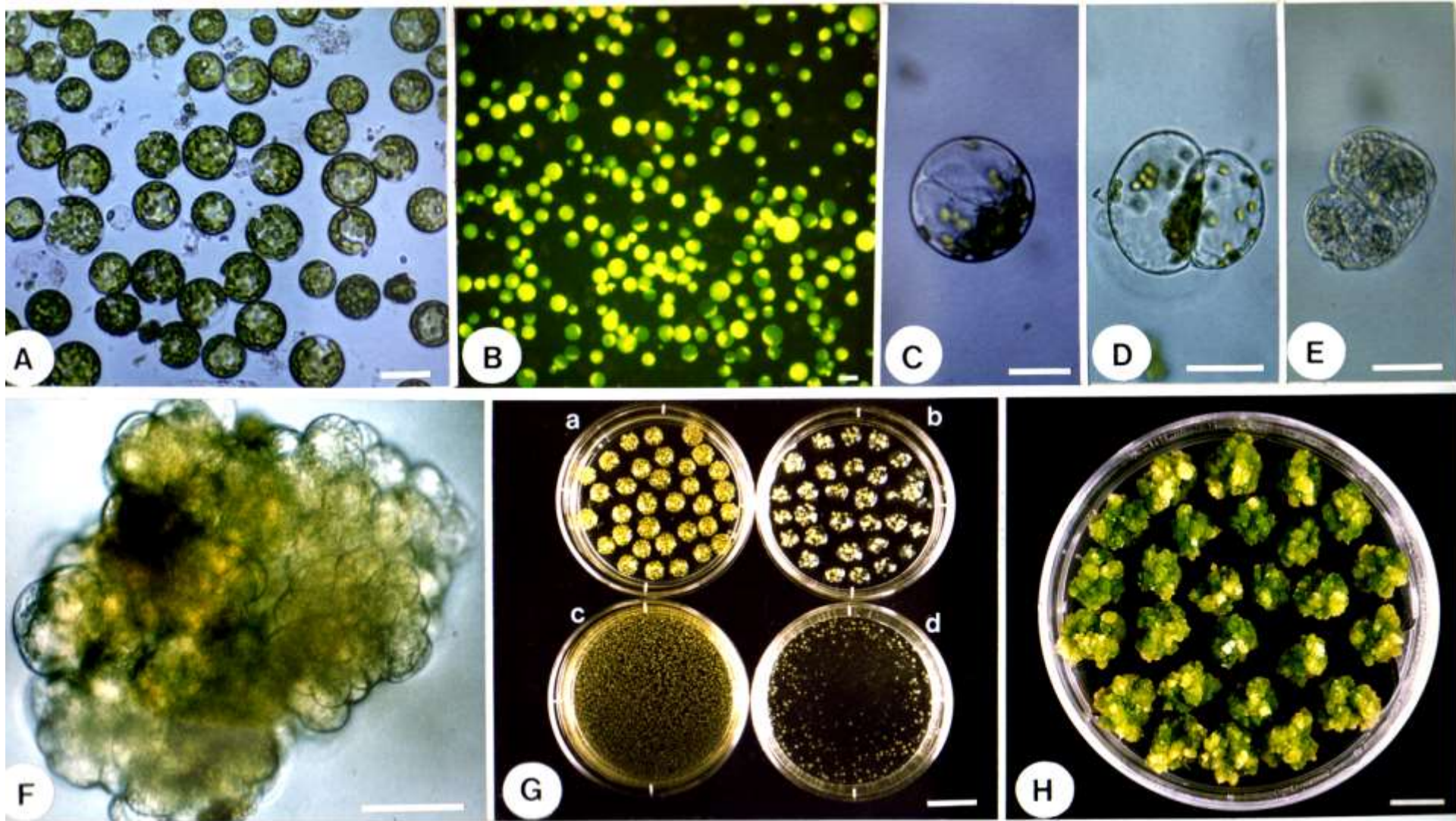
Laboratórios no Brasil

- ESALQ – Departamento de Genética
- EMBRAPA-CENARGEN
- CENA – ESALQ
- UFV
- UFAL
- IAC





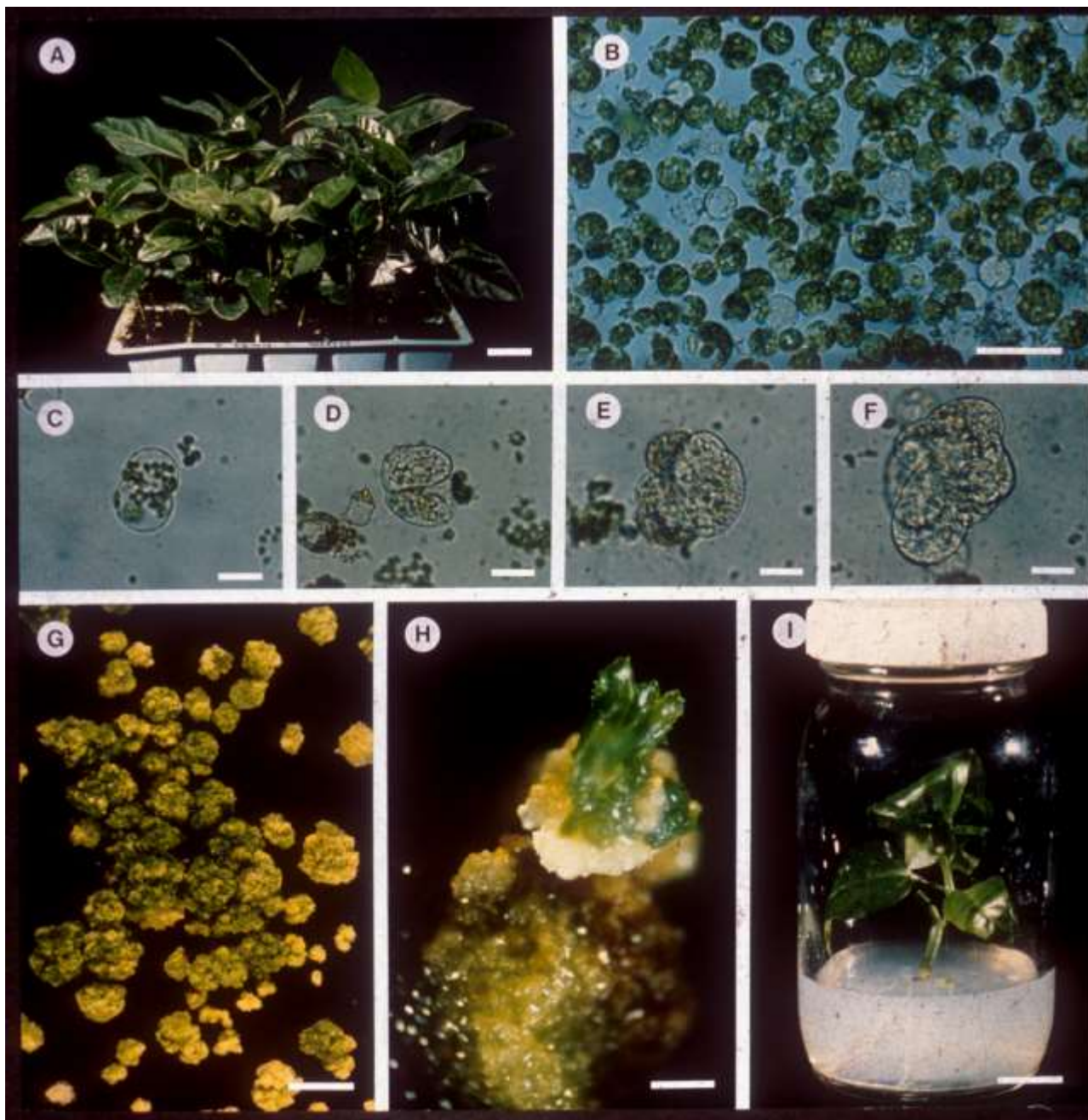




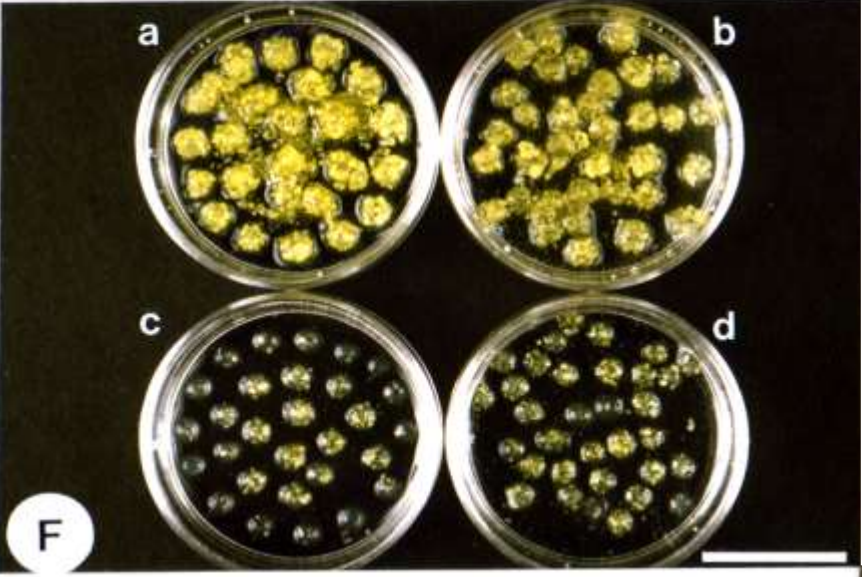
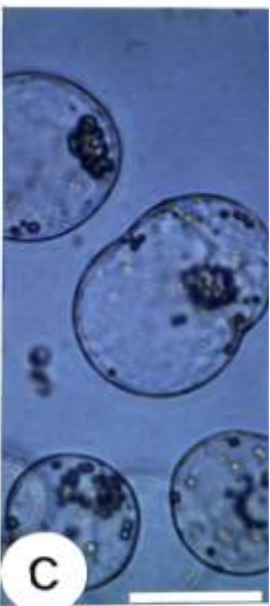
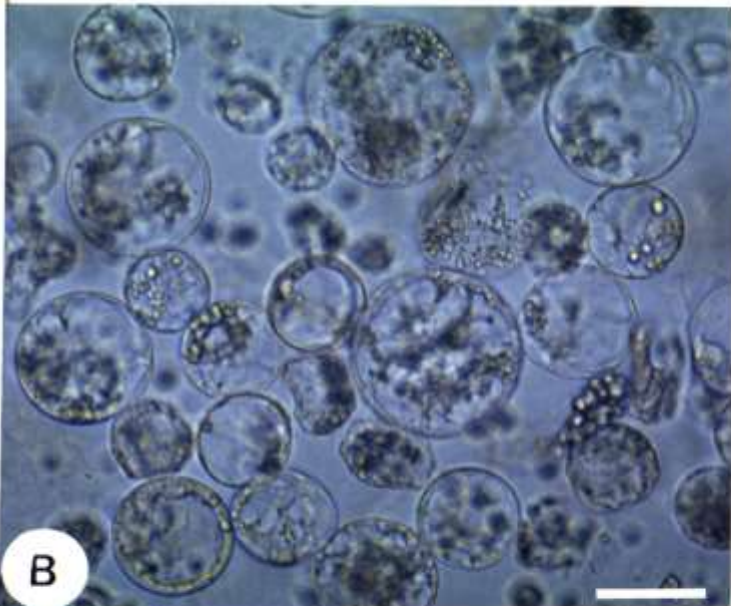
Otoni (1995)



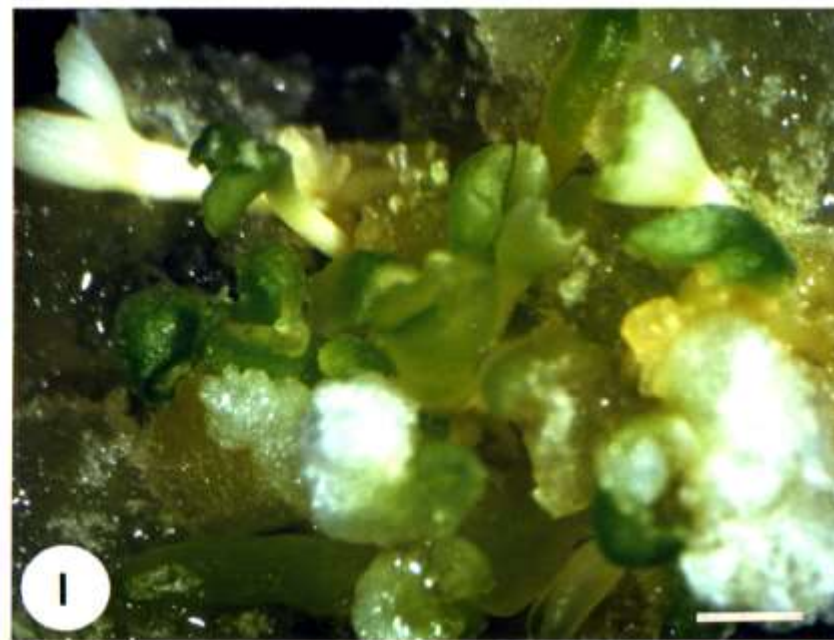
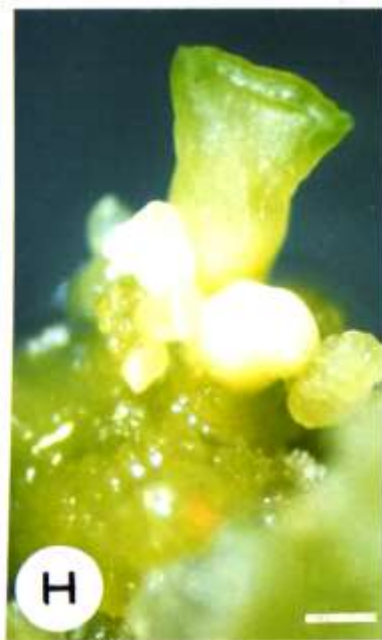
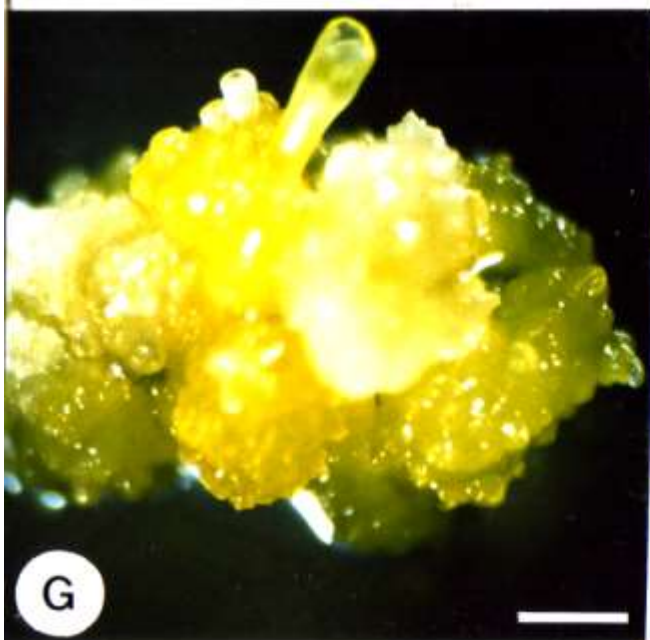
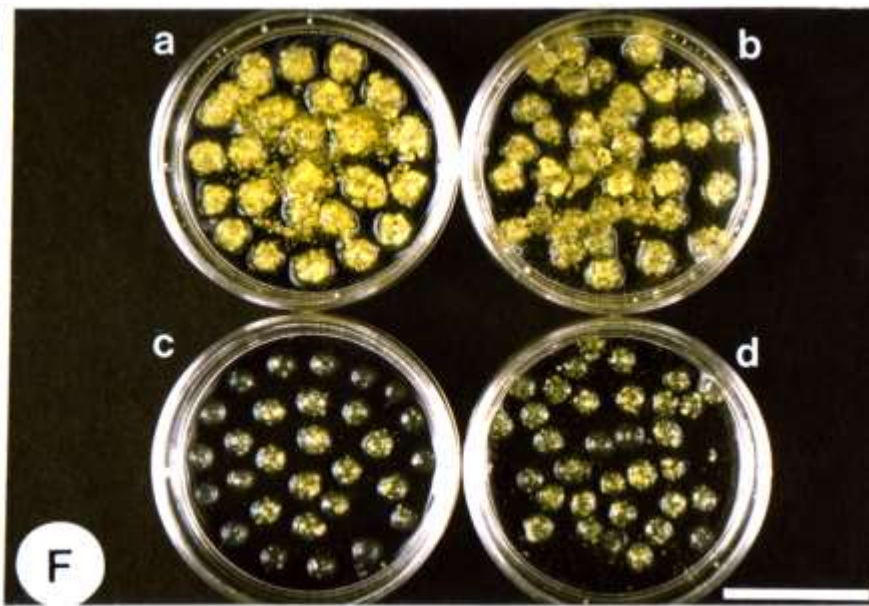
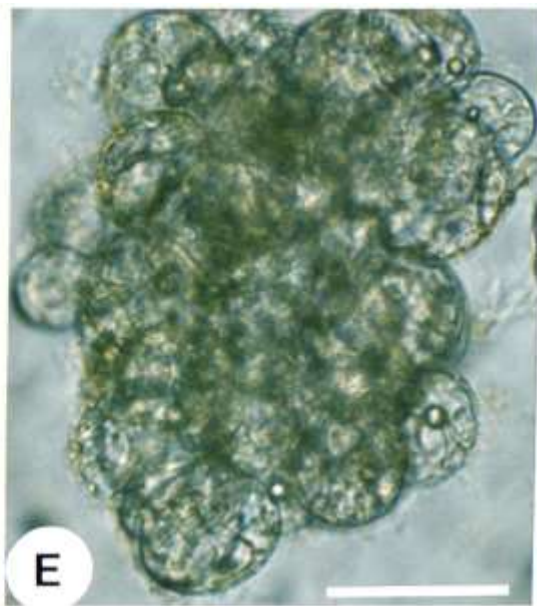
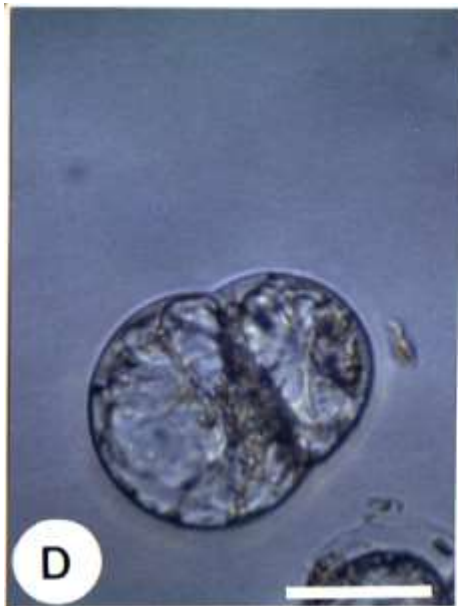
Otoni (1995)

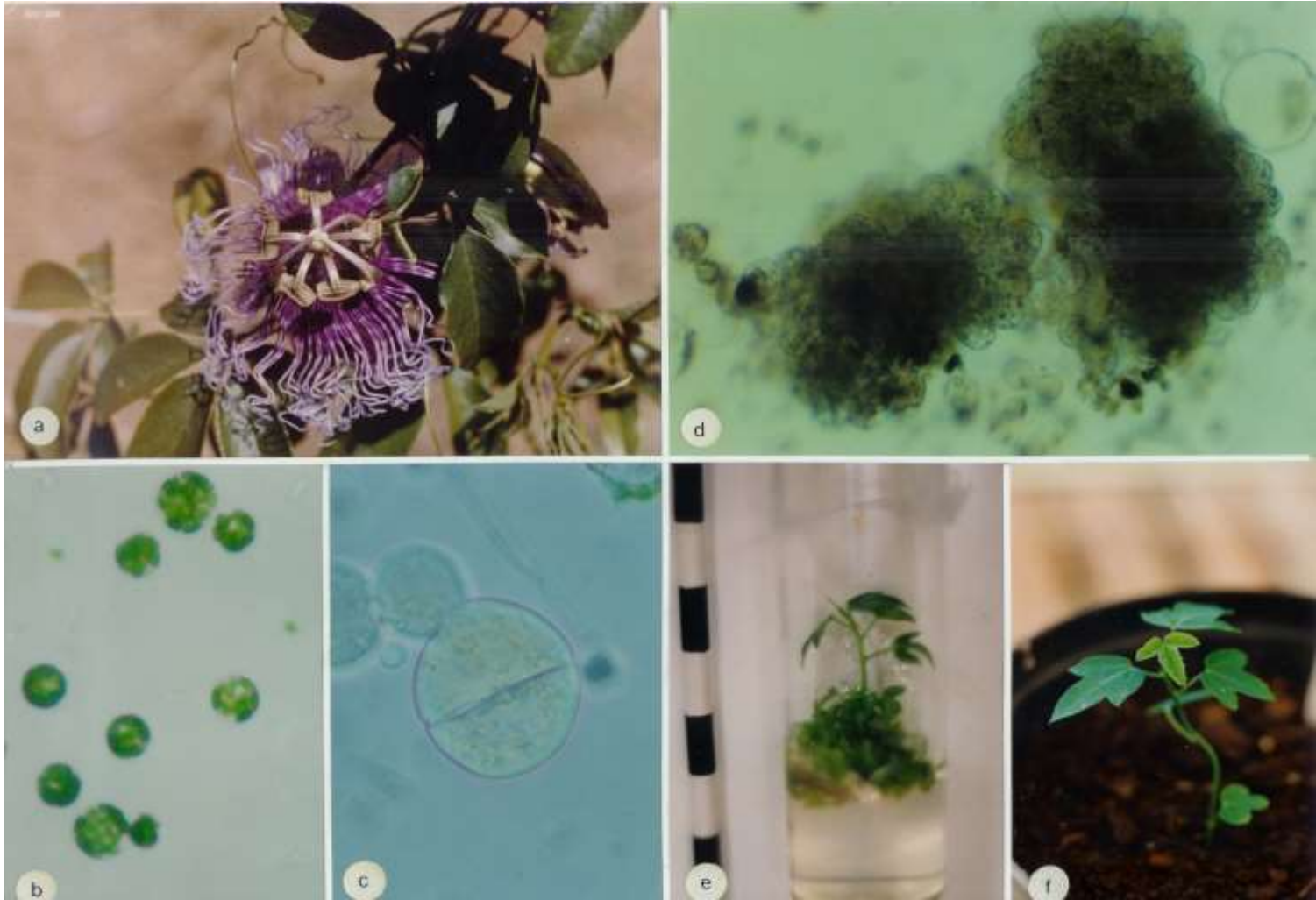


Anthony et al. (1999)



Otoni (1995)





Dornelas et al. (1994)

APLICAÇÕES

(i) Transformação genética

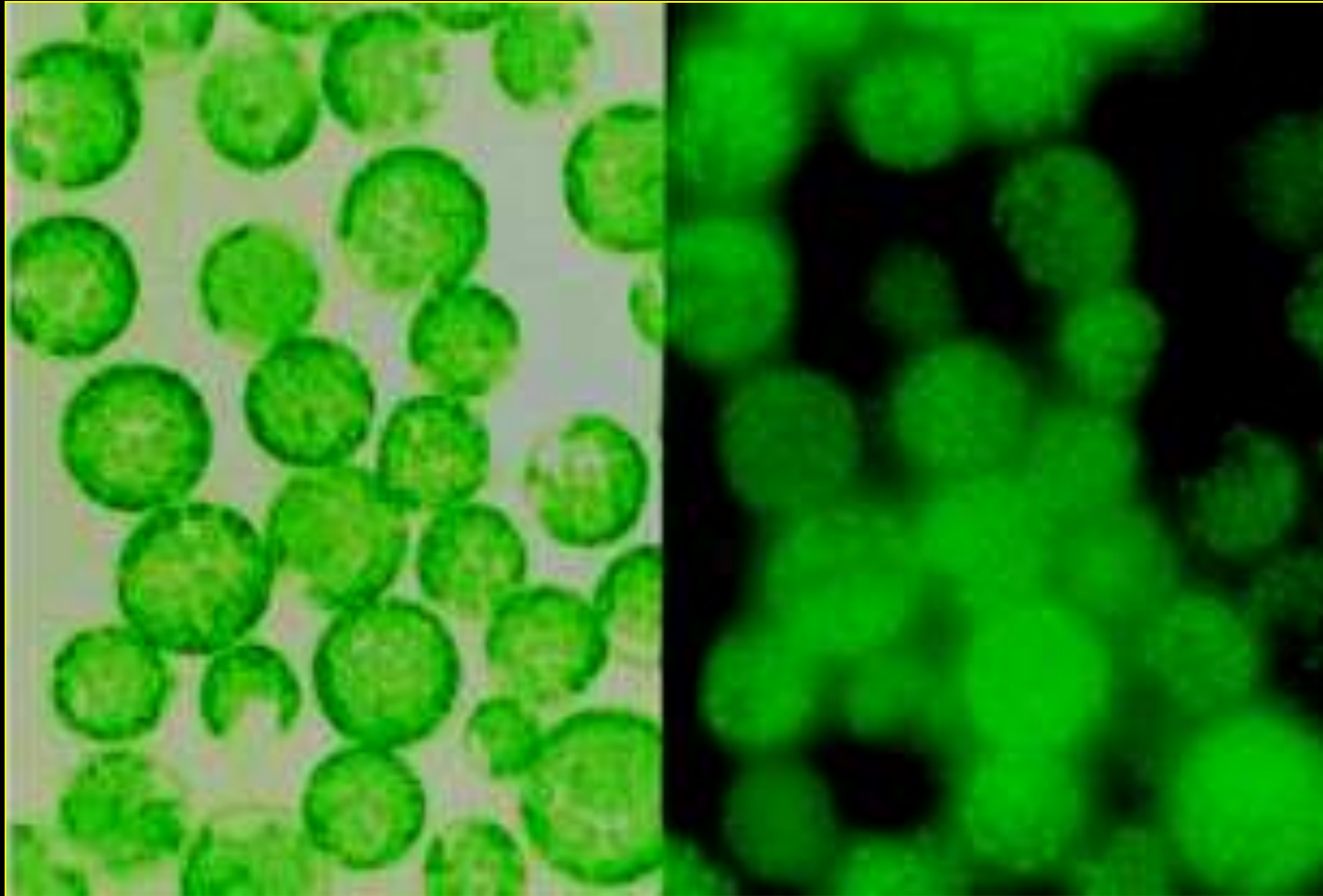
(ii) Ferramenta fundamental para o estudo da síntese de parede celular, transporte de membranas, e do citoesqueleto em relação ao ciclo e divisão celular

(iii) Hibridação somática (cibridização)

(v) Obtenção de mutantes/variantes somaclonais

(vi) Estudo da expressão de genes e sua regulação

Estudo da expressão de genes e sua regulação



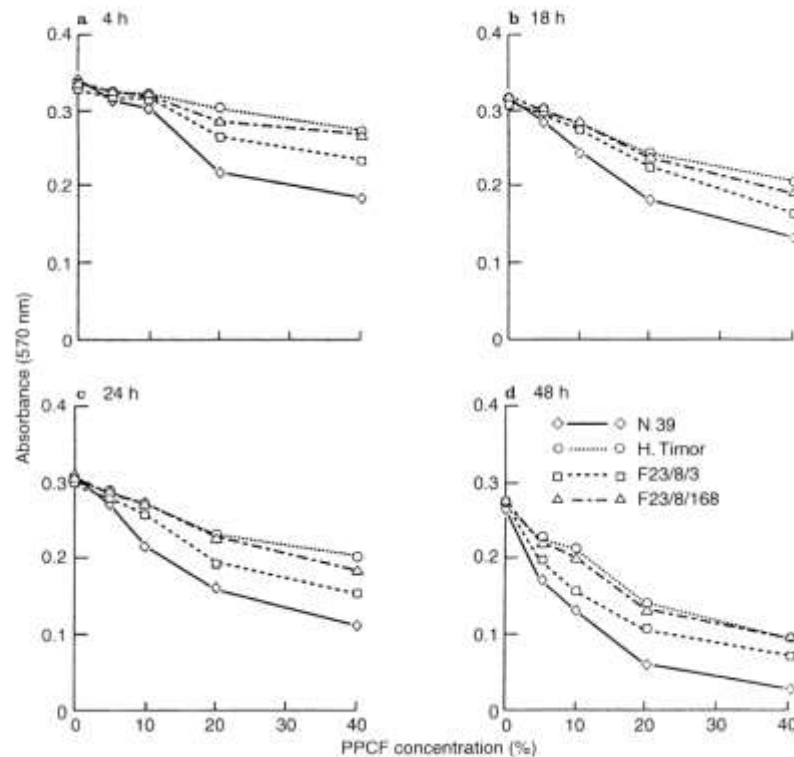
Sheen, 2001

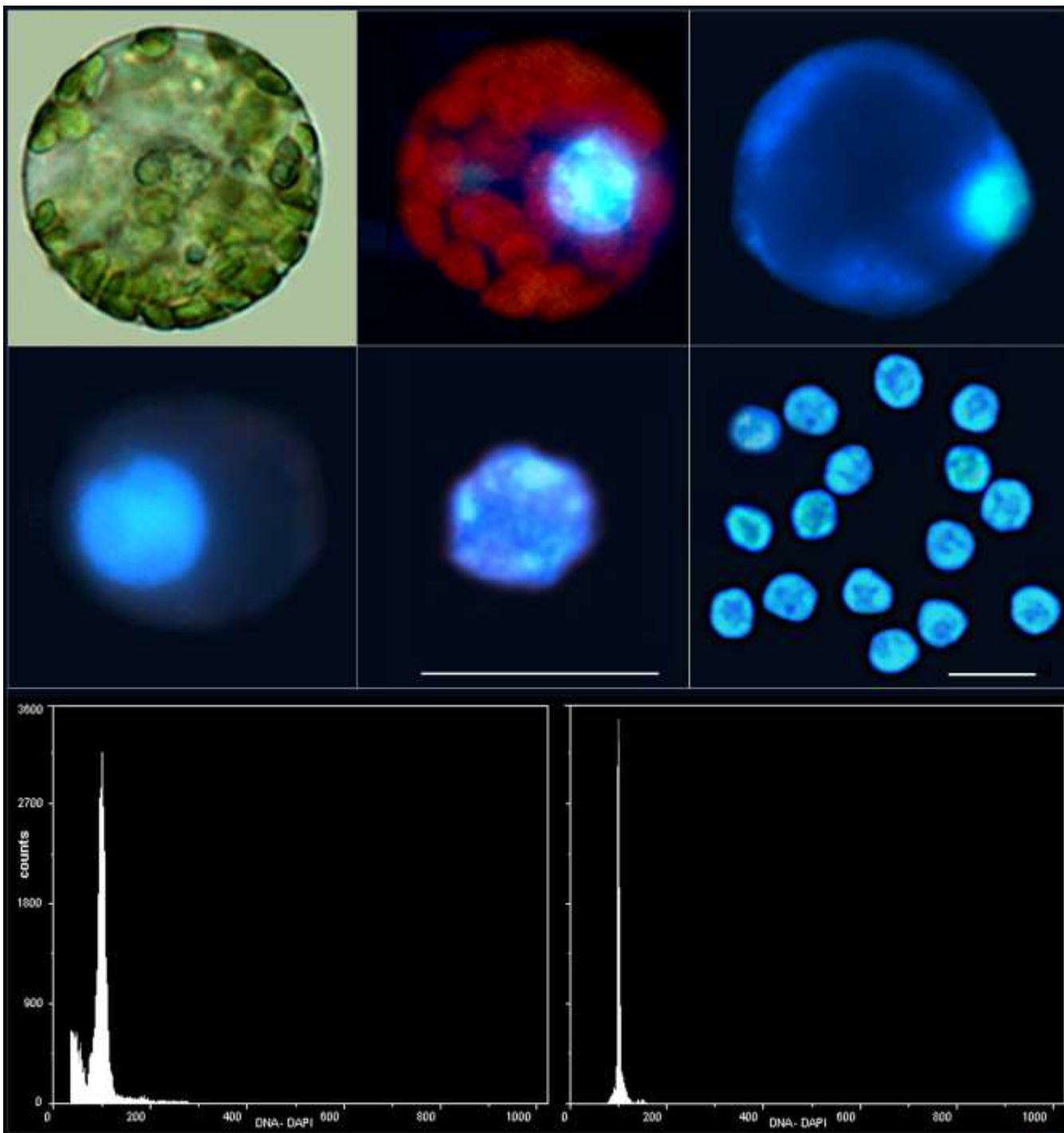
N. E. Nyange · B. Williamson · G. D. Lyon
R. J. McNicol · T. Connolly

Responses of cells and protoplasts of *Coffea arabica* genotypes to partially purified culture filtrates produced by *Colletotrichum kahawae*

767

Fig. 6a–d Effect of various concentrations of PPCFs from *C. kahawae* on the viability of protoplasts of *C. arabica* genotypes after 4, 18, 24 and 48 h. SED=0.007





GFLV replication in electroporated grapevine protoplasts

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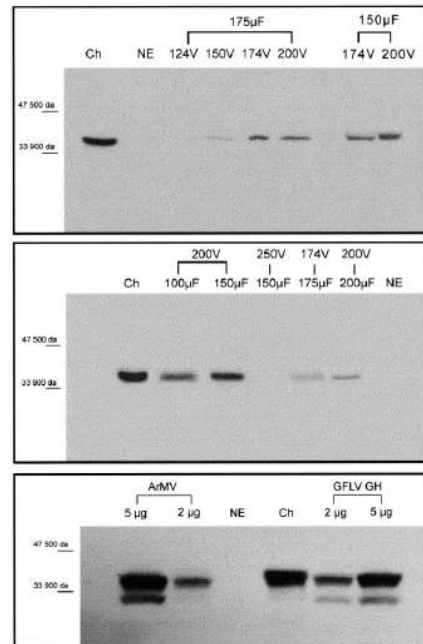


Fig. 3. Virus inoculation and replication in electroporated grapevine protoplasts. (A) Leaf protoplasts, effects of electrical parameters (2 µg of virus particles); (B) cell suspension protoplasts, effects of electrical parameters (2 µg of virus particles); (C) ArMV and GFLV-GH inoculation in suspension protoplasts (200 V–150 µF, 2 and 5 µg of particles). Western blotting with anti P38 antibody, 72 h after electroporation; Ch, GFLV-infected leaves of *Chenopodium*; NE, non-electroporated protoplasts incubated in electroporation mix.

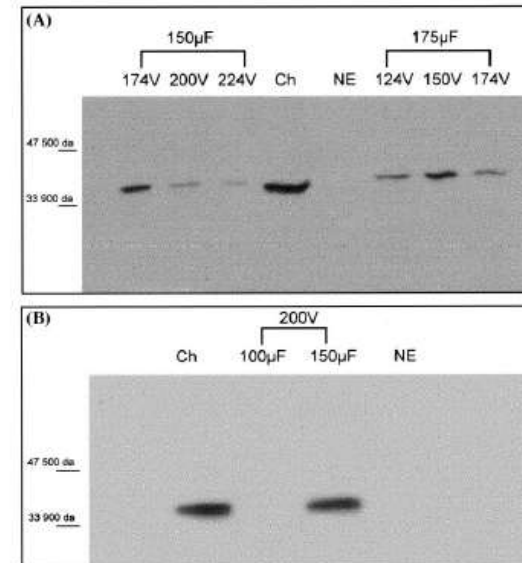


Fig. 4. Viral RNA inoculation and replication in electroporated grapevine protoplasts, effects of electrical parameters. (A) Leaf protoplasts (2 µg of virus particles); (B) Embryogenic cell suspension protoplasts (2 µg of virus particles). Western blotting with anti P38 antibody, 72 h after electroporation; Ch, GFLV-infected leaves of *Chenopodium*; NE, non-electroporated protoplasts incubated in electroporation mix.

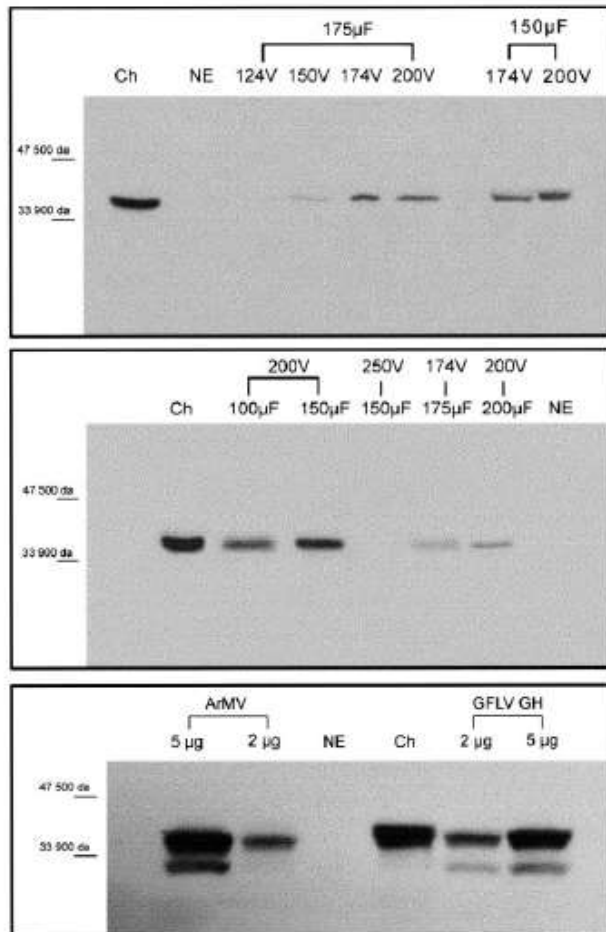


Fig. 3. Virus inoculation and replication in electroporated grapevine protoplasts. (A) Leaf protoplasts, effects of electrical parameters (2 μ g of virus particles); (B) cell suspension protoplasts, effects of electrical parameters (2 μ g of virus particles); (C) ArMV and GFLV-GH inoculation in suspension protoplasts (200 V–150 μ F, 2 and 5 μ g of particles). Western blotting with anti P38 antibody, 72 h after electroporation; Ch, GFLV-infected leaves of *Chenopodium*; NE, non-electroporated protoplasts incubated in electroporation mix.

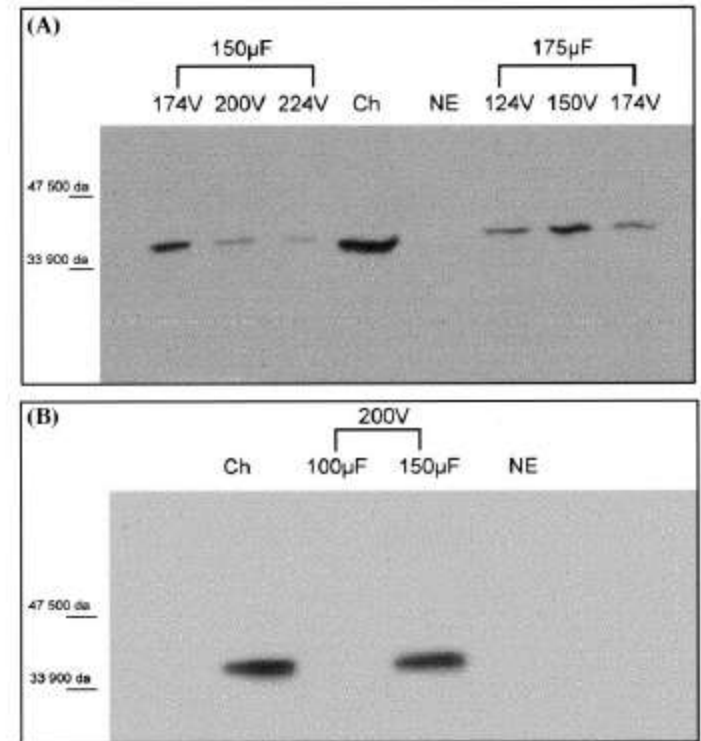


Fig. 4. Viral RNA inoculation and replication in electroporated grapevine protoplasts, effects of electrical parameters, (A) Leaf protoplasts (2 μ g of virus particles); (B) Embryogenic cell suspension protoplasts (2 μ g of virus particles). Western blotting with anti P38 antibody, 72 h after electroporation; Ch, GFLV-infected leaves of *Chenopodium*; NE, non-electroporated protoplasts incubated in electroporation mix.

Original article

Phytoalexin production in grapevine protoplasts during isolation and culture

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Received 14 October 2002; accepted 12 December 2002

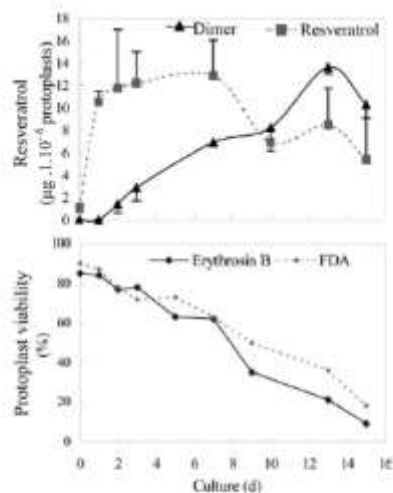


Fig. 4. Resveratrol and resveratrol dehydromer production and protoplast survival during the first 2 weeks of culture of CH leaf protoplasts. A, resveratrol production (means of three independent experiments); B, protoplast viability estimated by FDA or erythrosin B staining (mean of two independent protoplast populations).

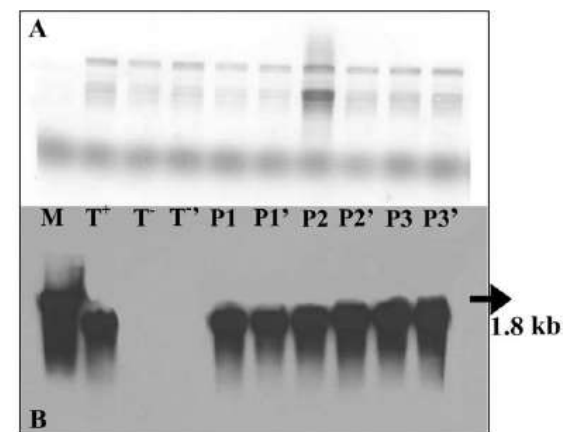


Fig. 3. Northern blot analysis of STS mRNAs. A, electrophoretic profile of total RNAs after methylene blue staining; B, northern blot using the cDNA *VstI* fragment as probe; M, molecular marker; T⁻, healthy fresh *in vitro* leaves; T⁺, fresh leaves infected with *Botrytis*; P1, 750 × 10³ protoplasts; P2, 1 × 10⁶ protoplasts; P3, 1.5 × 10⁶ protoplasts; P₂' and P₃', two independent experiments; 1–2 µg of total RNA were loaded in each lane.

CITRUS SOMATIC HYBRIDIZATION WITH POTENTIAL FOR IMPROVED BLIGHT AND CTV RESISTANCE

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¹Laboratório de Biotecnologia Vegetal, Centro de Energia Nuclear na Agricultura, 13416-970 Piracicaba/SP, Brazil

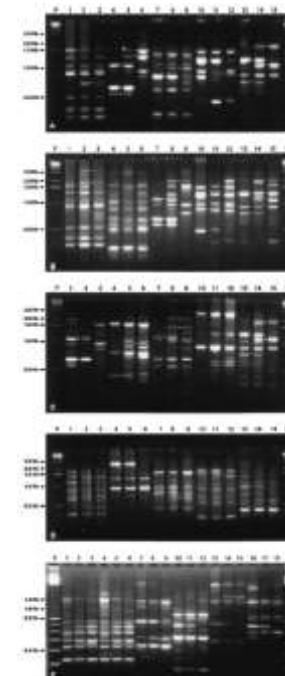
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(Received 9 November 2000; accepted 7 March 2001; editor S. S. Korban)

TABLE 1

PARENTAL COMBINATIONS AND PLANTS REGENERATED
FOLLOWING PROTOPLAST FUSION

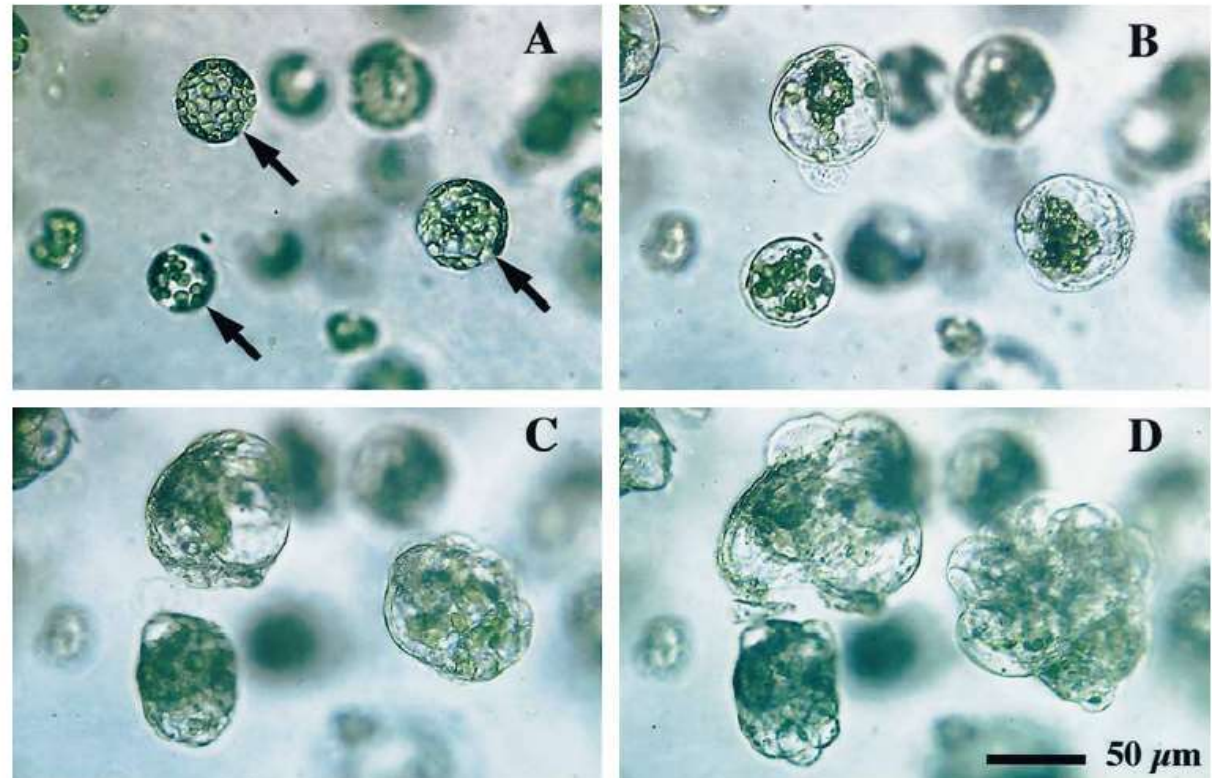
Embryogenic parent	Leaf parent	Number of regenerated somatic hybrid plants
<i>Citrus sinensis</i>	<i>C. reticulata</i>	
Caipira sweet orange	Cleopatra mandarin	9
<i>C. sinensis</i>	<i>C. volkameriana</i>	
Caipira sweet orange	Volkamer lemon	4
<i>C. sinensis</i>	<i>C. jambhiri</i>	
Caipira sweet orange	Rough lemon	4
<i>C. reticulata</i>	<i>C. aurantium</i>	
Cleopatra mandarin	Sour orange	23
<i>C. limonia</i>	<i>C. aurantium</i>	
Rangpur lime	Sour orange	7



N. Kuchuk · R. G. Herrmann · H.-U. Koop

Plant regeneration from leaf protoplasts of evening primrose (*Oenothera hookeri*)

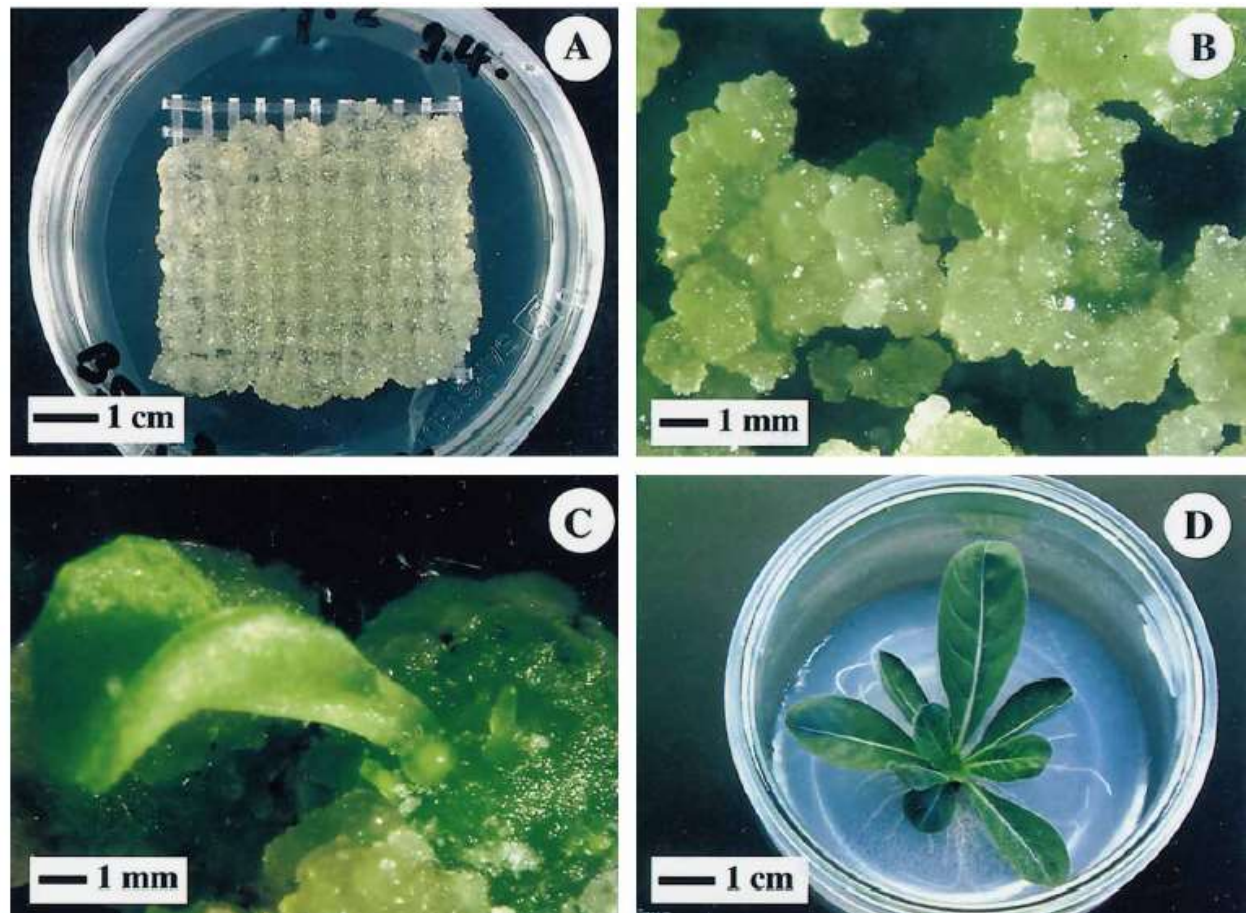
Fig. 1A–D Development of leaf protoplasts from *Oenothera hookeri* embedded in a thin layer of alginate. **A** Three randomly chosen protoplasts (*arrows*) photographed on the 1st day of culture. **B** Regenerants of these cells on the 5th day of culture. **C** The 7th day of culture. **D** The 10th day of culture. Note that all of the randomly chosen protoplasts developed into multicellular colonies, reflecting a high plating efficiency



N. Kuchuk · R. G. Herrmann · H.-U. Koop

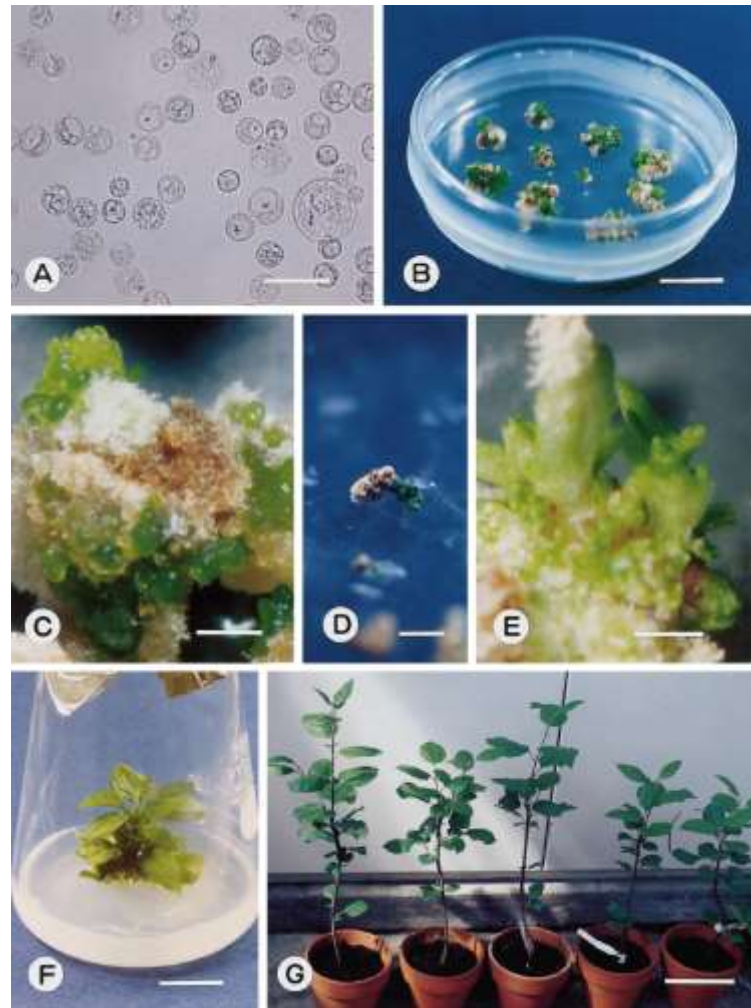
Plant regeneration from leaf protoplasts of evening primrose (*Oenothera hookeri*)

Fig. 2A–D Plantlet development from protoplast-derived colonies of *O. hookeri*. **A** After 3 weeks of culture, a cell lawn has grown in the alginate layer supported by a polypropylene mesh. At this stage, the colonies are recovered by dissolving the alginate gel, and are plated onto solid medium. **B** Green friable callus after 4–5 weeks of culture. **C** Shoot primordia developing after



A. Saito · M. Suzuki

Plant regeneration from meristem-derived callus protoplasts of apple (*Malus × domestica* cv. ‘Fuji’)



Levantamento de Trabalhos

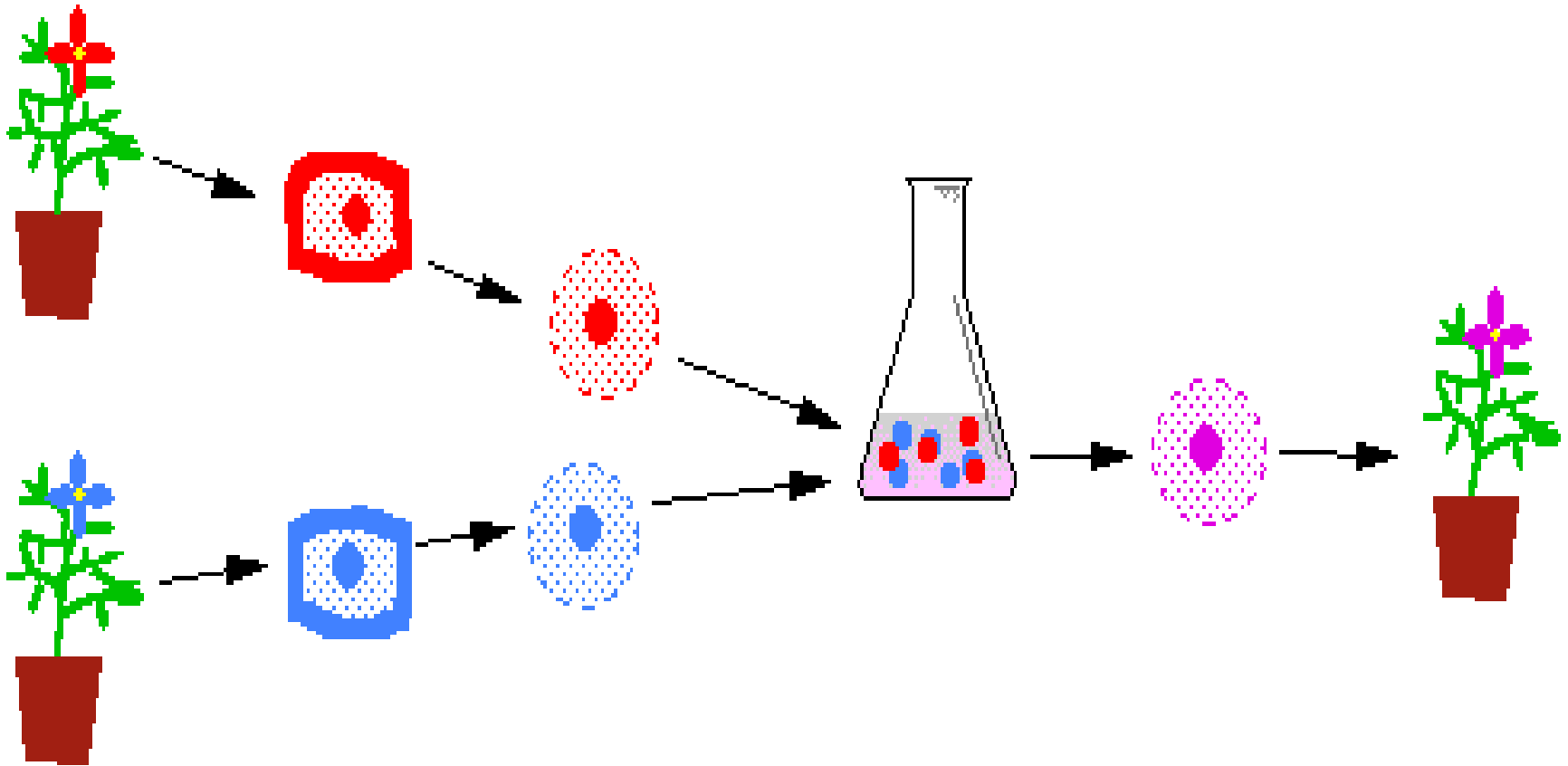
- IVCDB-P, PCTOC, PCR (1990-Agosto 2005)
- Queda considerável de publicações envolvendo protoplastos.

Davey M.R., Anthony P., Power J.B. and Lowe K.C. (2005) Isolation and culture of plant protoplasts : Historical perspectives and current status (Chapter 2). In: Journey of a Single Cell to a Plant. Murch S.J. and Saxena P.K. (Eds.) Science Publishers, Inc., Enfield (NH), USA, Plymouth, UK., pp. 37-57.

Davey, MR, Anthony, P, Power, JB and Lowe, KC (2005) Research Review Paper: Plant protoplasts: status and biotechnological perspectives. Biotechnology Advances 23, 131-171.

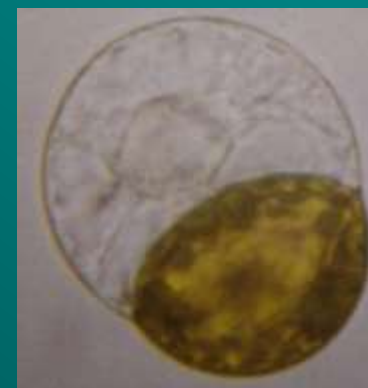
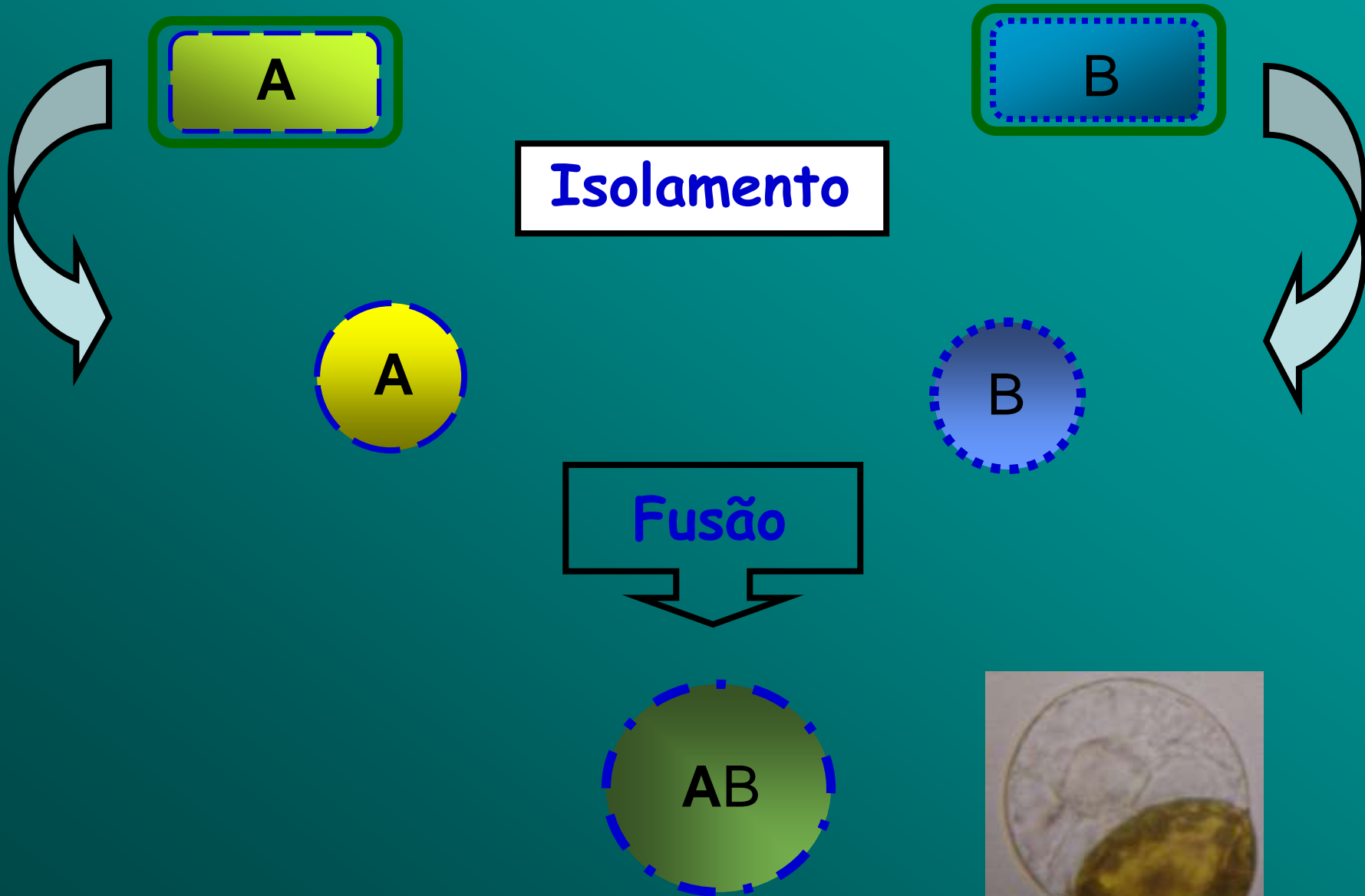


Hibridação Somática



Agentes Fusogênicos ("Fusão química")

- Nitrato de sódio.
- Combinação de elevado pH (10,5), altas concentrações de cálcio e alta temperatura (37°C)
- Polietileno glicol (PEG) é o agente de uso mais amplo.



Eletrofusão



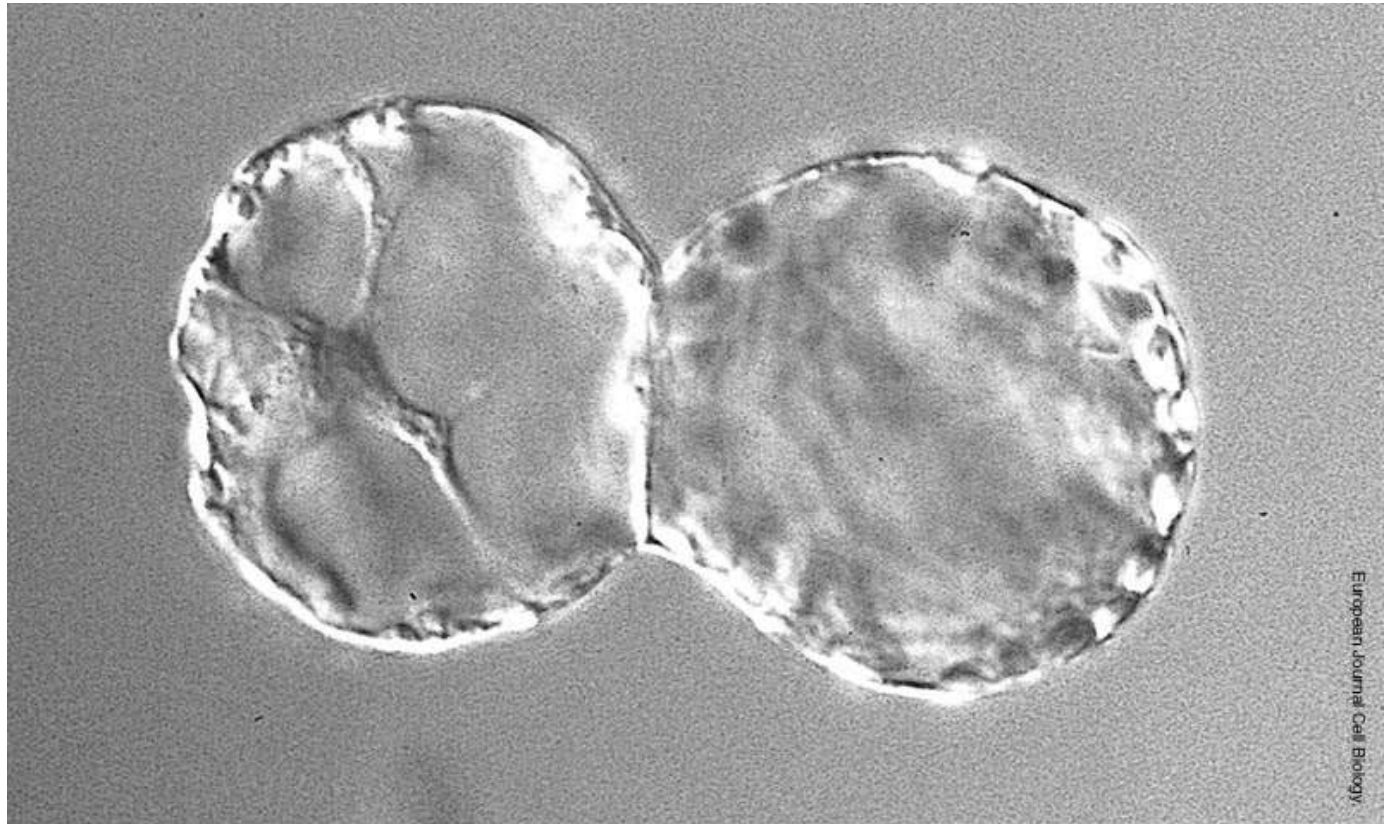
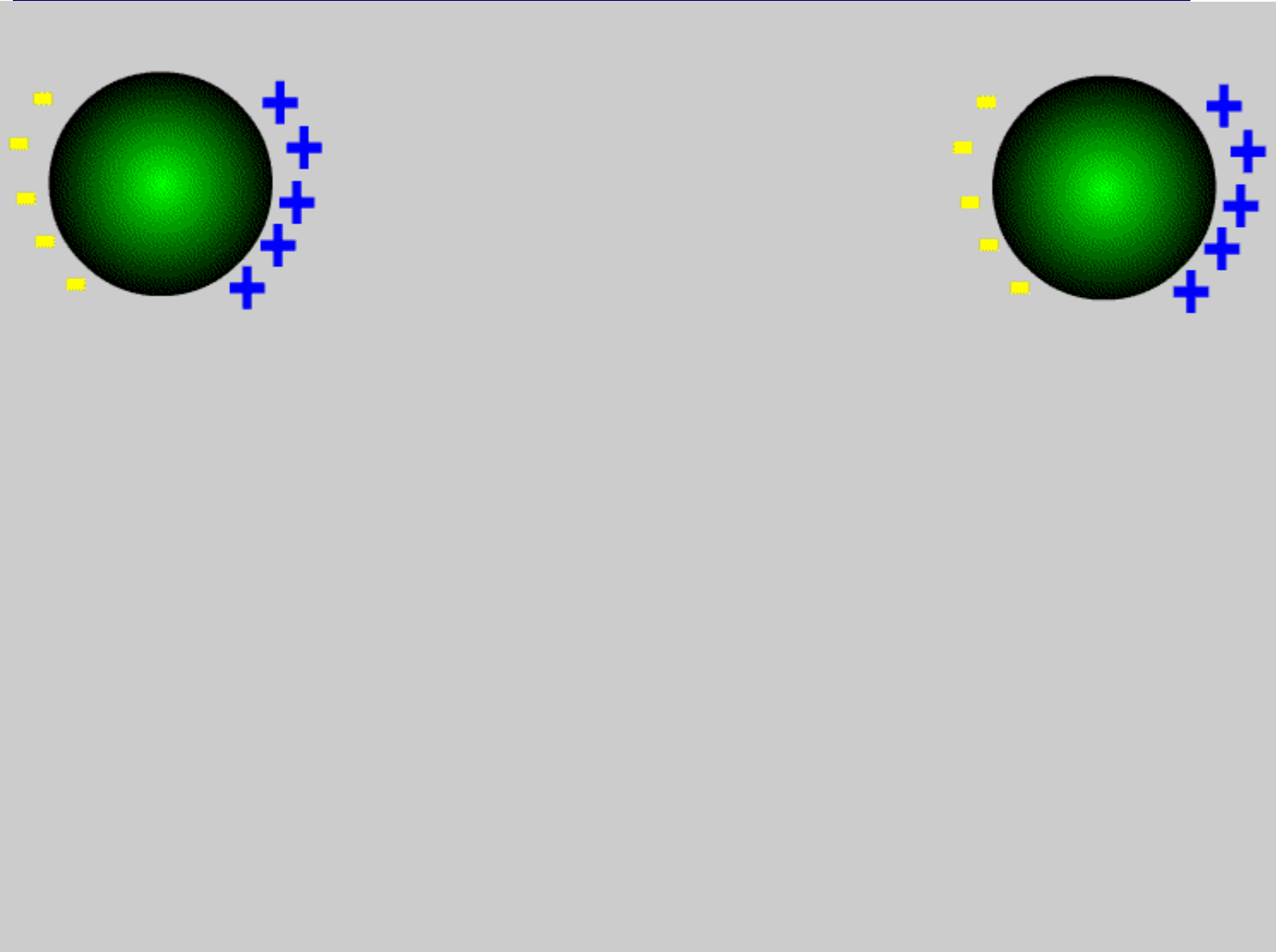
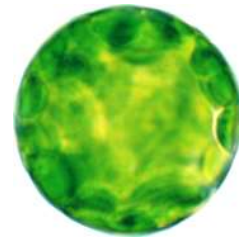
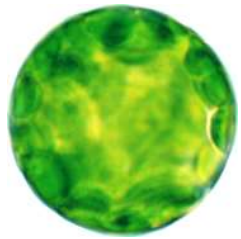
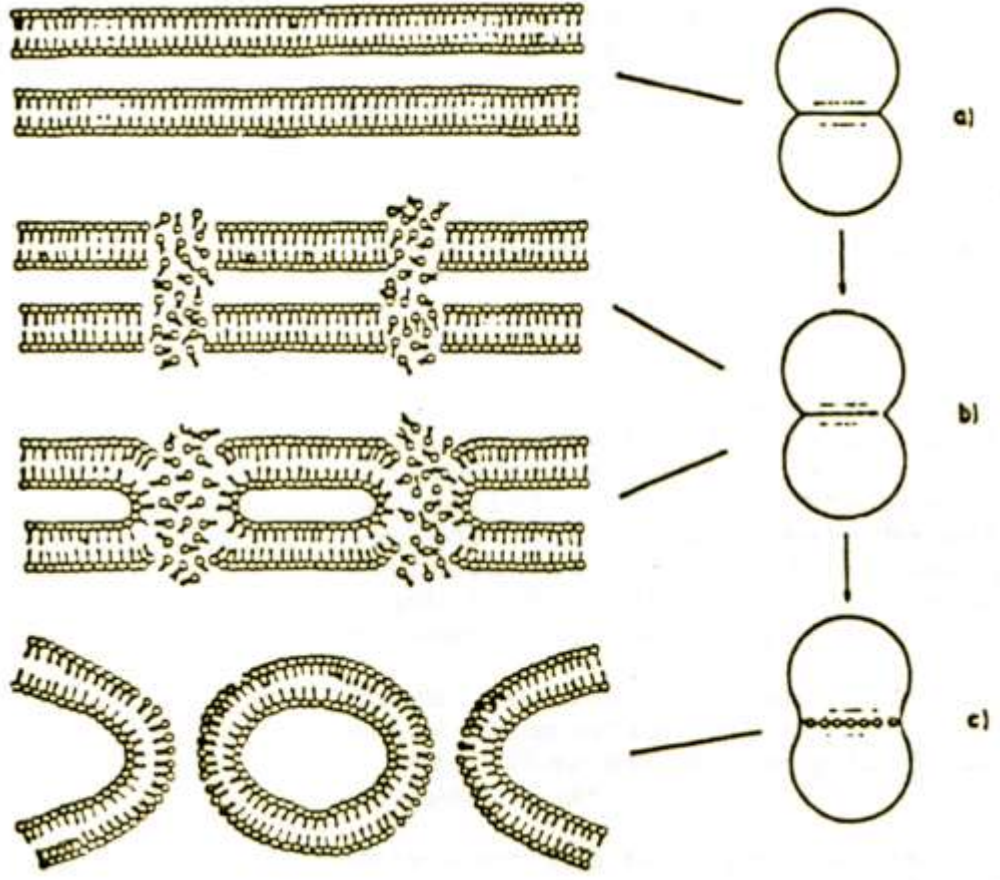
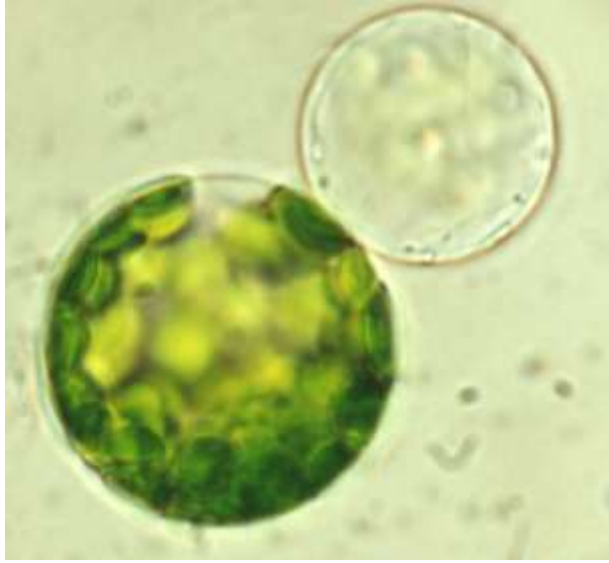
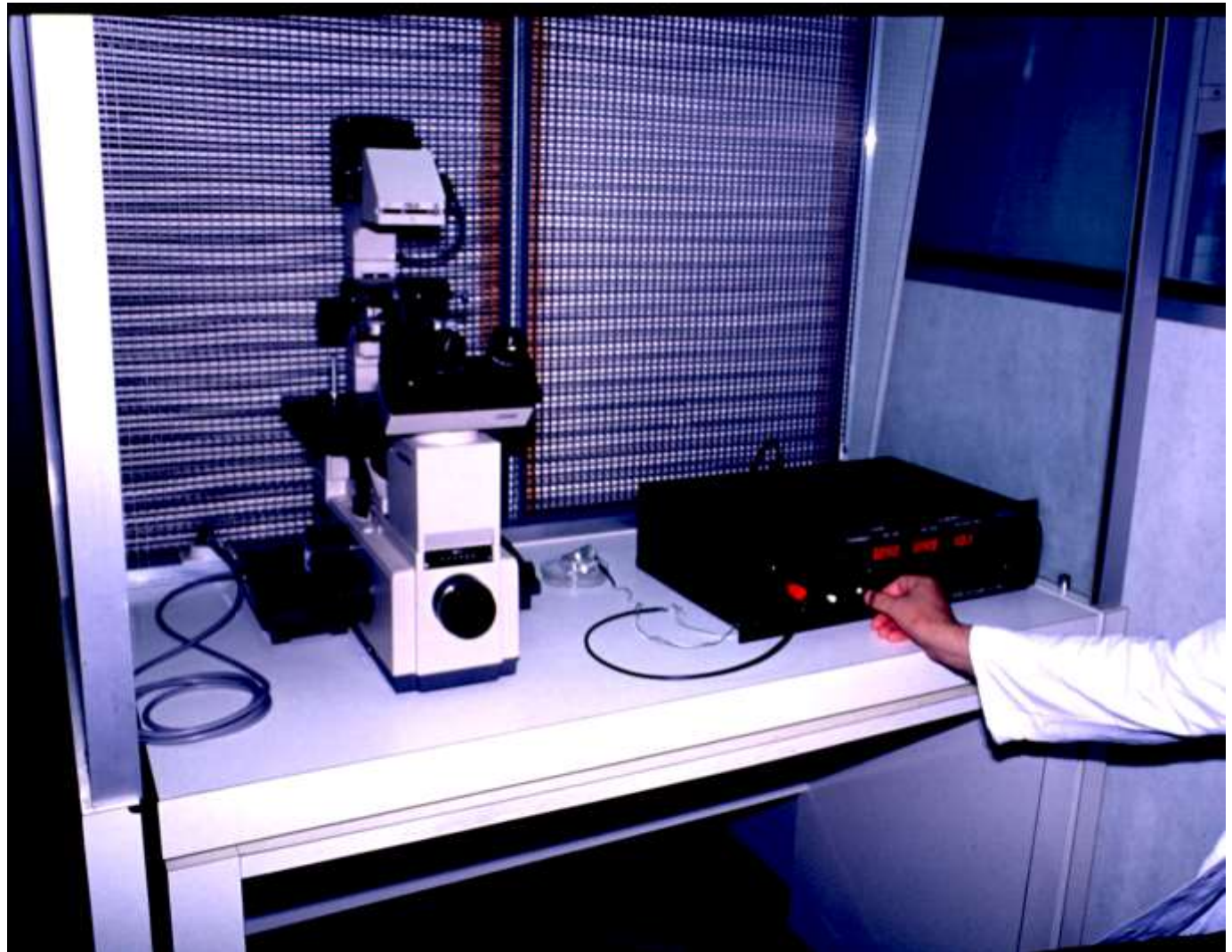
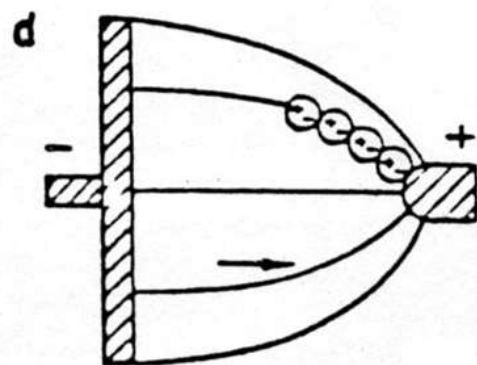
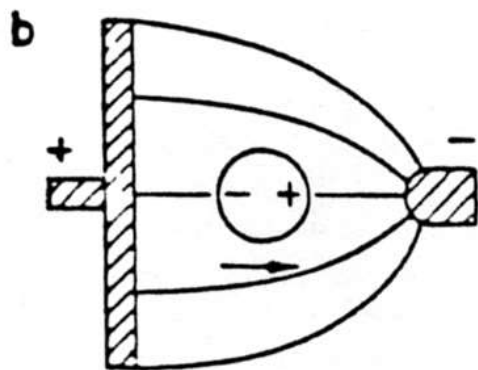
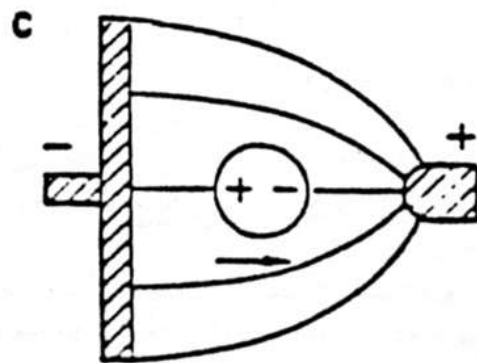
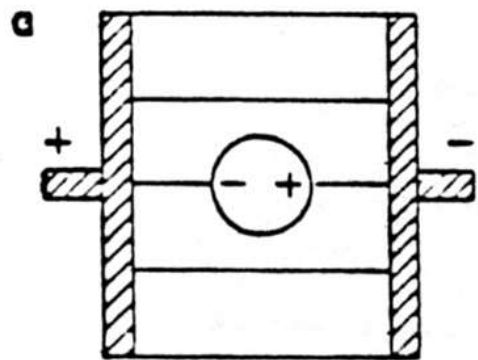


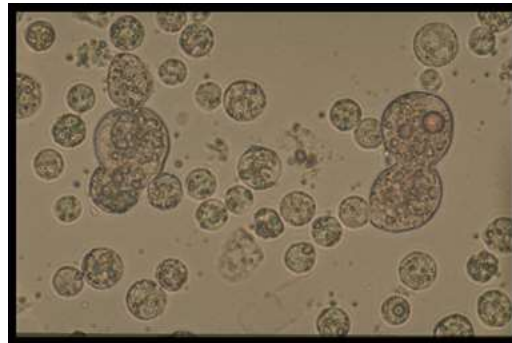
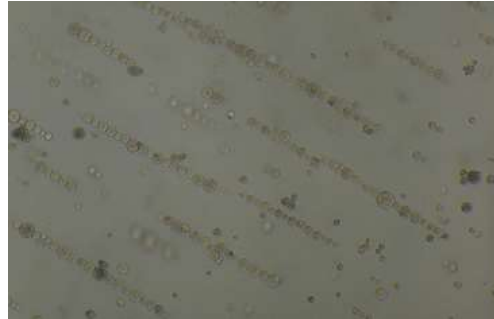
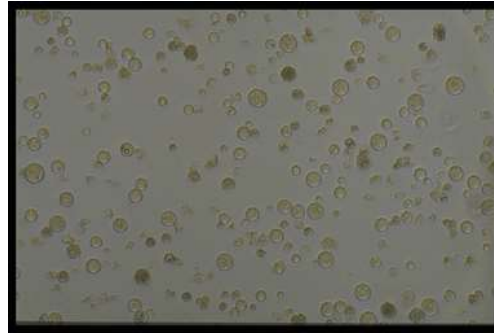
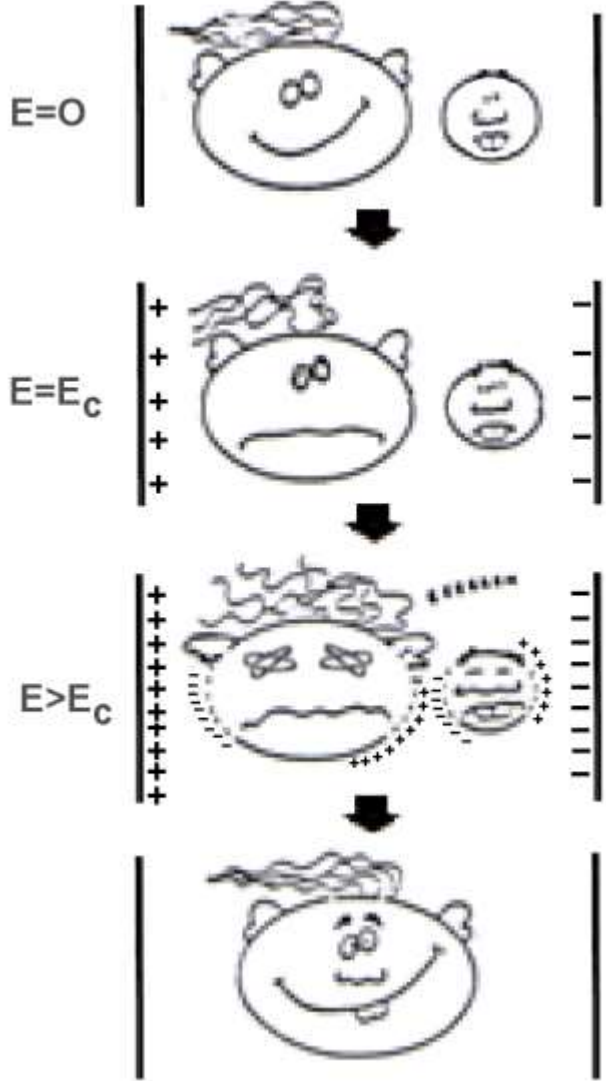
FIGURE 6.6a Two tobacco plant protoplasts, obtained by digesting away the cell wall, are fused (6a and 6b) to produce a cell that acquires some of the characteristics of both genetic backgrounds and can be regenerated into a plant with some traits from both parental plants.

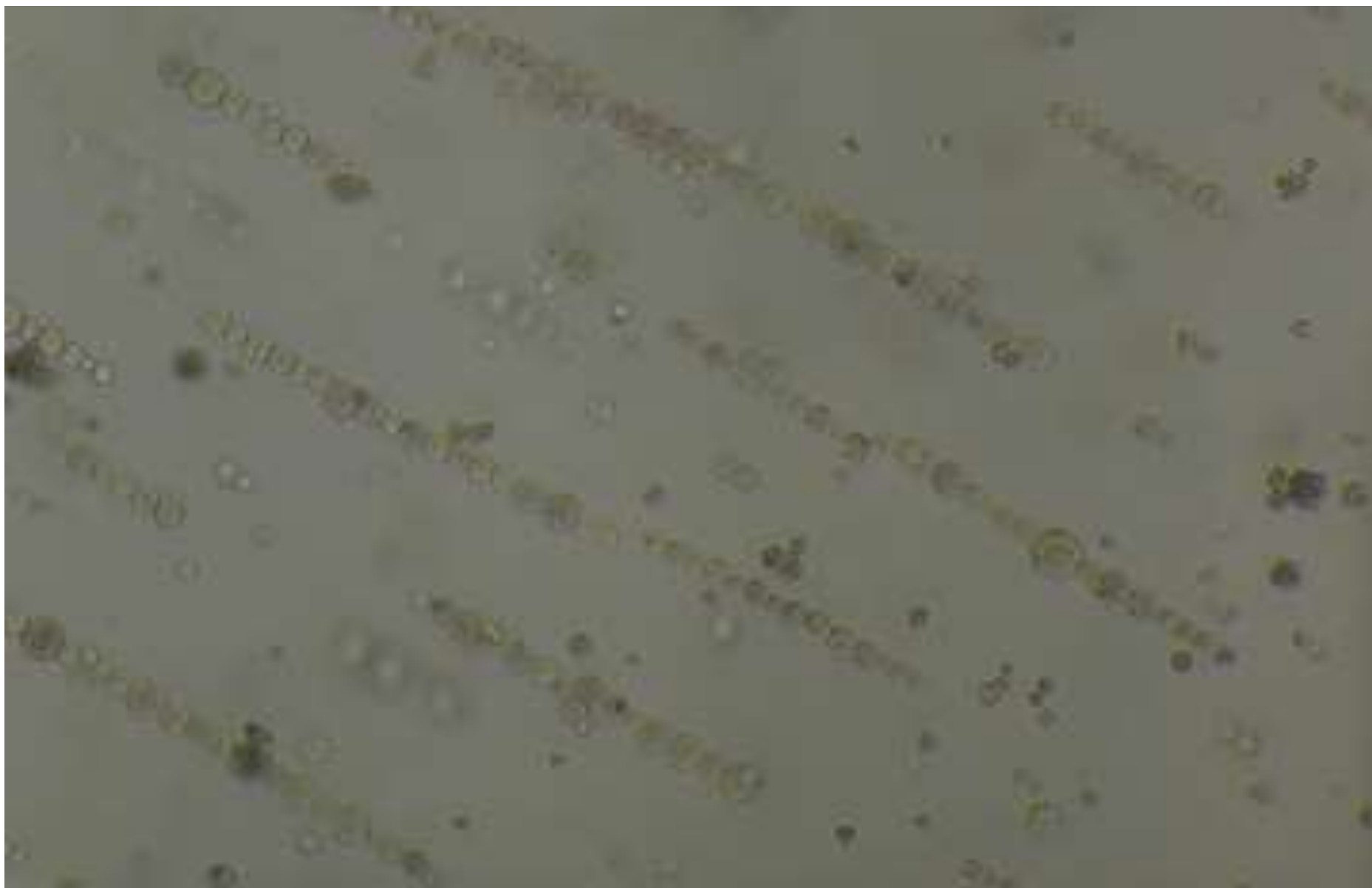


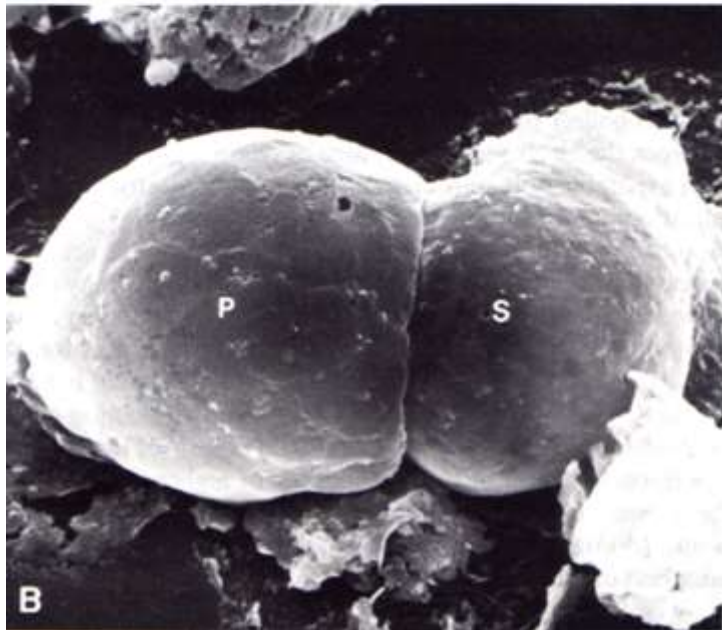
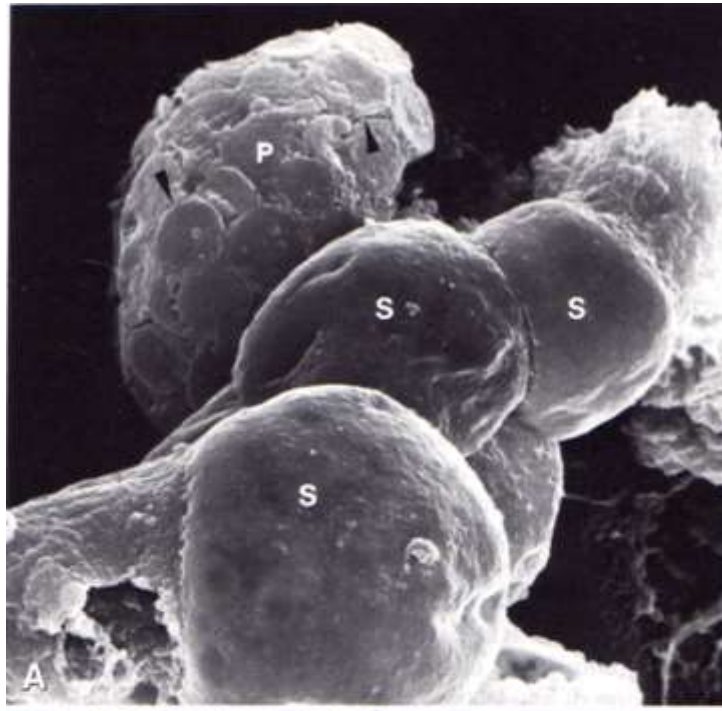


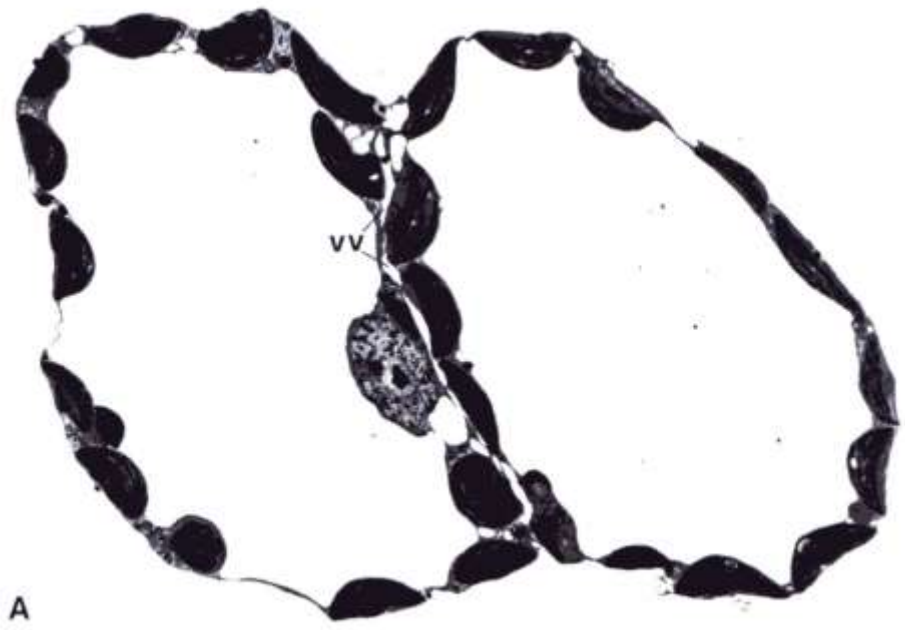




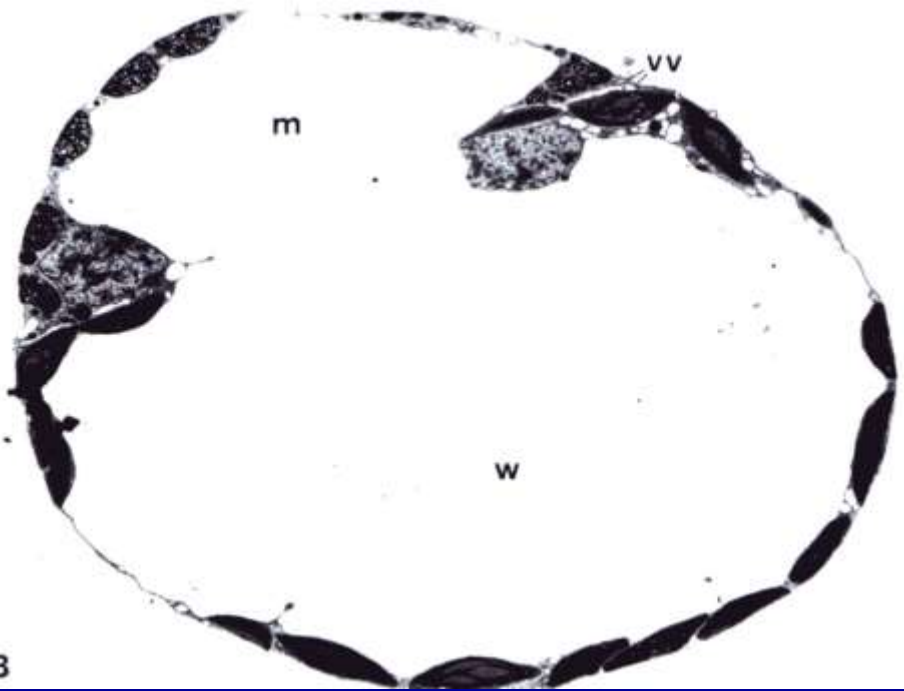




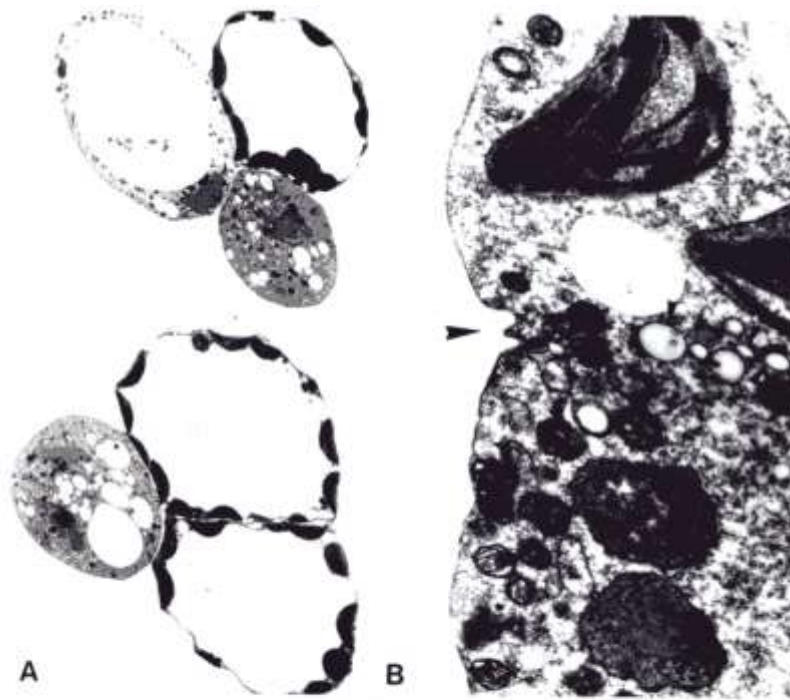




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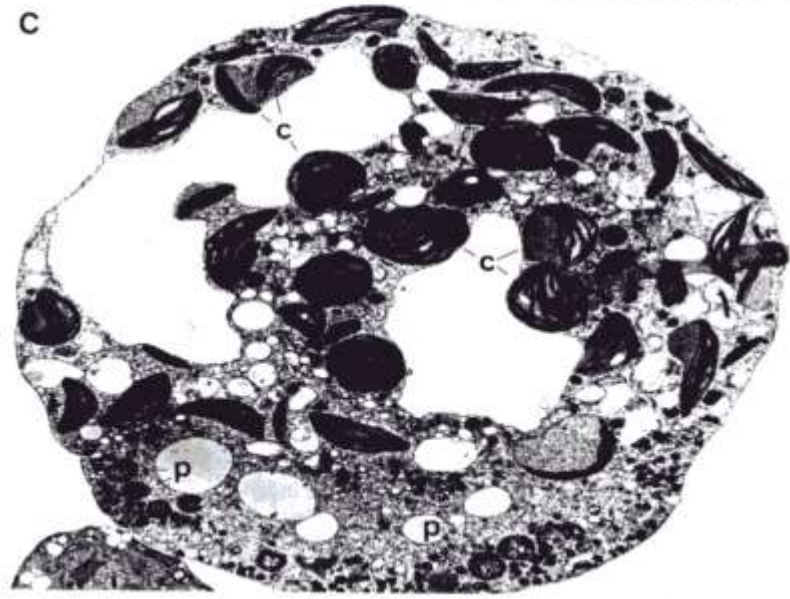
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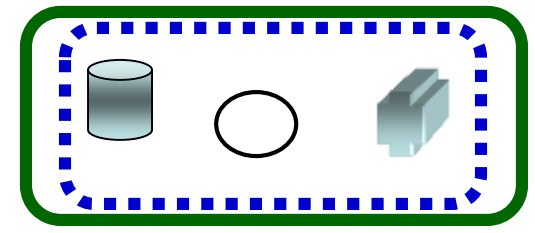
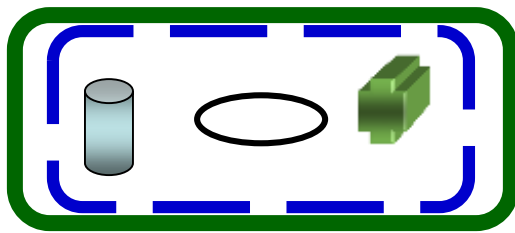


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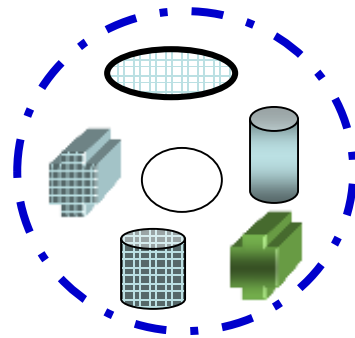




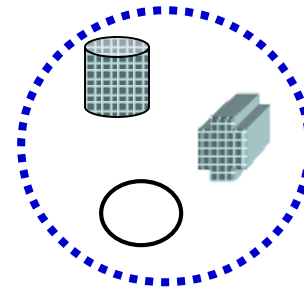
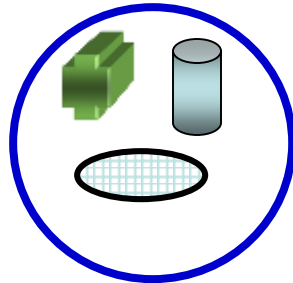
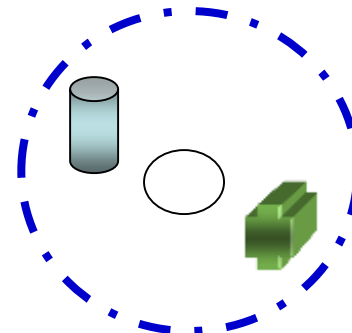
Isolamento



Fusão



Cíbrido



 Inativação

Fig: 1a

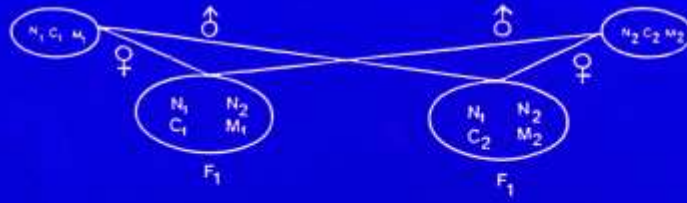
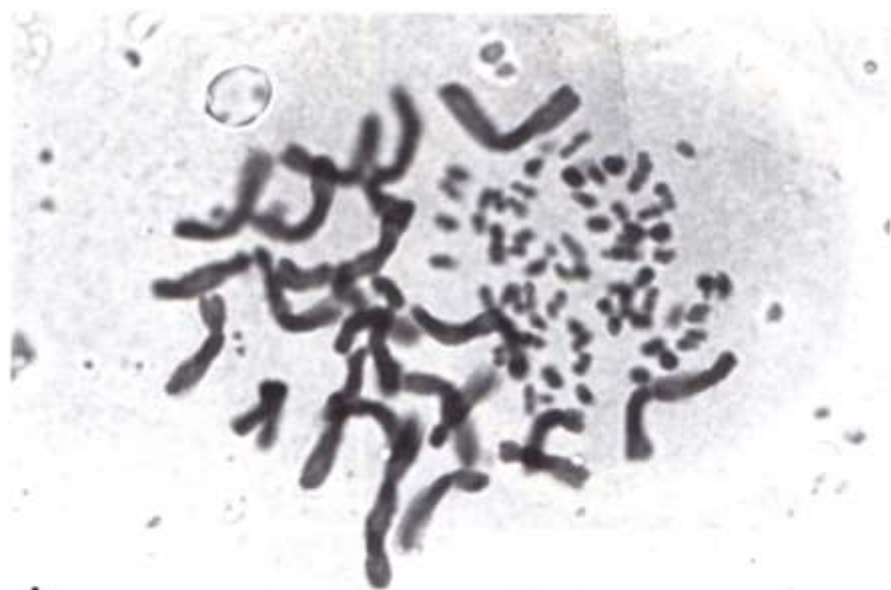


Fig: 1b

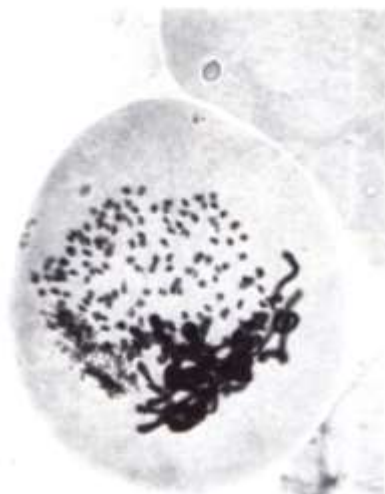


CARACTERIZAÇÃO

- Padrões eletroforéticos e de isoenzimas
- Análises morfológicas
- Análises cromossômicas
- Citometria de fluxo
- RAPD, AFLP
- Fitoquímica



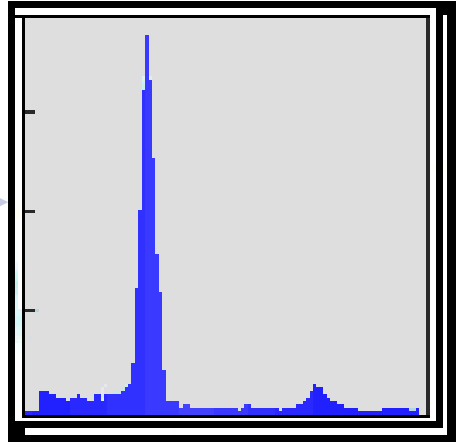
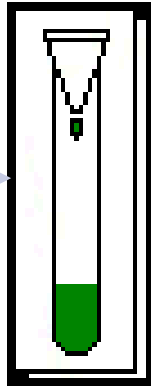
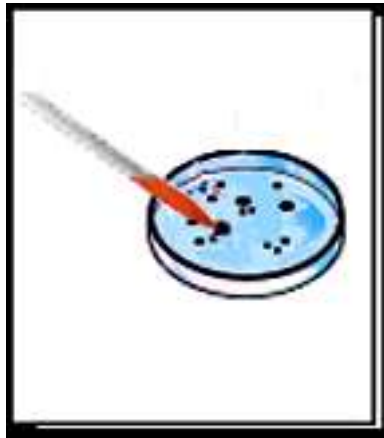
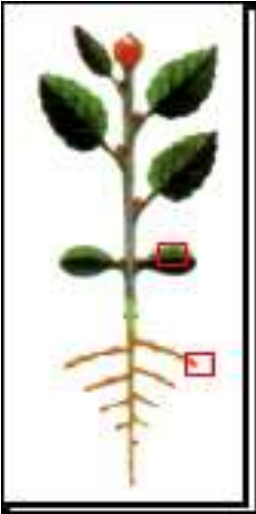
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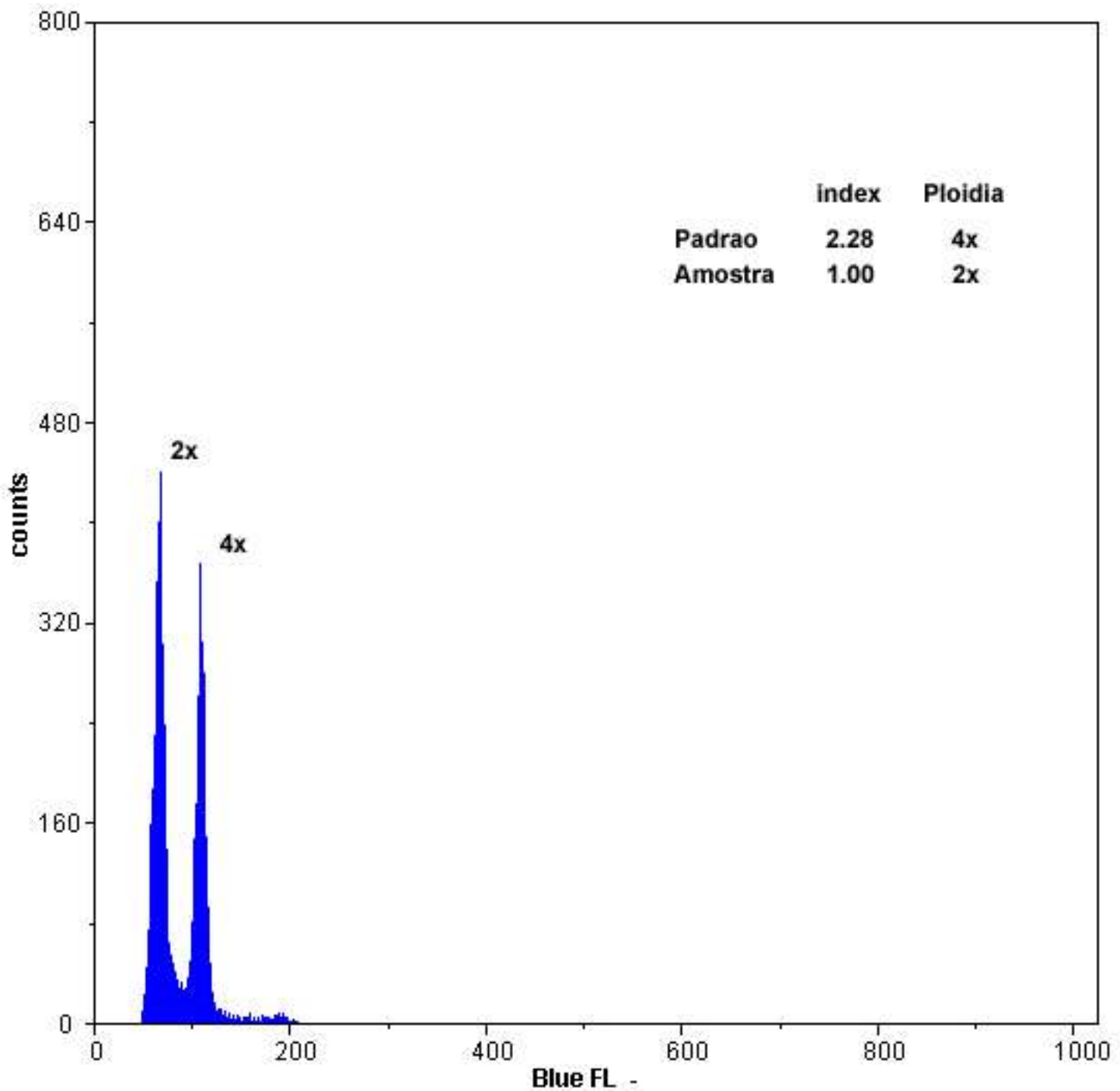


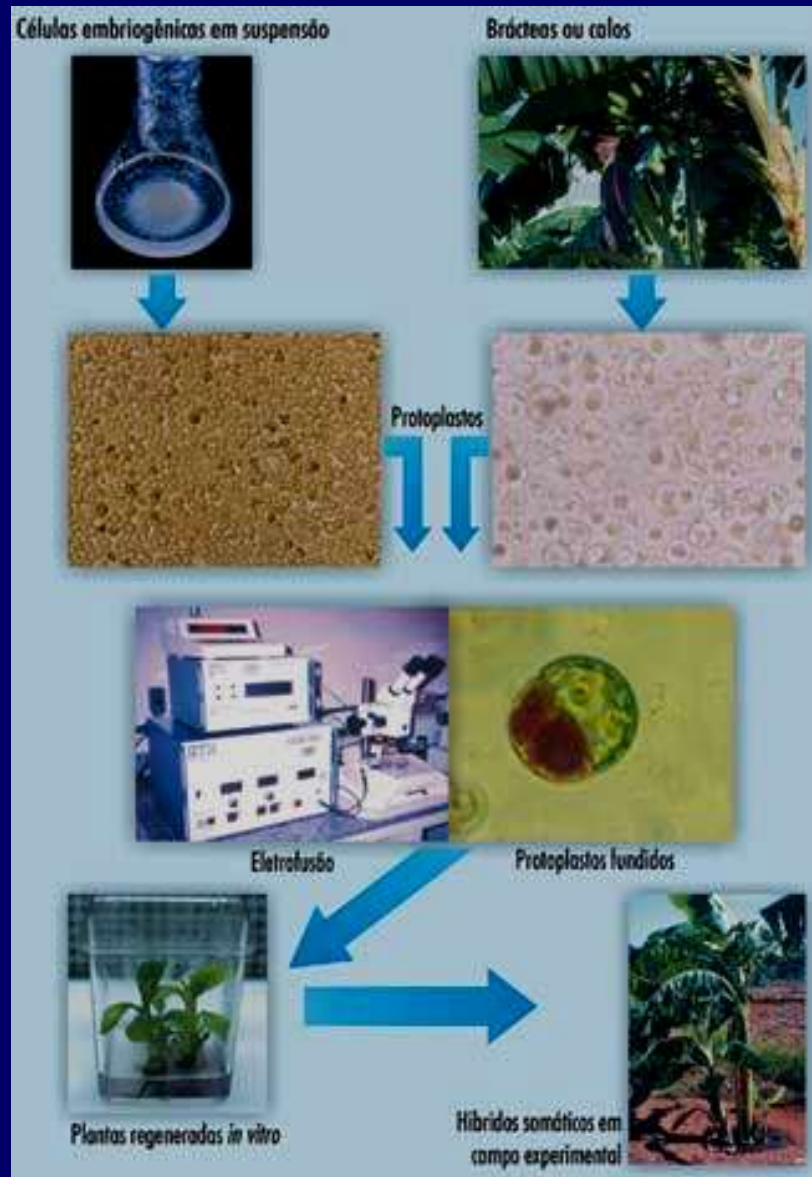
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C







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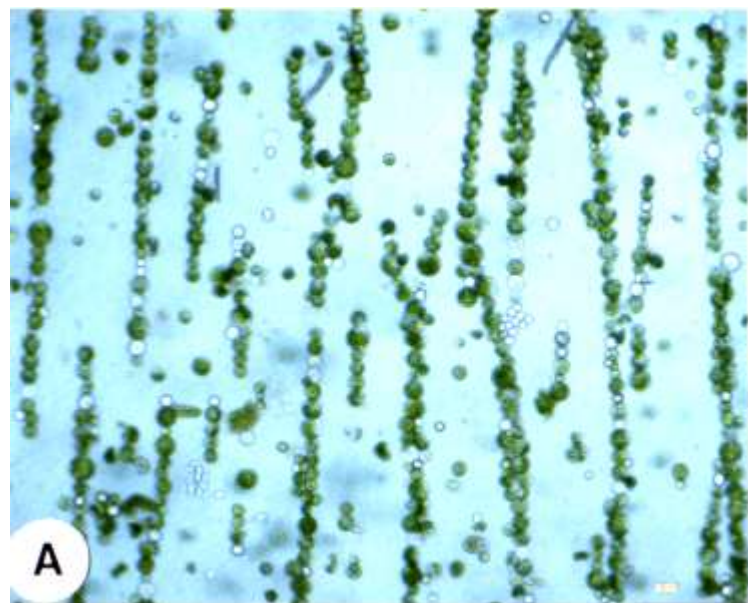


Somatic hybridization of the *Passiflora* species, *P. edulis* f. *flavicarpa* Degener. and *P. incarnata* L.

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Plant Genetic Manipulation Group, Department of Life Science, University of Nottingham, University Park, Nottingham NG7 2RD, UK

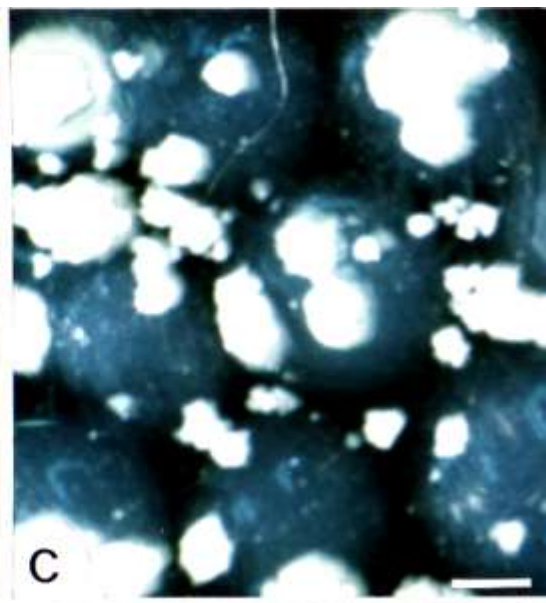
Received 5 September 1994; Accepted 10 March 1995



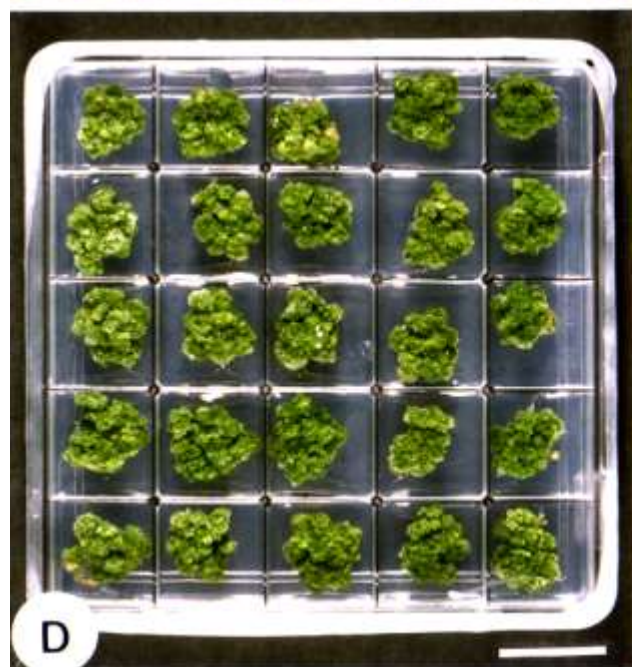
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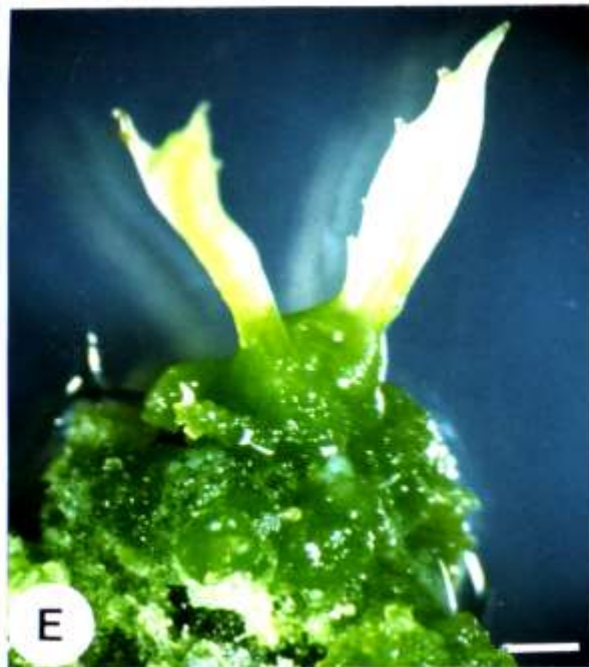
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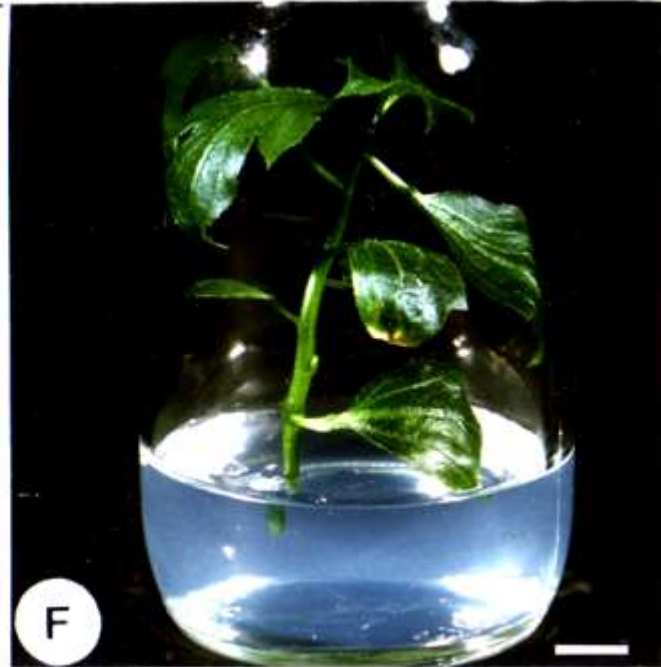
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F

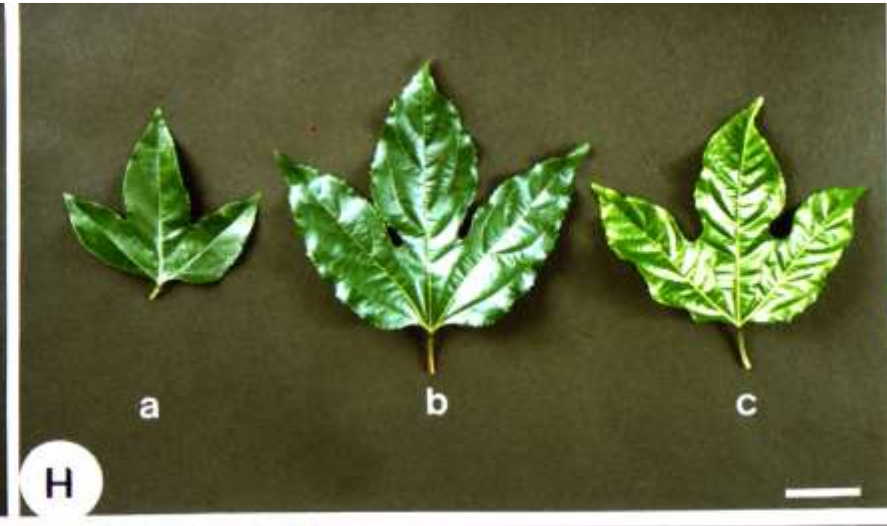
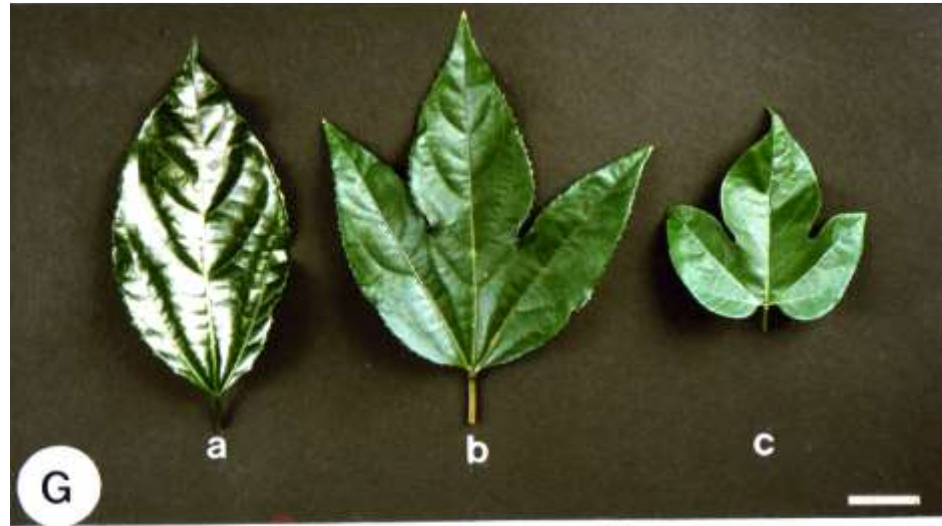
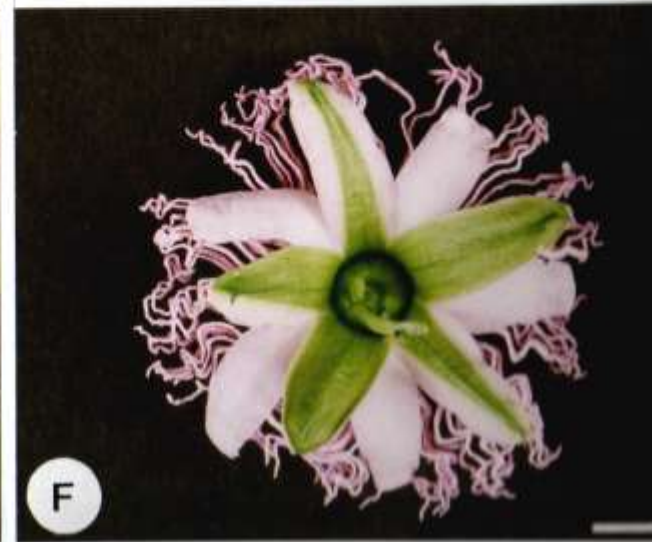
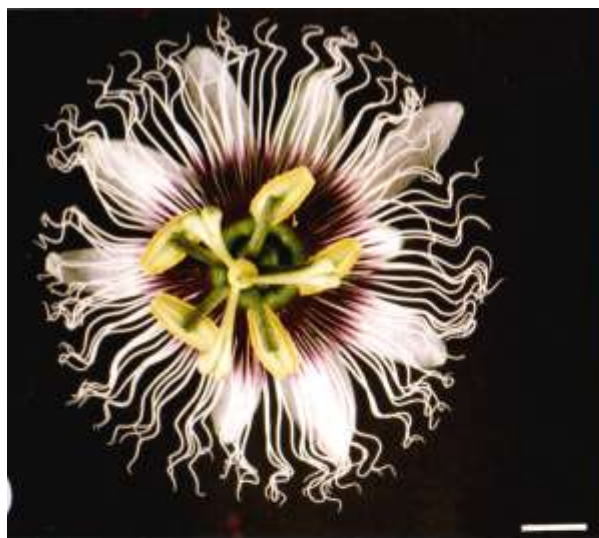
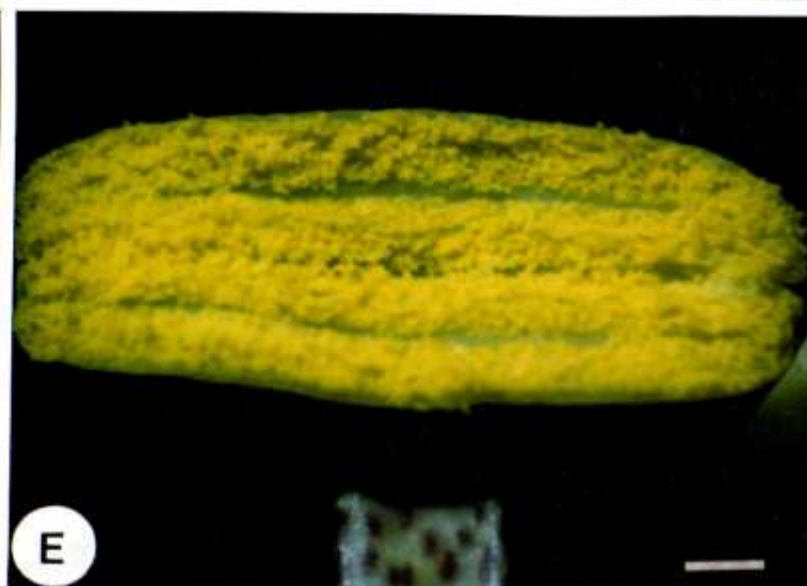
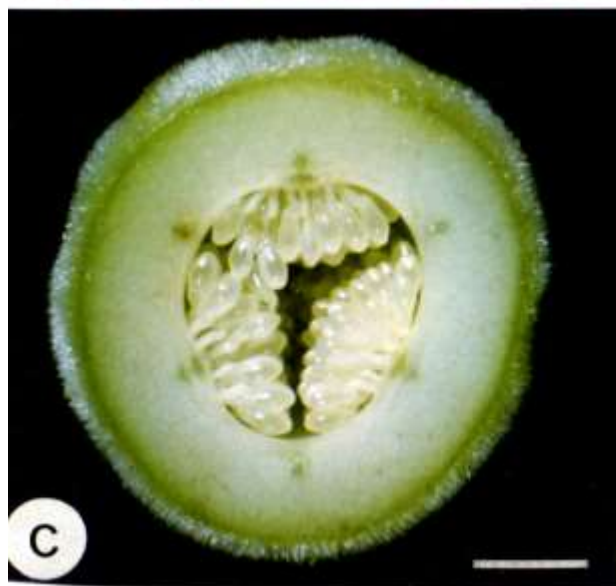


Table 1. Morphological and floral characteristics of *P. edulis* f. *flavicarpa*, *P. incarnata* and the putative somatic hybrids SH1-4

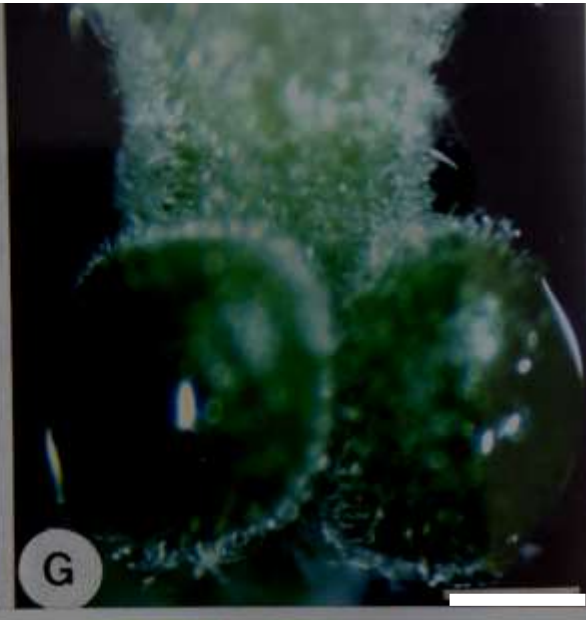
Genotype	Anthocyanin pigmentation (veins, adaxial leaf surface)	Mean number of stomata in 9940 μm^2 of adaxial leaf epidermis	Mean number of chloroplasts per guard cell	Floral bracts	Glands on floral bracts	Glands on sepals	Flower diameter (cm)	Pollen viability (%)	Anthocyanin pigmentation (anther filaments)	Anthocyanin pigmentation (stigma filaments)	Trichomes on ovary
<i>P. edulis</i> f. <i>flavicarpa</i>	+	69.7 \pm 6.8	10.7 \pm 1.5	3 large bracts in close contact to bud	-	+	6.0-7.0	81 \pm 8	++	-/+	Few, short trichomes
<i>P. incarnata</i>	-	51.4 \pm 5.3	14.4 \pm 1.6	3 small bracts located below floral bud	+	-/+	5.5-6.5	86 \pm 7	++	+	Abundant, long trichomes
SH1	+	44.4 \pm 7.8	22.3 \pm 2.0	Similar to <i>P. edulis</i> f. <i>flavicarpa</i> , but with glands	++	+	8.5-9.0	16 \pm 4	+++	+	Abundant, long trichomes
SH2	+	29.4 \pm 3.1	20.4 \pm 2.8	Similar to <i>P. edulis</i> f. <i>flavicarpa</i> , but with glands	++	+	8.0-8.5	14 \pm 4	+++	+	Abundant, long trichomes
SH3	+	21.7 \pm 1.9	21.4 \pm 1.9	Similar to <i>P. edulis</i> f. <i>flavicarpa</i> , but with glands	++	+	8.6-9.8	15 \pm 5	+++	+	Abundant, long trichomes
SH4	+	19.8 \pm 1.1	18.6 \pm 1.9	np	np	np	np	nt	np	np	np

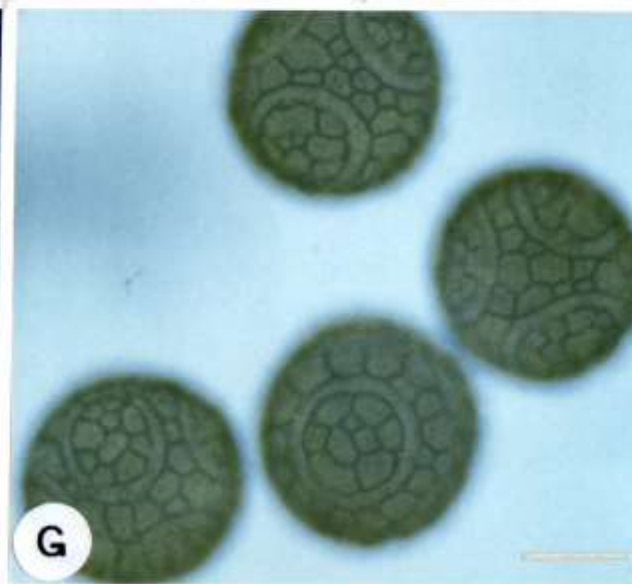
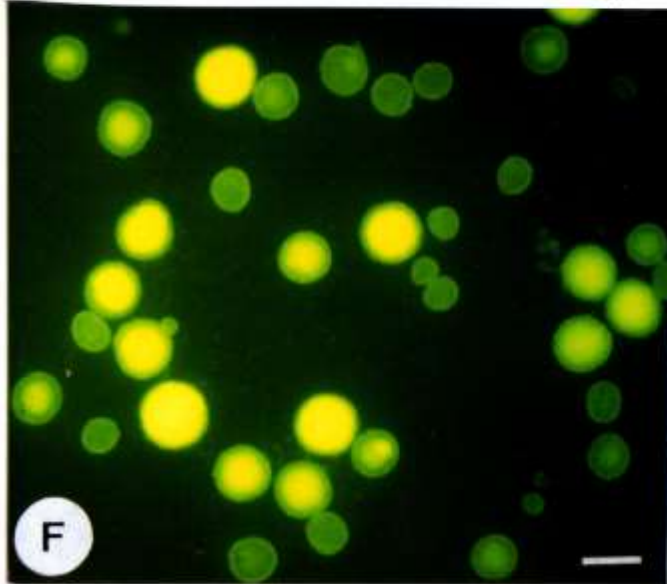
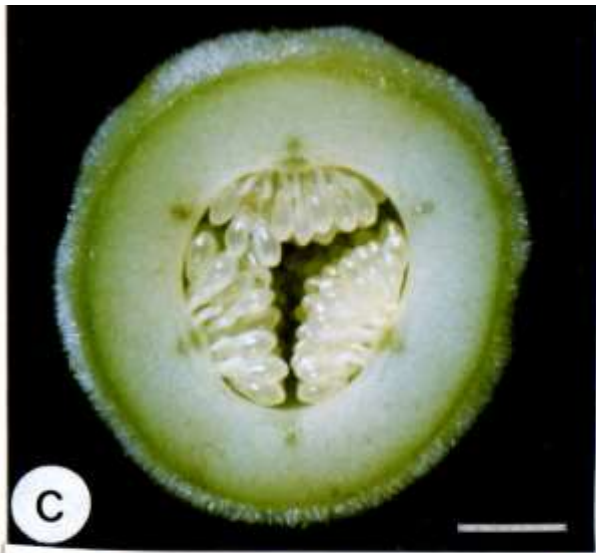
np: not produced; nt: not tested; - absent; +, ++, +++: present in increasing number/intensity.



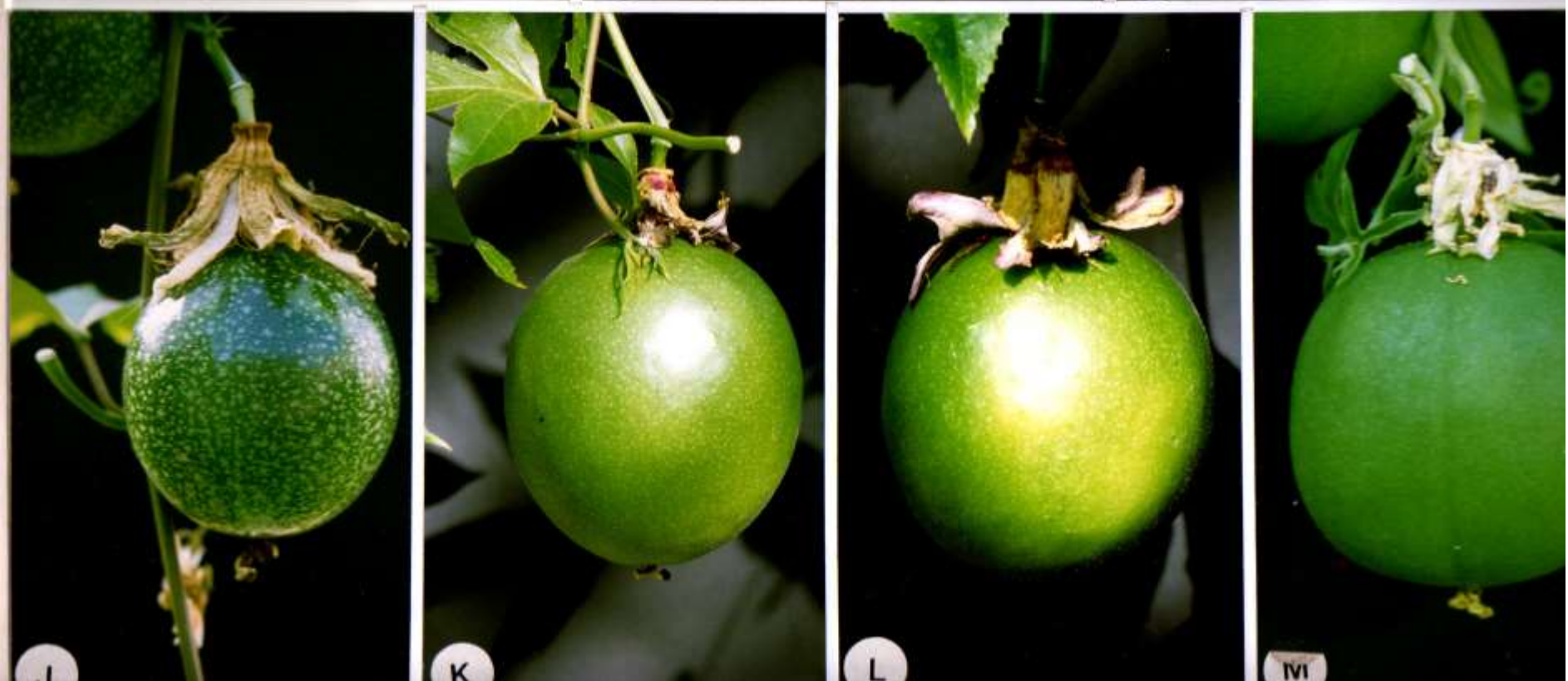
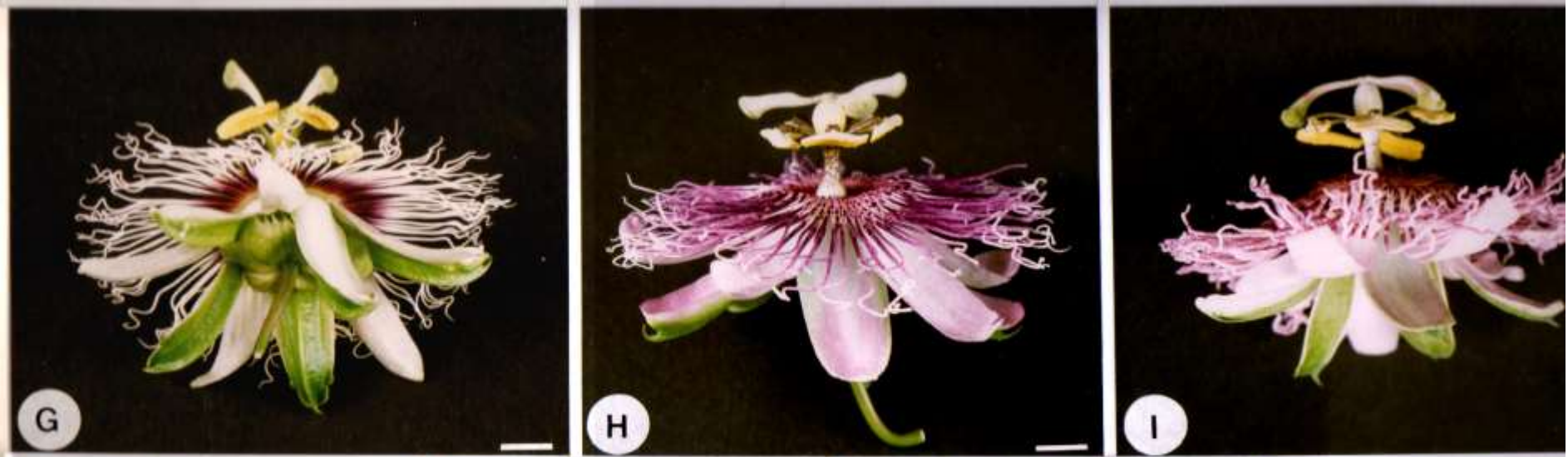


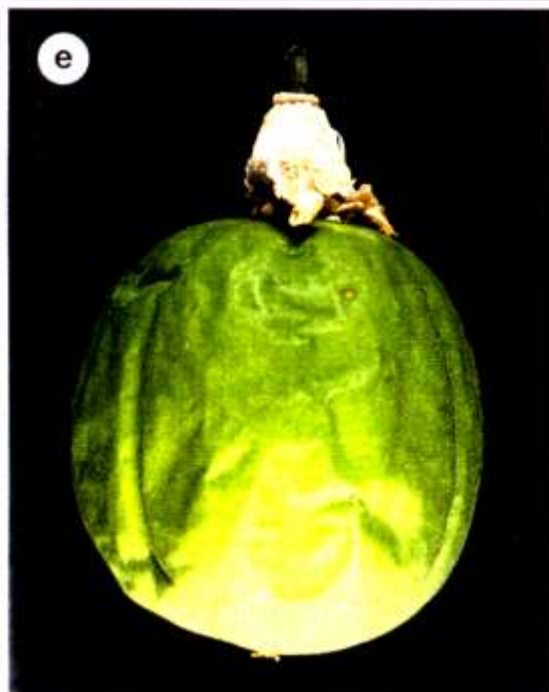


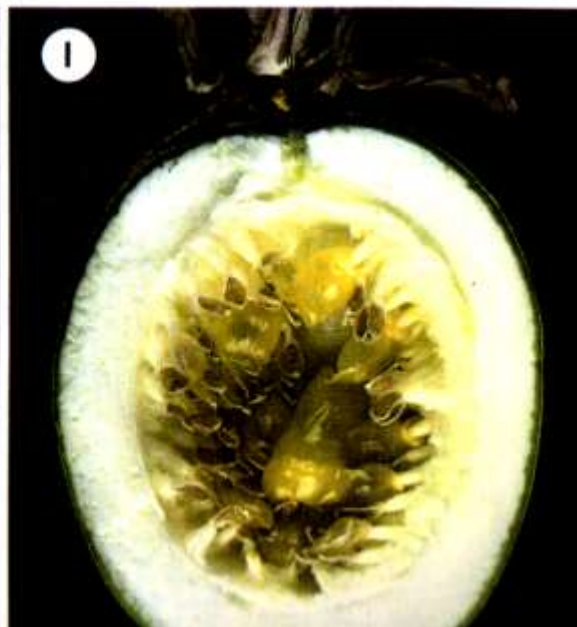
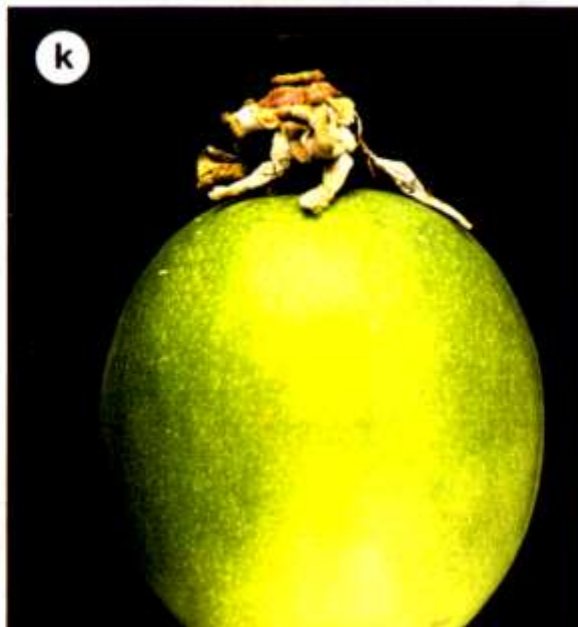
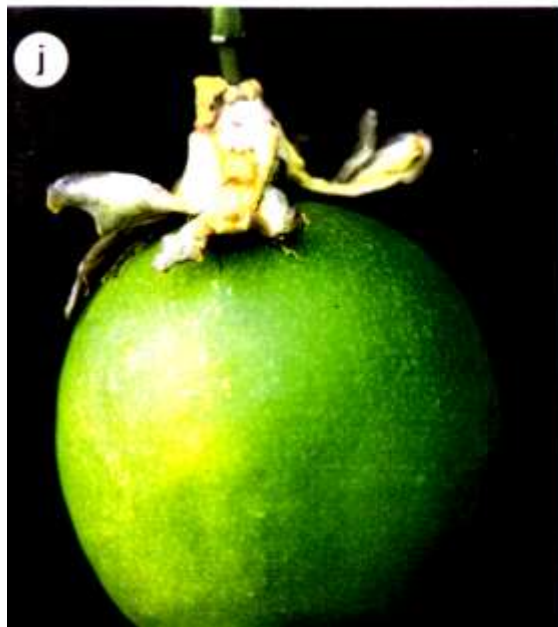
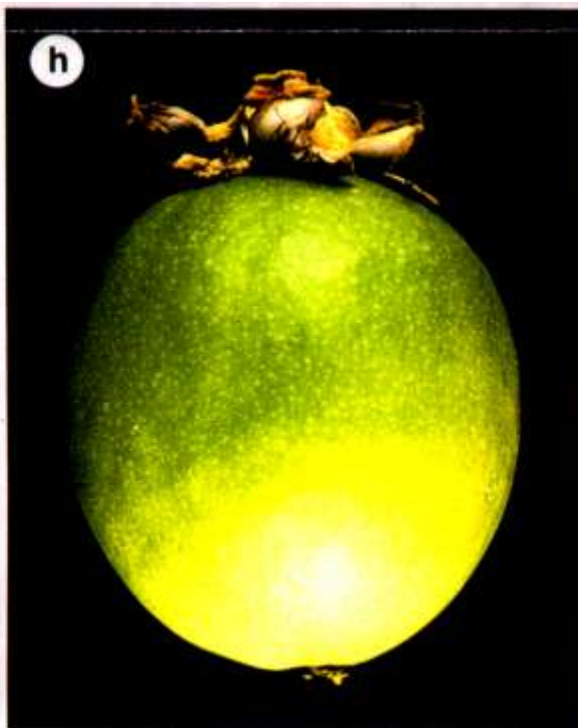


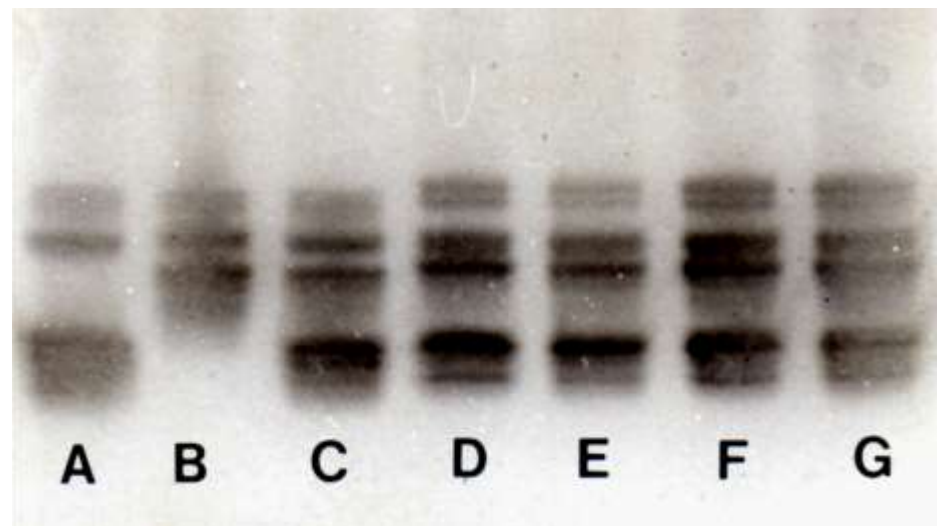
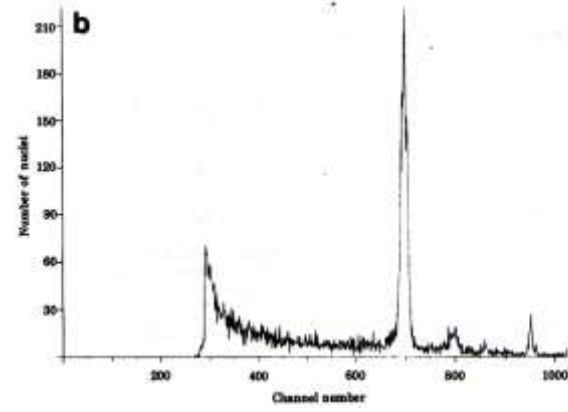
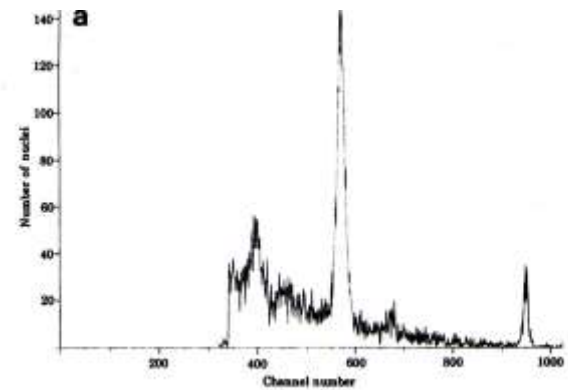
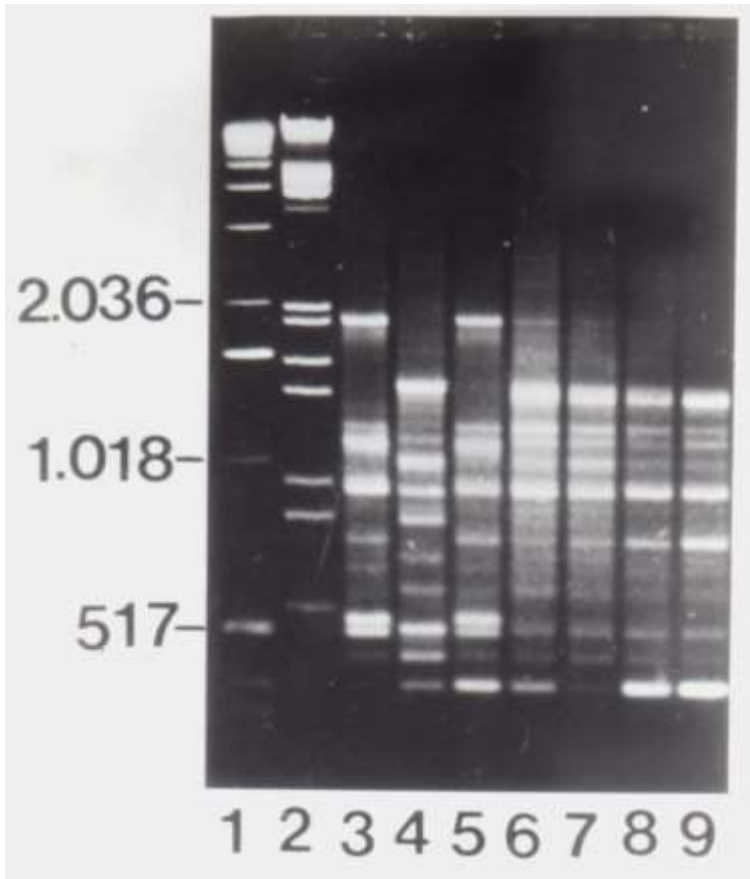


Otoni et al. (1995)

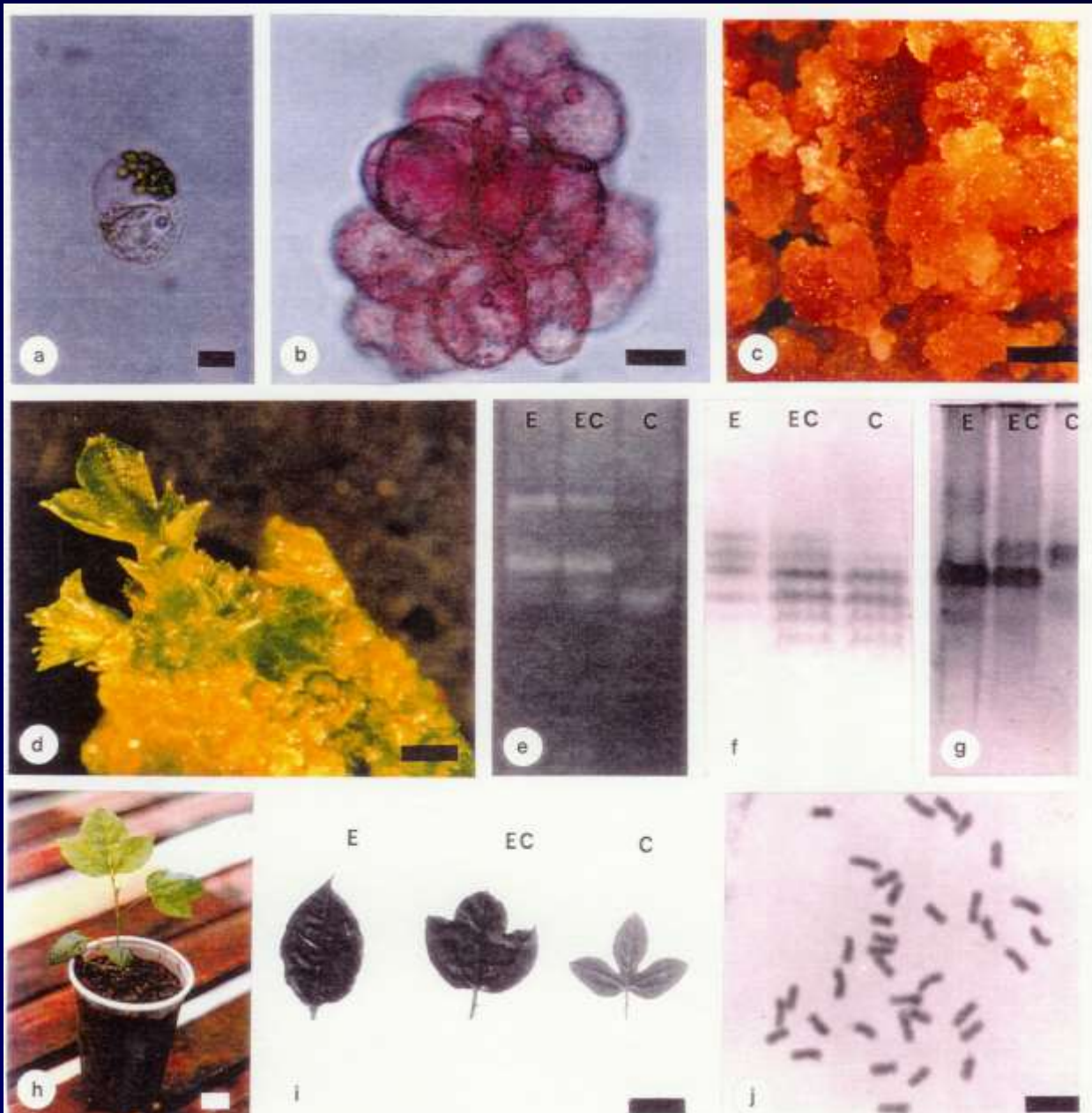


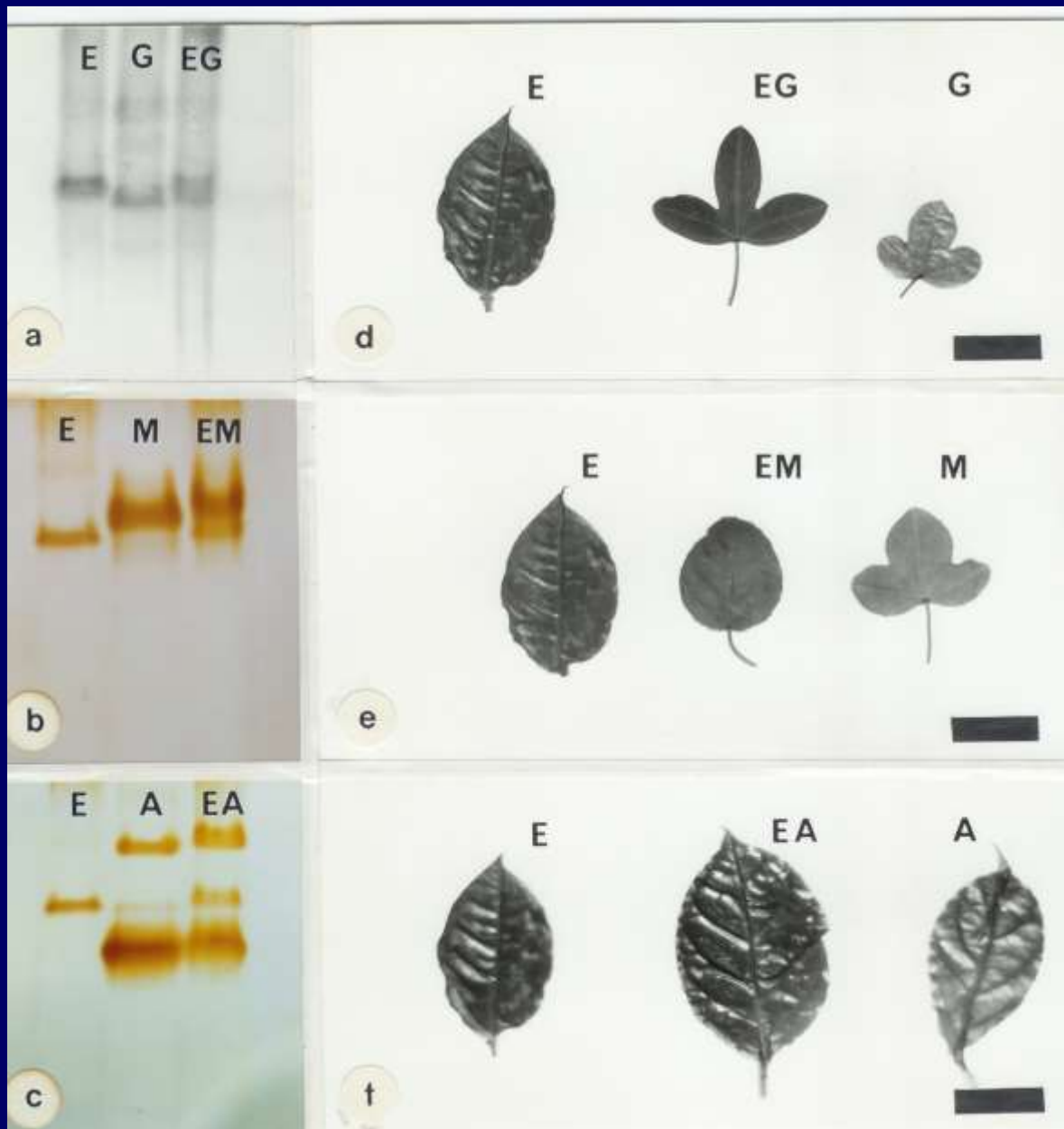






Otoni et al. (1995)







Vieira & Carneiro (2004)



P. edulis f. *flavicarpa* enxertado em *P. edulis* f. *flavicarpa* + *P. cincinnata*

Vieira & Carneiro (2004)

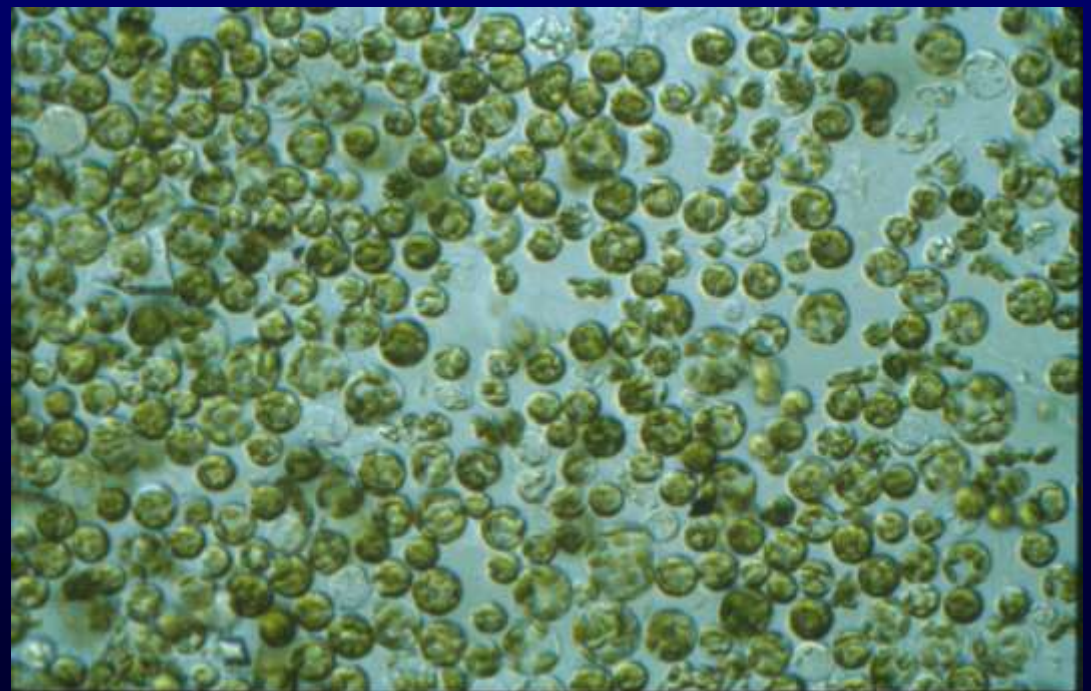
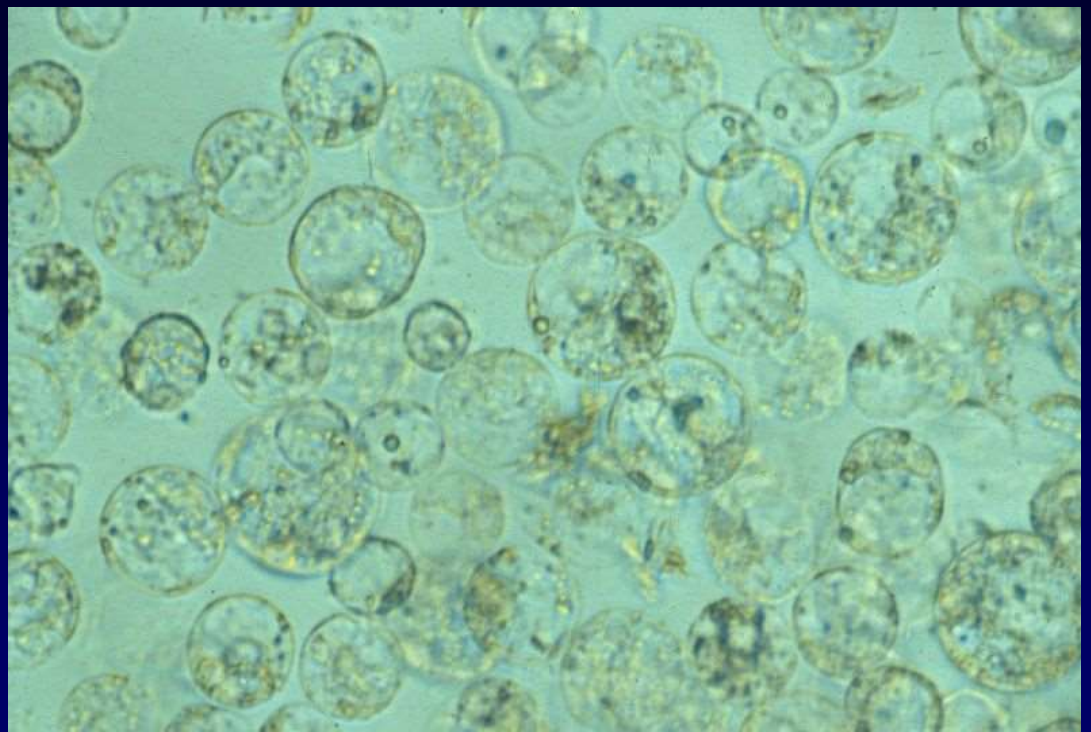
Jude W. Grosser

University of Florida - IFAS

Citrus Research and Education Center

Lake Alfred, FL 33850







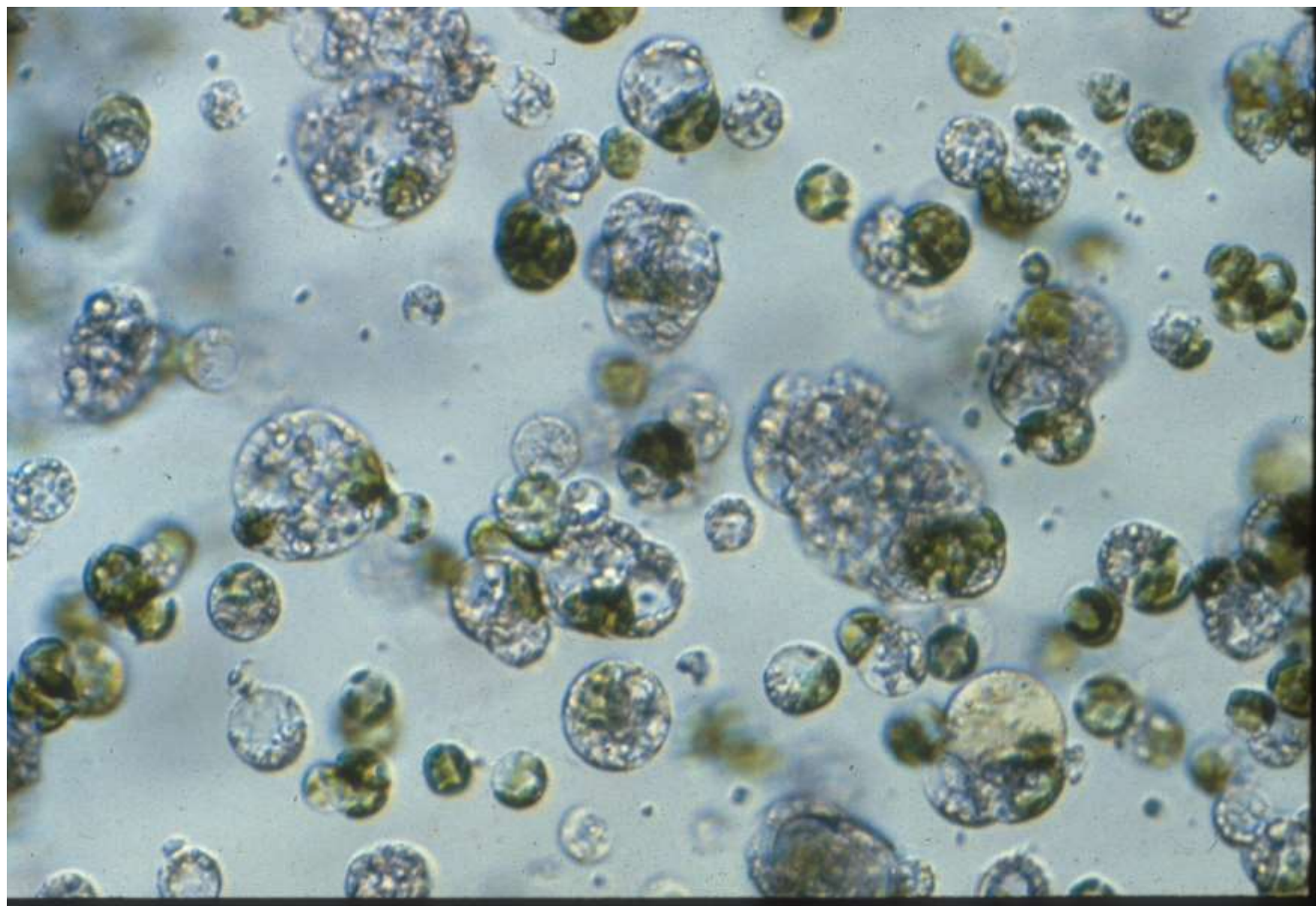
Enzyme

Callus



plant regeneration







Sunburst

M + S

Murcott

M + (C x St)

Clem x Satsuma

G-96

Itaborai + G-96

Itaborai

Navel

M + N

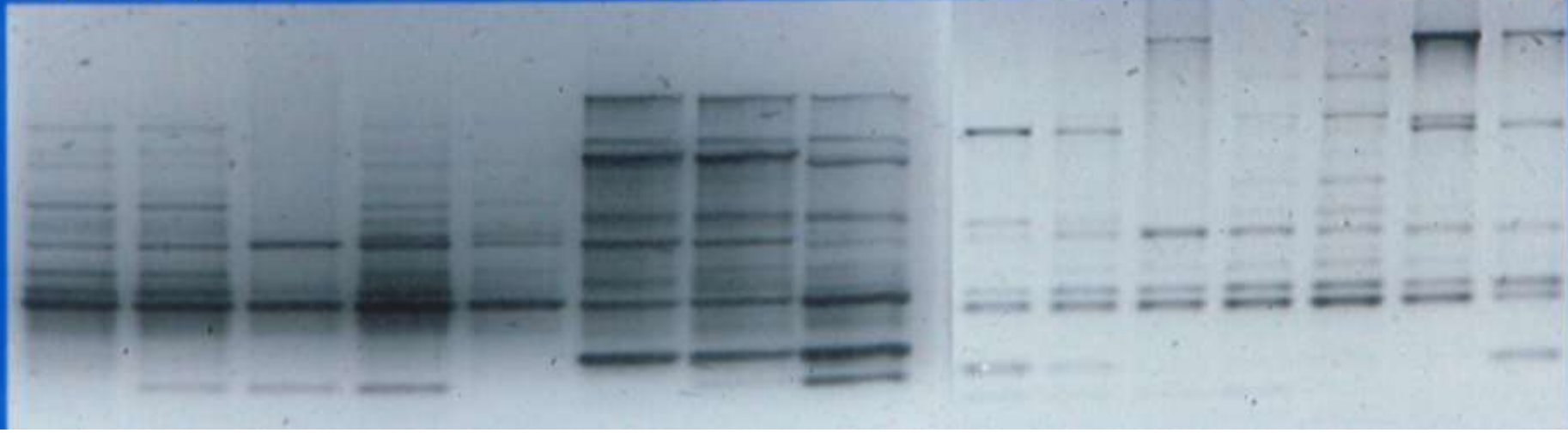
Murcott

M + O

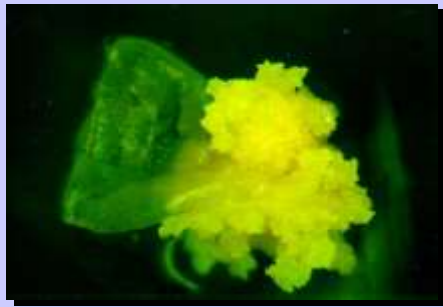
Osceola

No + O

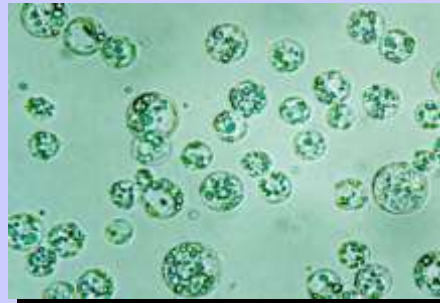
Nova



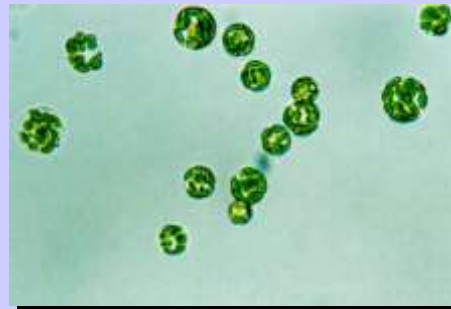
Protocolo para Hibridação Somática



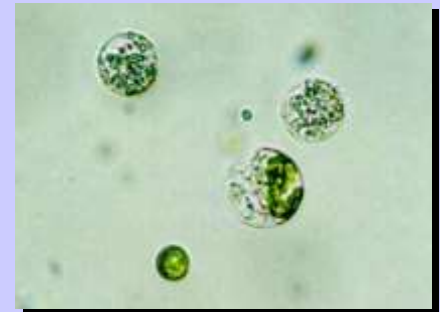
Callus



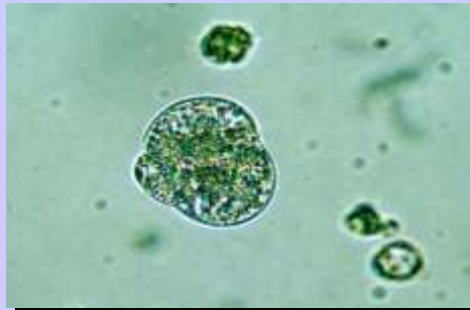
Protoplastos - C



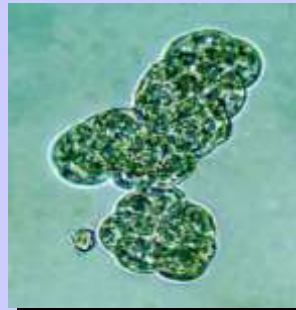
Protoplastos - M



Fusão C-M



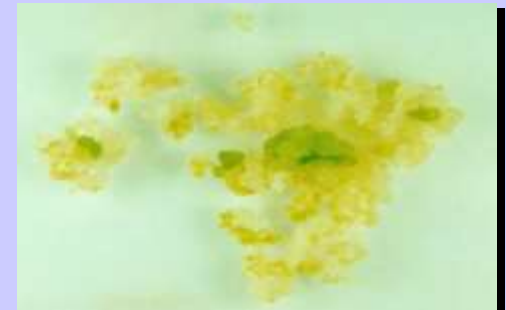
Mitose



Colônias



Calogênese



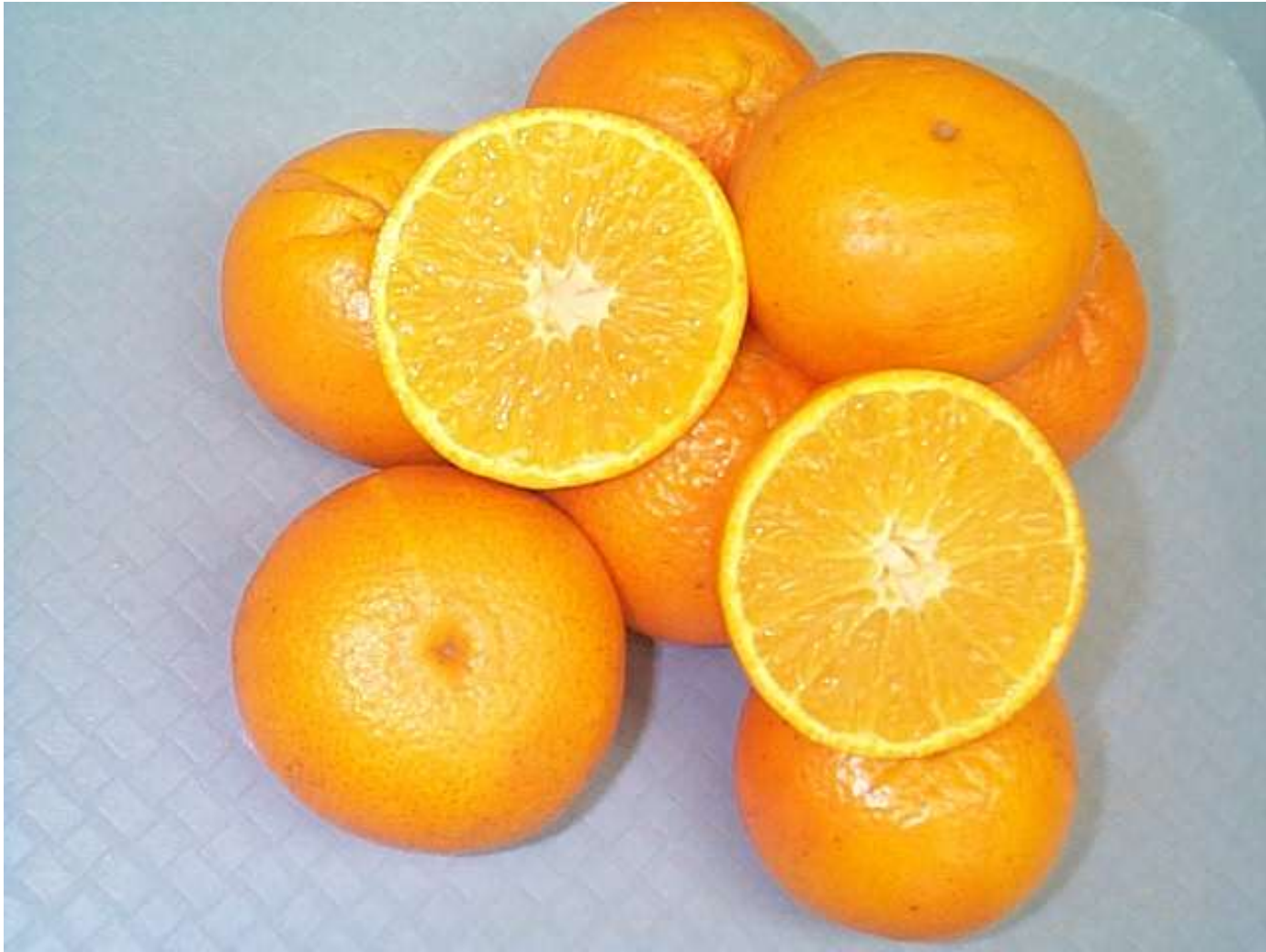
Embriogênese



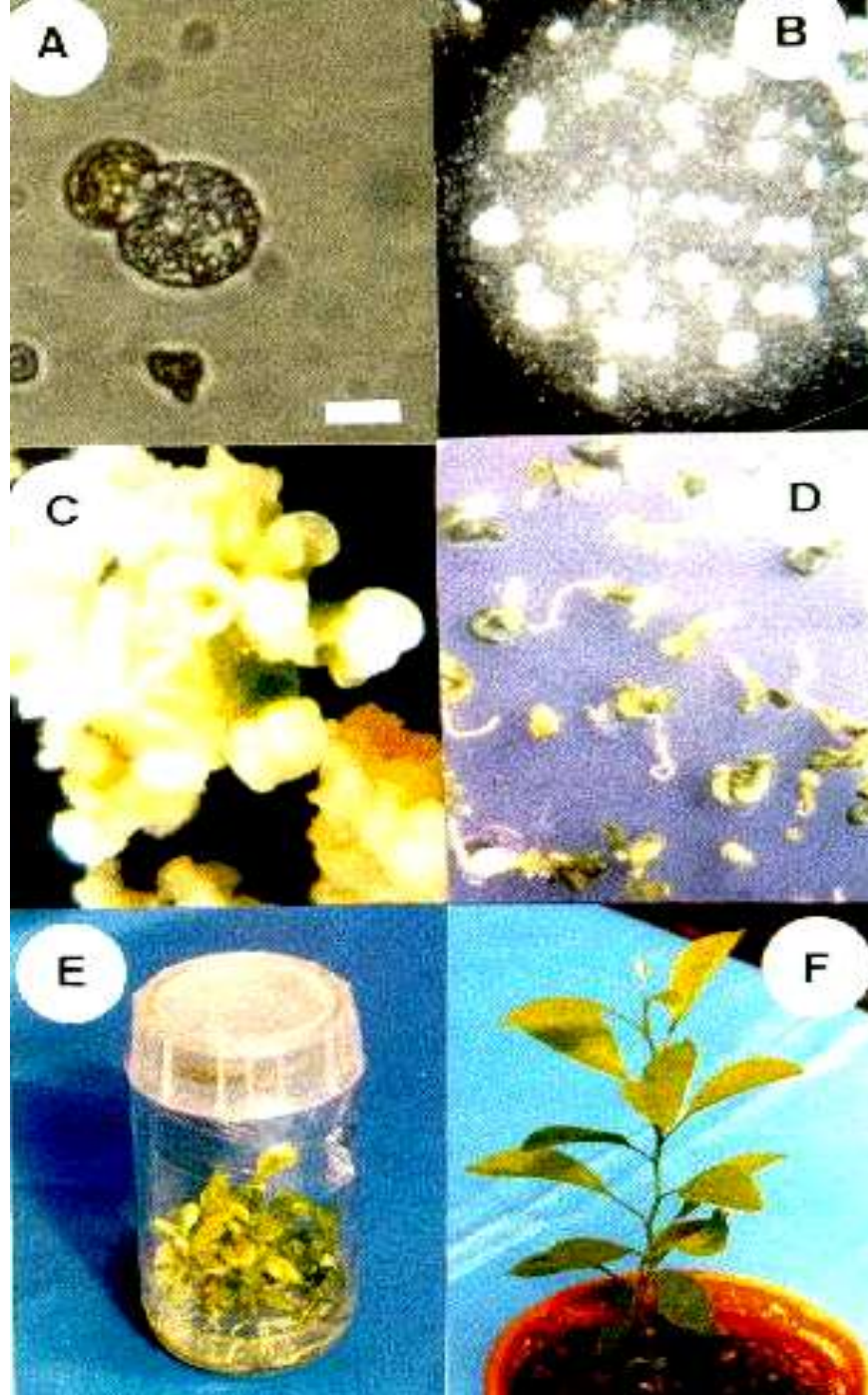
Enraizamento Híbrido Somático



'Roble'/ SO + Carrizo, 6 anos



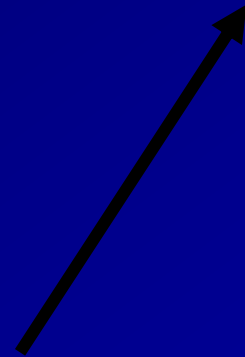
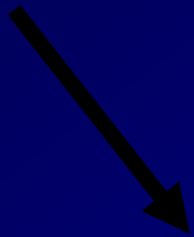
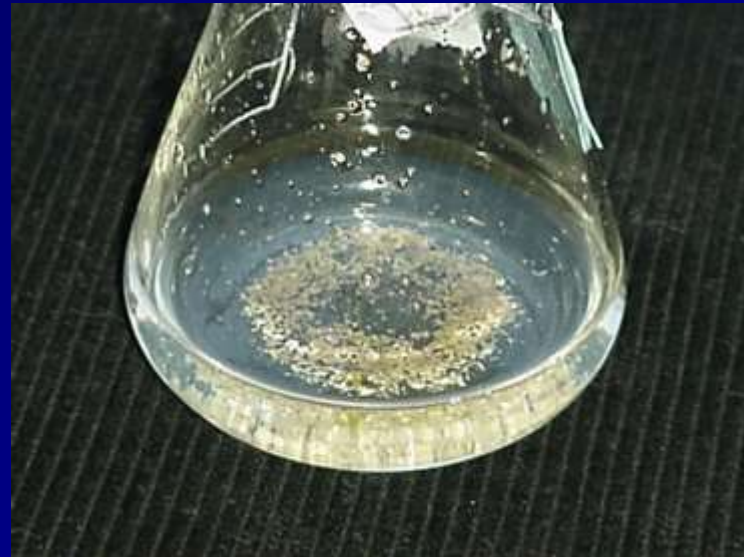
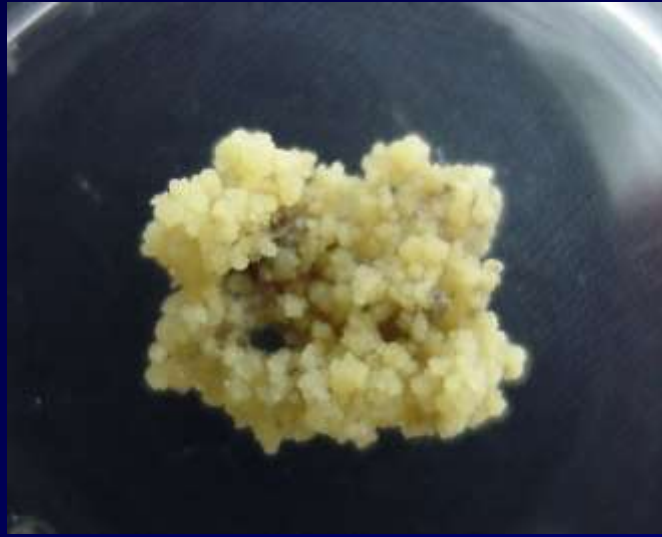
PINK MARSH GRAPEFRUIT + MURCOTT TANGOR



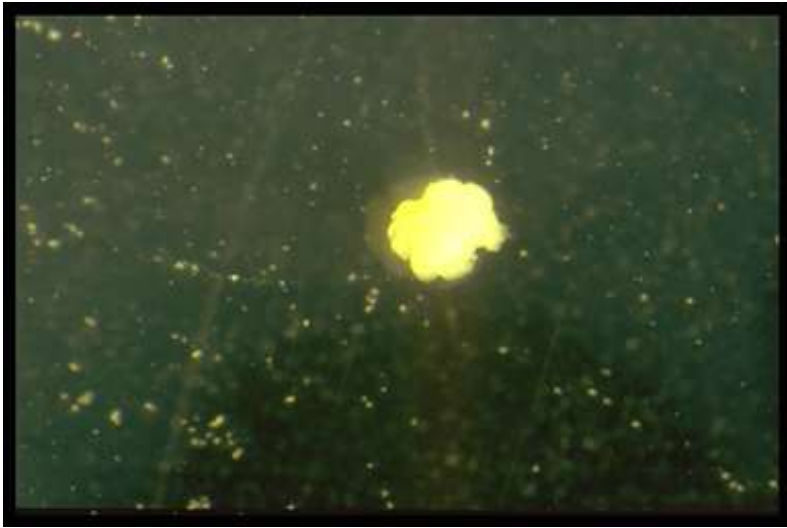
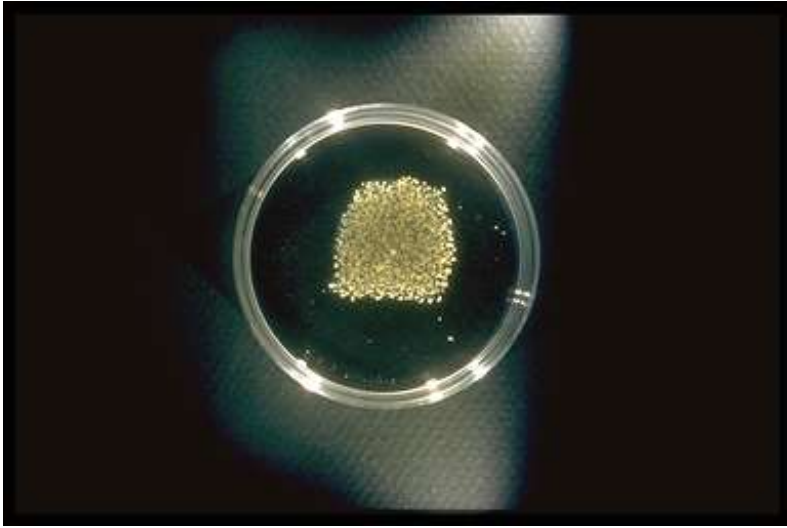
Fontes: Latado (1998)



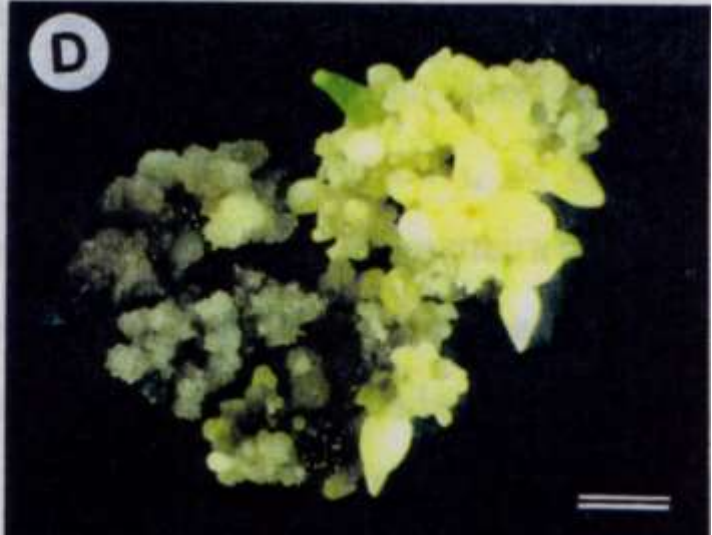
Rohde Red Valencia + Palestine sweet lime somatic hybrid - 6 anos de idade







Cordeiro, 1999



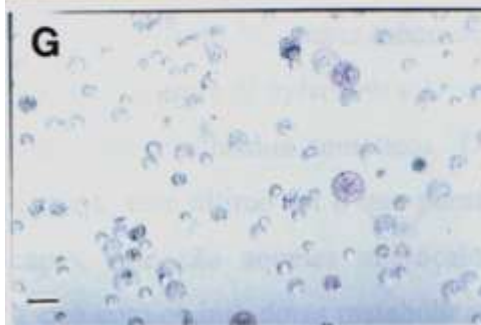
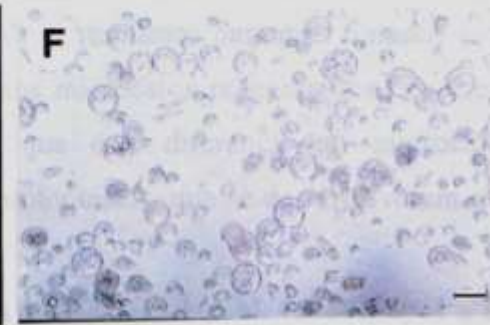
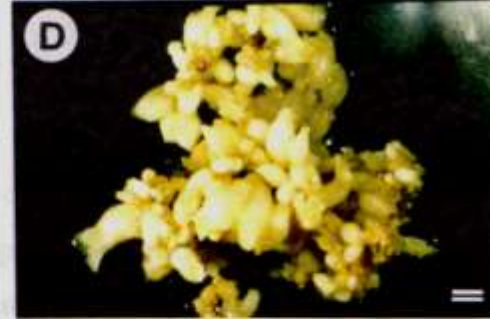
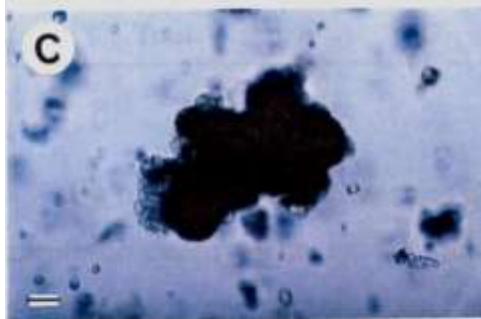
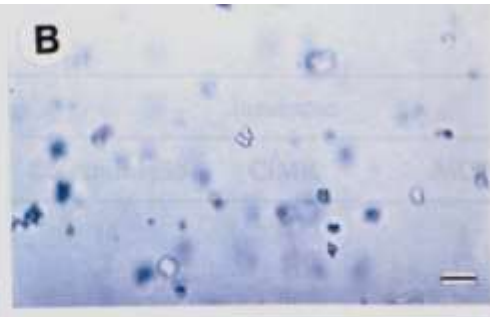




Foto: Marina de Oliveira Cruz



Foto: Maria Elizabete Pena Freitas



Foto: Marcus Vinicius Sant'Anna









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