Somatic embryogenesis – more than cloning

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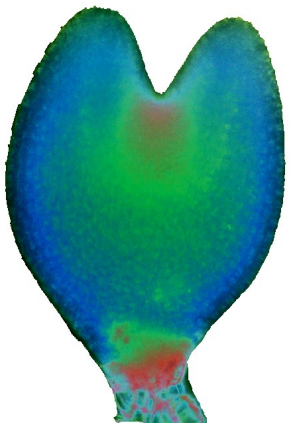
Summer Course – Plant Biotechnology

1

Somatic embryogenesis

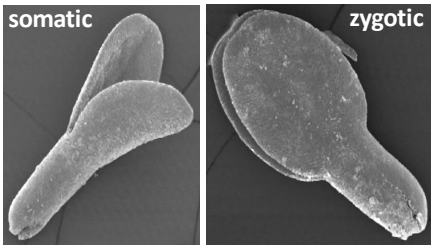
A type on non-sexual embryogenesis in which cells of the plant body (somatic) are stimulated to develop into embryos



2

Somatic embryos

Somatic embryos are morphologically similar to their zygotic counterparts and genetically identical to the mother plant



Plant cloning

3

Pollen embryogenesis

A type on non-sexual embryogenesis in which microspores (future pollen grains) are stimulated to develop into embryos

Haploid plants

Chromosome doubling

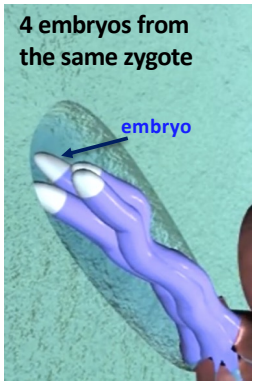
Homozygous diploid plants

4

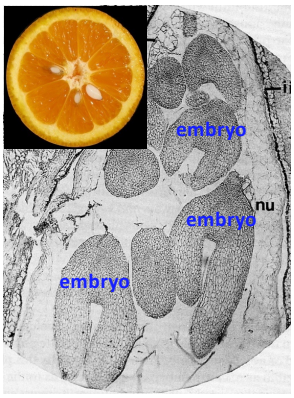
Non-zygotic embryogenesis occurs naturally

In some species, embryos arose from cells other than the zygote – non zygotic embryogenesis

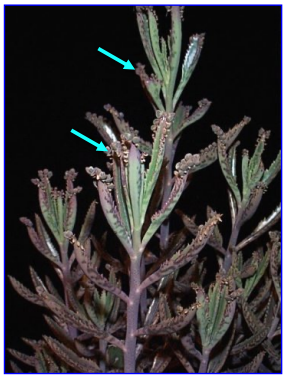
Cleavage polyembryony (*Pinus* sp.)



Adventitious apomixis (*Citrus* sp.)



Leaf embryos (*Bryophyllum* sp.)



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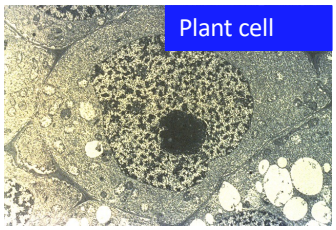
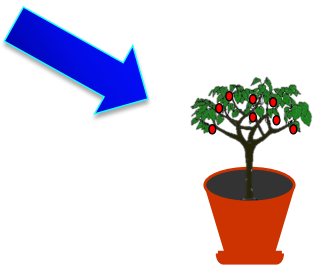
Totipotency (1902)

Non-zygotic embryogenesis is the expression of totipotency exhibited by some plant cells

wikipedia
Gottlieb Haberlandt (1854-1945)





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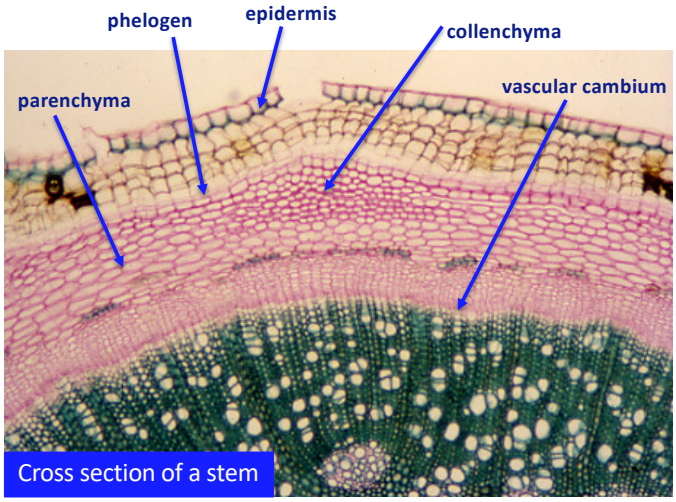
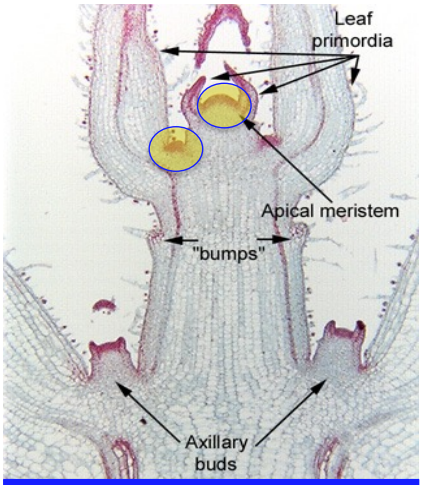
Which plant cells?

Meristematic and poorly specialised cells



Cross section of a stem



Longitudinal section of a stem apex

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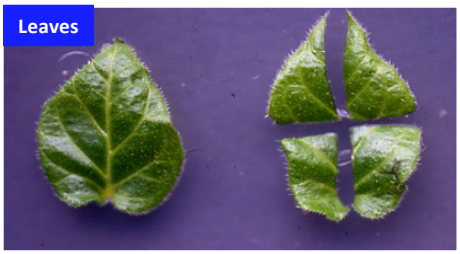
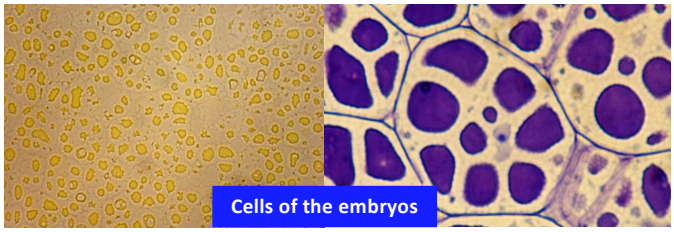
Zygotic embryos and young leaves are used

Somatic embryogenesis is usually induced from embryonic organs such as whole zygotic embryos or young leaves

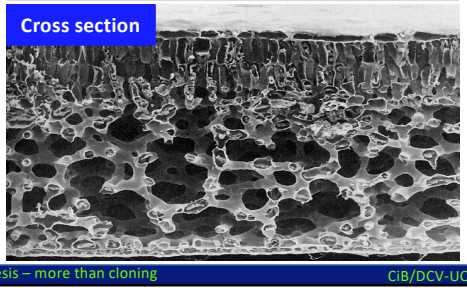
Zygotic embryo



Leaves



Cells of the embryos



Cross section

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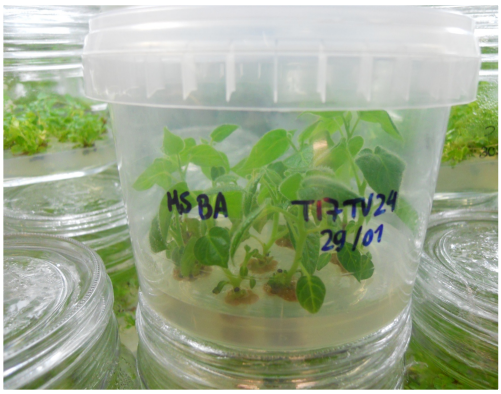
Problems of the young explants

When starting from young explants, such as zygotic embryos, the phenotype is unknown

Leaves from elite plants previously established *in vitro* are a reliable alternative



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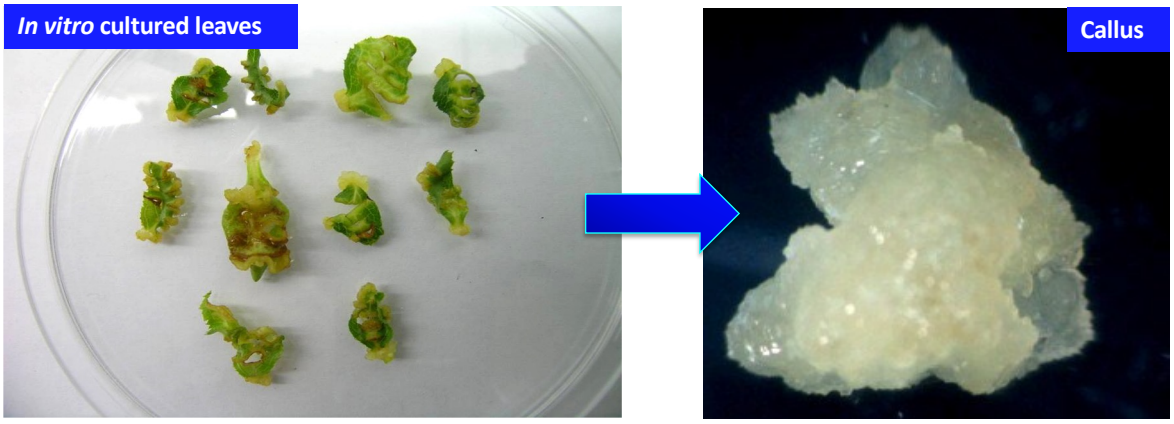
Callus formation is usually an intermediate step

Cells of the explant only acquire totipotency following a previous step of proliferation



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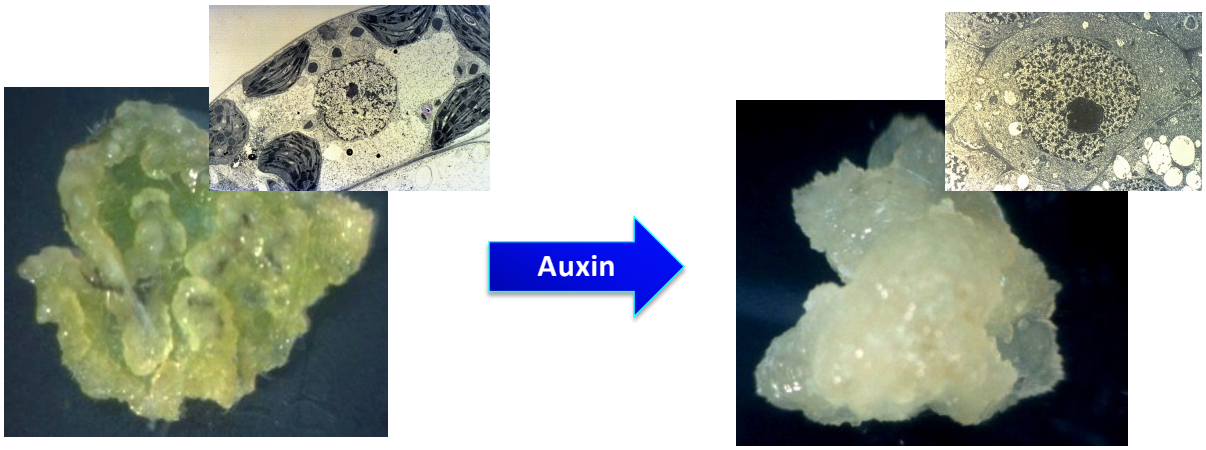
Auxins promote dedifferentiation

Cells of the explant only acquire totipotency following a previous step of proliferation



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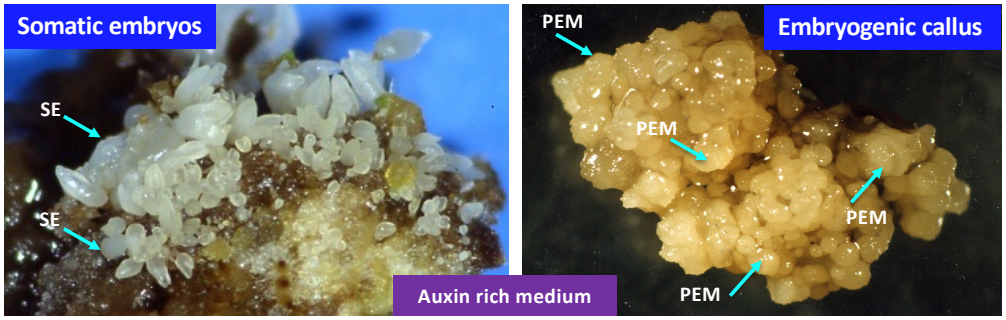
SE may (or may not) appear in the auxin medium

In some species somatic embryos are able to develop in the presence of an auxin; in others no



SE – somatic embryos; PEM – proembryogic masses

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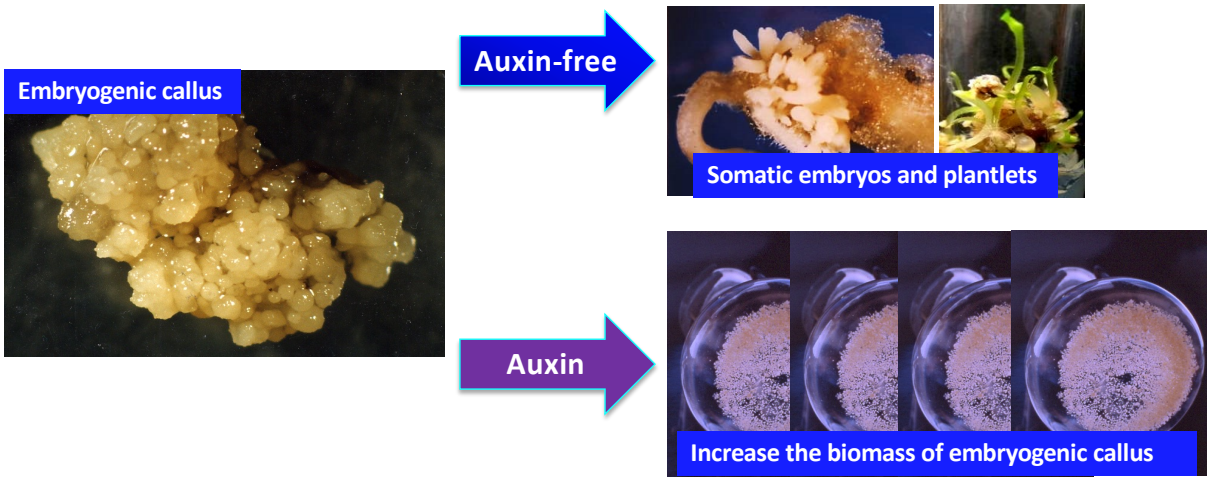
Embryogenic callus originate SE in an auxin-free medium

When embryogenic calli are transferred to an auxin-free medium, somatic embryos develop from the proembryogenic masses



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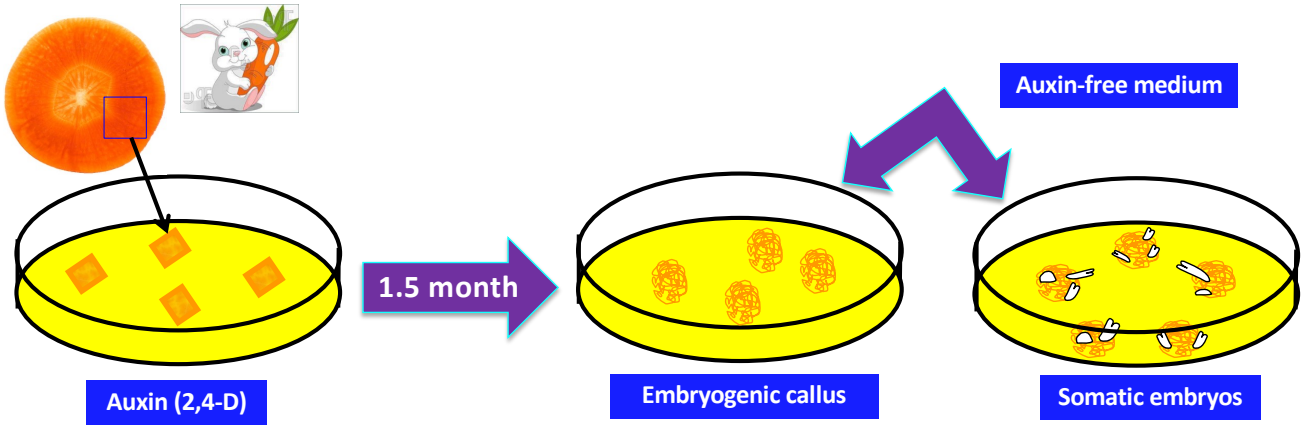
Carrot was the first species in which SE has been induced

In 1958, Steward et al. and Reinert first induced somatic embryogenesis in callus tissue of carrot



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SE has been achieved in many species

Arabidopsis thaliana

Acca sellowiana
(pineapple guava)

Arbutus unedo
(strawberry tree)

Solanum betaceum
(tamarilho)

Ceratonia siliqua
(carob)

Pinus halepensis
(Aleppo pine)

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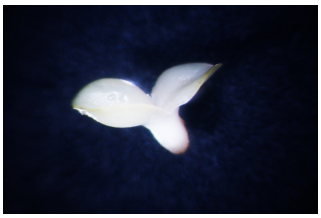
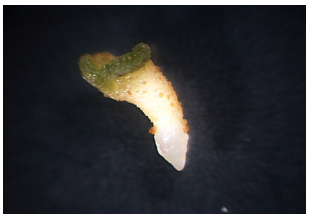
Somatic embryogenesis for what?

Somatic embryogenesis can be used for different purposes, namely:

- Cloning
- Genetic transformation
- Synthetic seeds
- Conservation of endangered species
- Propagation of hybrids or dioecious species
- Production of tetraploids
- Embryo development





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Cloning

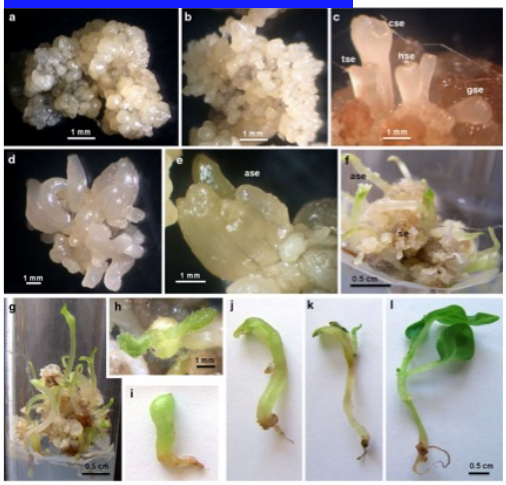
Through somatic embryogenesis a great number of SE and plantas can be obtained

Mass proliferation (measured as fresh weight—FW) and total number of somatic embryos (SE) and emblings obtained after 1 and 3 months on the embryo development medium (MS medium, 0.07 M sucrose) and under photoperiod conditions

Explant	Initial embryogenic tissue FW (g)	Callus + SE FW after 1 month (g)	Number of SE per explant (after 1 month of culture)		Number of emblings per explant (after 3 months)
			Normal/cotyledonary SE	Abnormal SE	
1	0.049	1.06	8	8	2
2	0.052	1.32	2	13	7
3	0.026	1.16	3	33	23
4	0,041	0.96	10	28	41
5	0.040	1.57	8	15	23
6	0.050	1.06	6	28	32
Mean ± SE*	0.043 ± 0.004	1.19 ± 0.1	6.2 ± 1.3	20.8 ± 4.1	21.3 ± 6.0
Number of SE/emblings per g of initial embryogenic tissue FW*			143.9 ± 30.2	482.6 ± 95.3	494.2 ± 139.5

*Mean number ± standard error

Tamarillo (*Solanum betceum*)



Cloning - acclimatisation

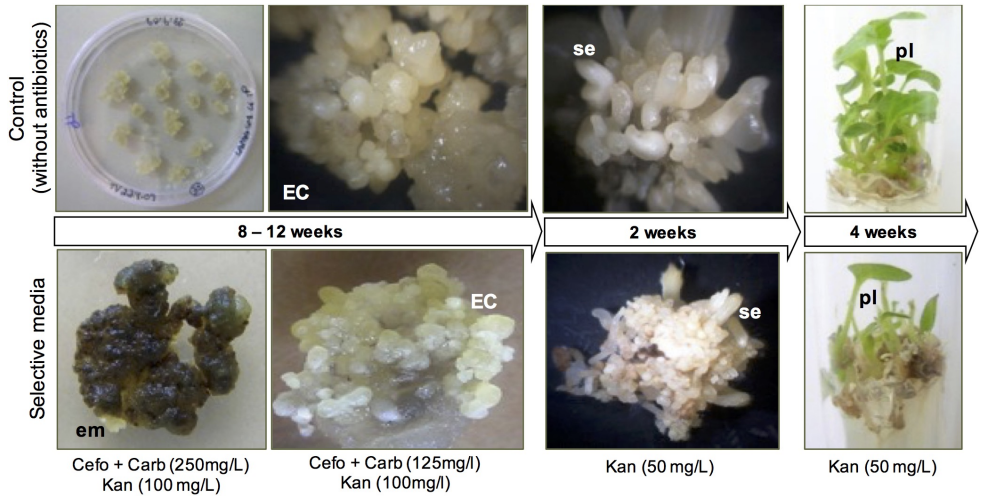
Transfer to *ex vitro* conditions is particularly problematic

In vitro

Ex vitro

Genetic transformation

Prior regeneration through somatic embryogenesis, cells can be genetically transformed



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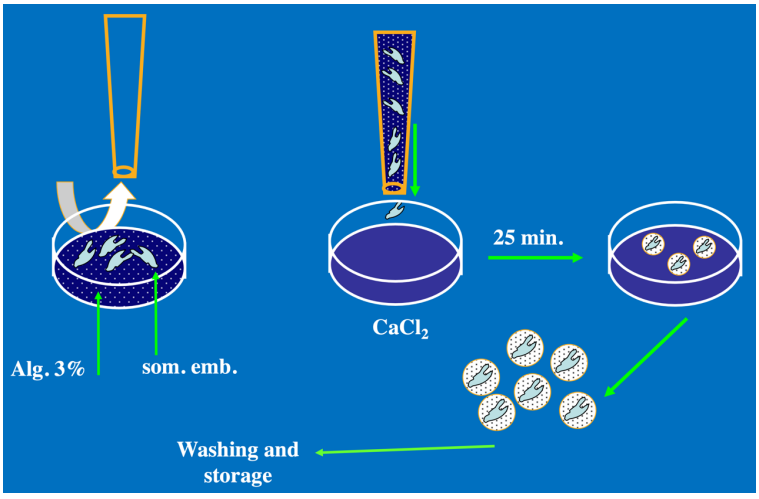
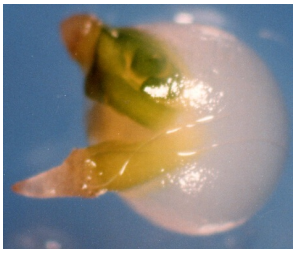
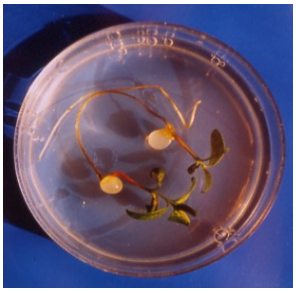
Synthetic seeds

Somatic embryos can be encapsulated and used to produce artificial seeds







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Synthetic seeds

Forestry companies are producing artificial seeds of several coniferous species

Pinus halepensis somatic embryos

Weyerhaeuser

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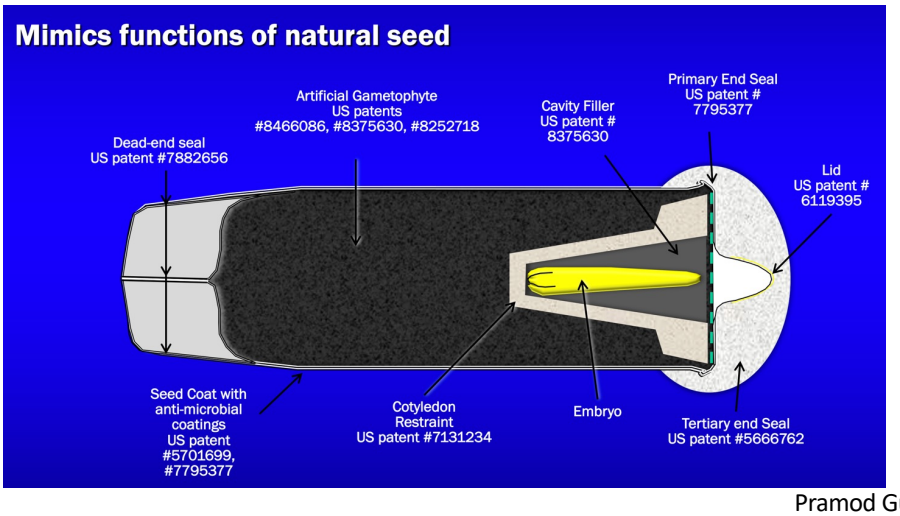
Synthetic seeds

Forestry companies are producing artificial seeds of several coniferous species



Pramod Gupta Weyerhaeuser

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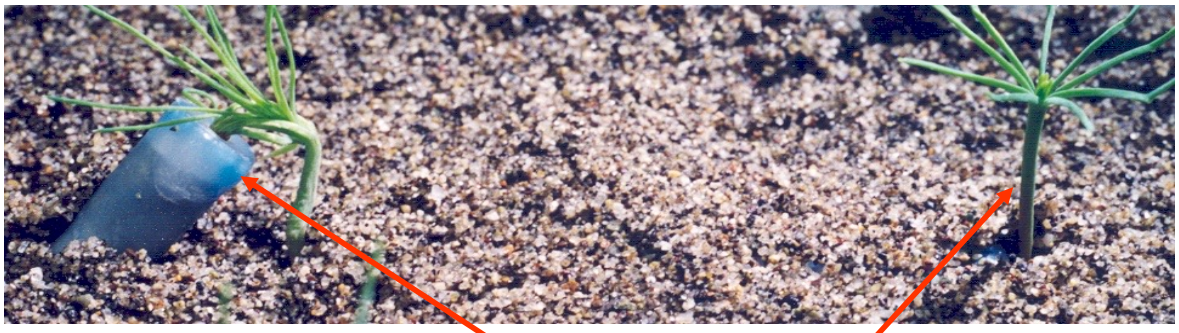
Synthetic seeds

Forestry companies are producing artificial seeds of several coniferous species



Loblolly pine (*Pinus taeda*) somatic embryo germinated from manufactured seed and zygotic embryo

Pramod Gupta Weyerhaeuser

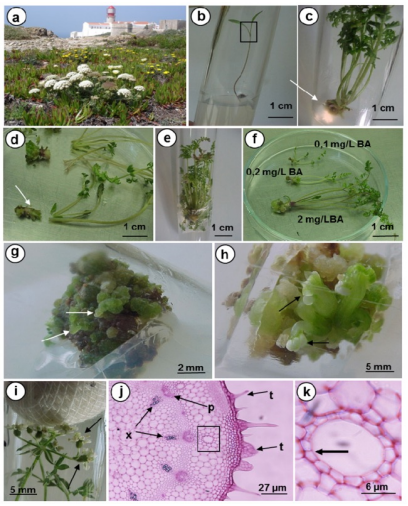
Conservation of endangered species

Somatic embryogenesis can be used to propagate species that are endangered

In Vitro Cell.Dev.Biol.—Plant (2010) 46:47–56
DOI 10.1007/s11627-009-9258-y

MICROPROPAGATION

***In vitro* propagation of the wild carrot *Daucus carota* L. subsp. *halophilus* (Brot.) A. Pujadas for conservation purposes**



Conservation of endangered species

Bellevalia dubia (Guss.) Schult.f.

Jacinto-azul-do-Barrocal

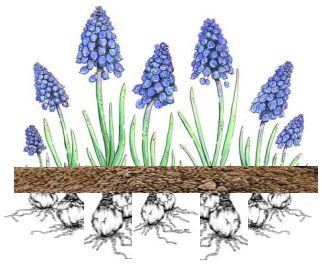
Fruits

Seeds

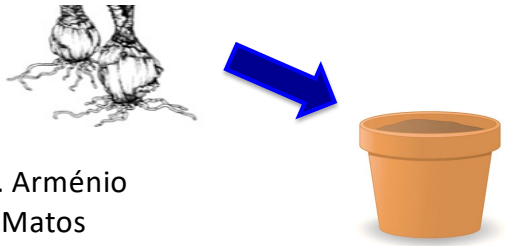
25

Conservation of endangered species

Bulbs of *B. dubia* were collected in the field and potted in the Botanic Garden of the University of Coimbra



March/April/May



Botanic Garden/Field

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Conservation of endangered species

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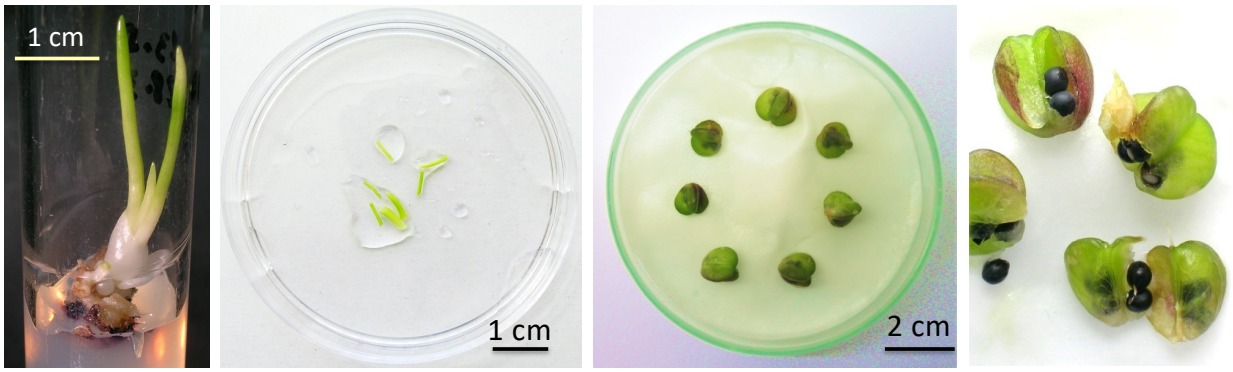
Somatic embryogenesis in *B. dubia*

Leaf segments from *in vitro* growing shoots and mature zygotic embryos were used to induce somatic embryogenesis



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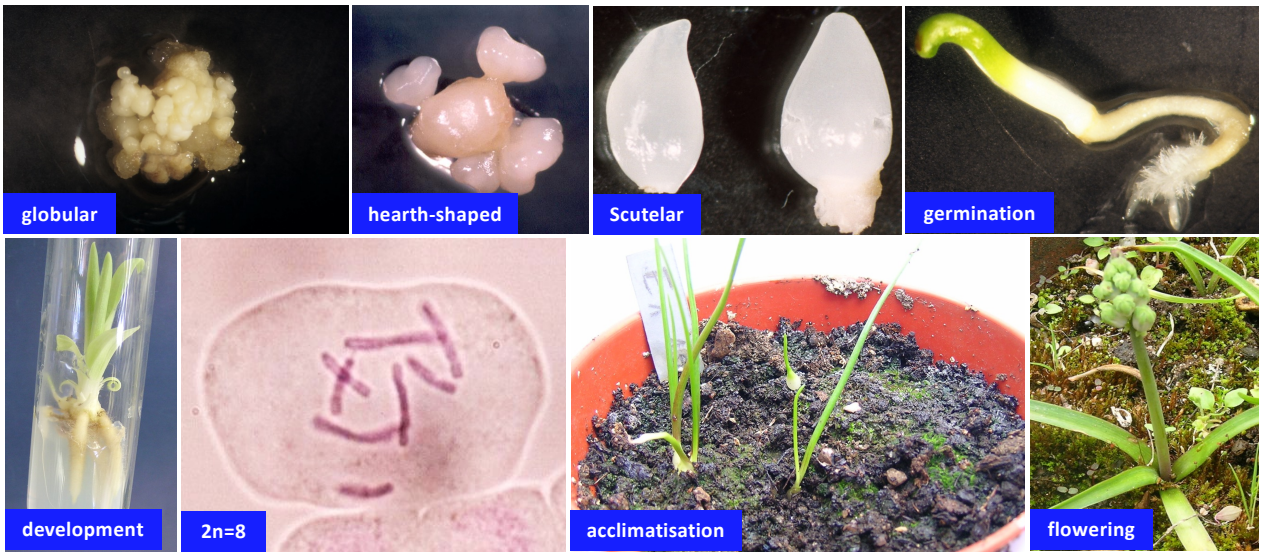
Somatic embryogenesis in *B. dubia*



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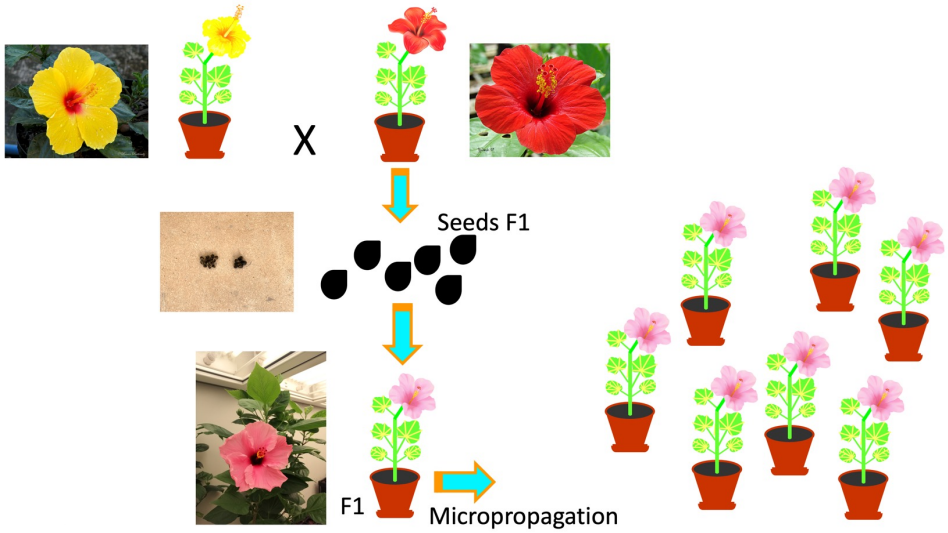
Propagation of hybrids or dioecious species

To keep the features of the hybrids they must be propagated asexually, for example, through somatic embryogenesis



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Propagation of hybrids or dioecious species

To same is true for dioecious species (kiwi)

Female flower

Male flower

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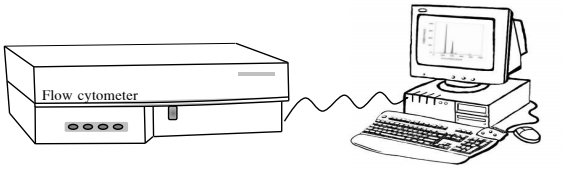
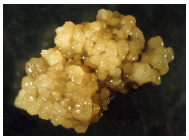
Production of tetraploids



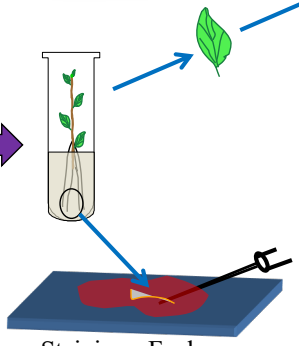
Flow cytometry analysis



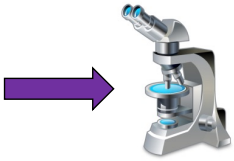
Embryogenic callus

Treatment with a c-mitotic agent

Δt



Staining - Feulgen reagent



Chromosome counting

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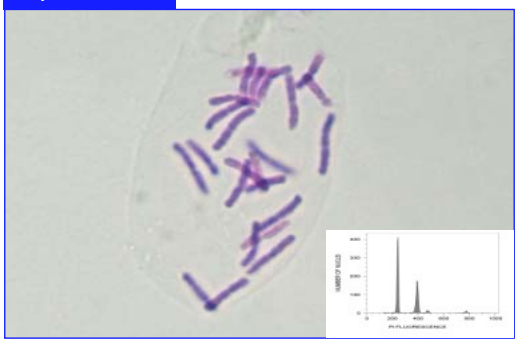
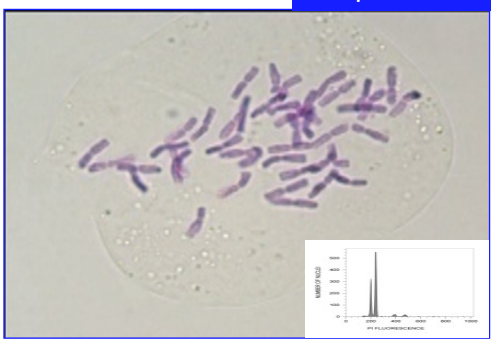
Production of tetraploids

Diploids 2n=24



Tetraploids 2n=48



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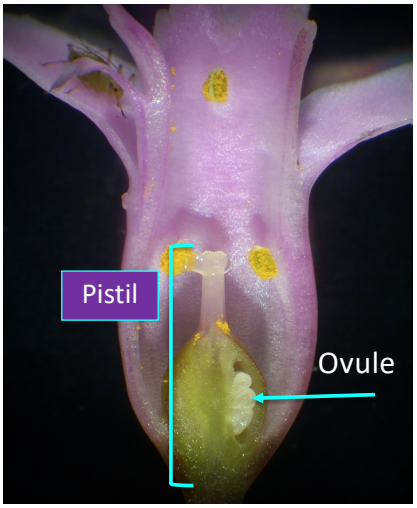
Embryo development

Zygotic embryos develop inside the ovule and ovary being surrounded by several mother tissues



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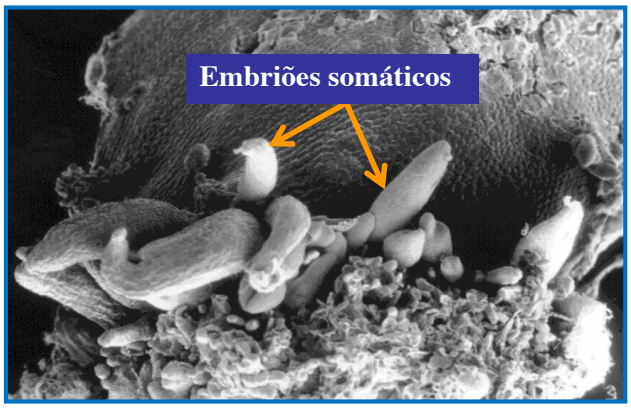
Embryo development

On the contrary, in a single explant hundreds of somatic embryos may be produced



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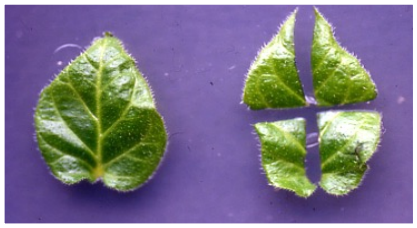
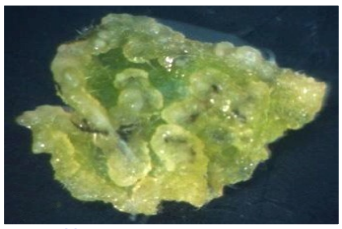
Embryo development



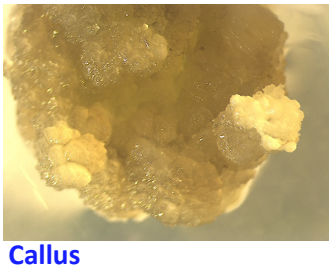
Leaves

4-6 weeks



Dedifferentiation

6 weeks



Callus

2,4-D or picloram (auxin)
Sucrose
Dark
25 ^{0}C

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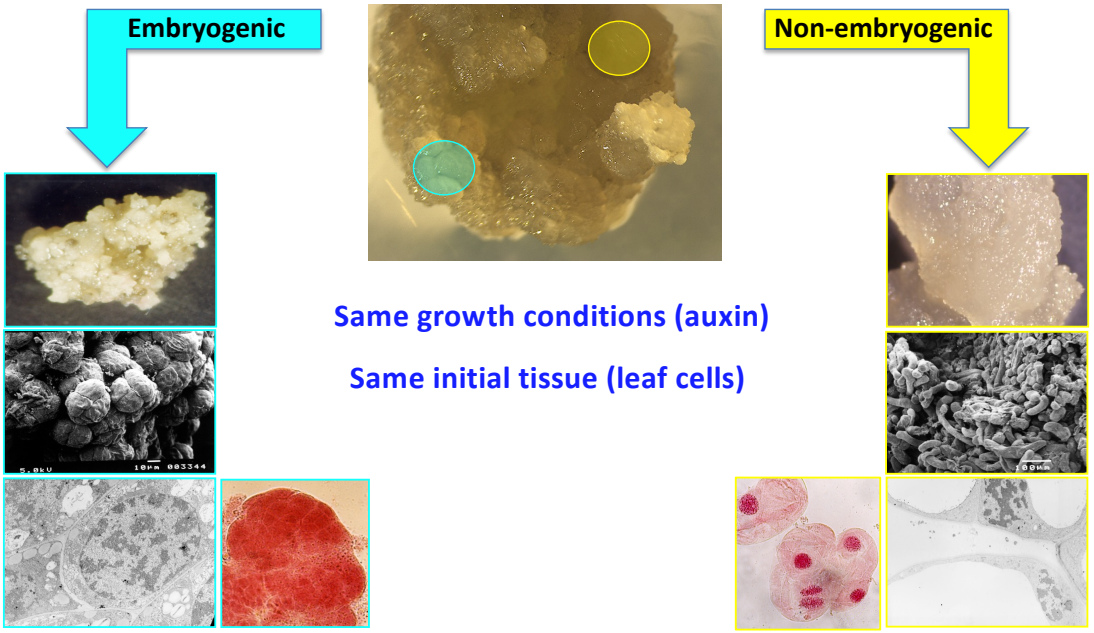
Tamarillo as a model system



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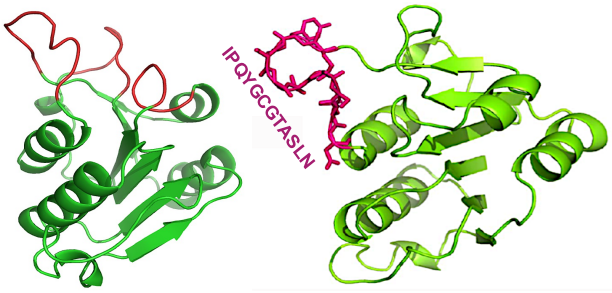
Proteomics to compare the two types of calli

A RNA methyl transferase is consistently expressed in non-embryogenic callus (NEP-TC)

Encodes 221 amino acids with a theoretical mass of 24.74 kDa and a theoretical isoelectric point of 6.09



A somatic embryogenesis inhibitor?

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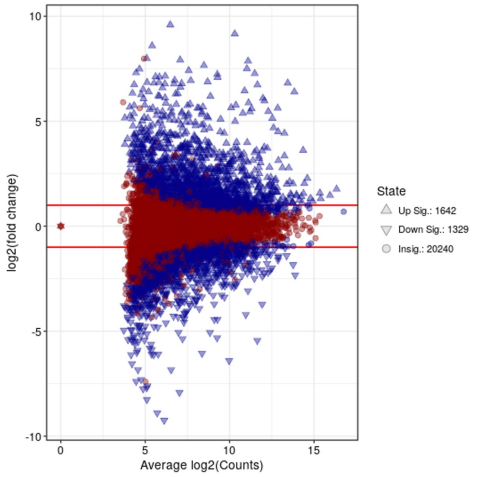
Transcriptomics



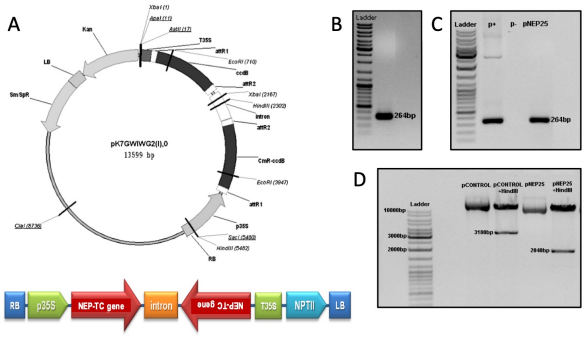
Embryogenic

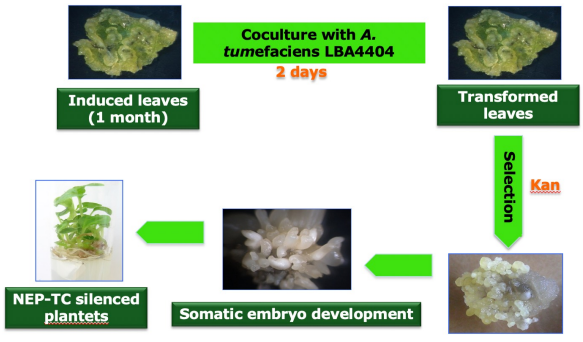
Non-embryogenic

BlastX Name	logfc
LOB domain-containing protein 22 TF	5.37
Ninja-family protein AFP3 TF	5.13
Protein FEZ TF	4.32
Transcription factor bHLH30 TF	3.91
Late embryogenesis abundant protein EM	3.45
Transcription factor bHLH14 TF	2.81
Transcription factor bHLH112 TF	2.68
Protein flowering locus (Histone deacetylase) EP	2.50
Lisine-specific demethylase (Histone demethylase) EP	2.27
DNA (cytosine-5)-methyltransferase 1 EP	2.26
Auxin-responsive protein IAA17 TF	-1.17
Transcription factor bHLH68 TF	-5.46
Auxin-responsive protein IAA14 TF	-5.74
LOB domain-containing protein 18 TF	-6.07
Ethylene-responsive transcription factor 13 TF	-7.49

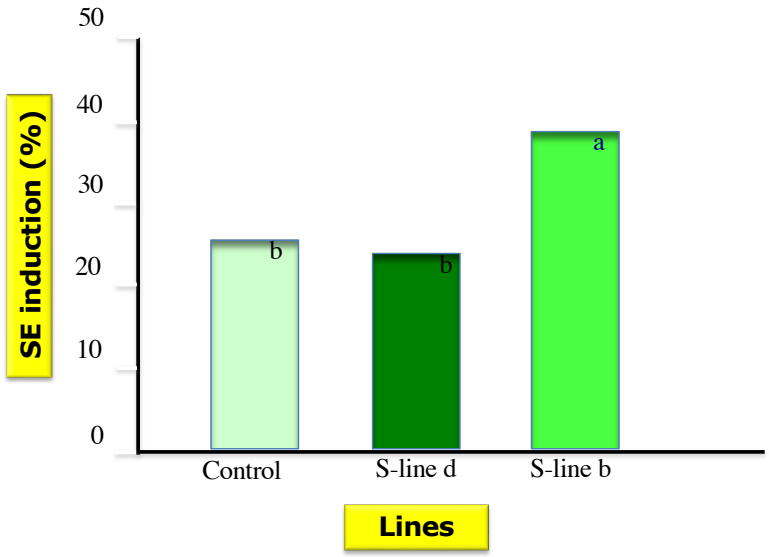
Comparative global transcriptional expression patterns of tamarillo genes. MA plot depicting the log2 fold change of genes against the average expression level in embryogenic and non-embryogenic samples.

Silenced lines of tamarillo for NEP-TC





Some silenced lines show na increase on SE induction



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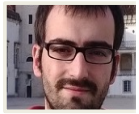
Ongoing works

Daniela Cordeiro (PhD student)
Functional characterization of epigenetic markers during plant regeneration



André Caeiro (PhD student)
NMR-based analyses to identify metabolomic markers of somatic embryogenesis in tamarillo (*Solanum betaceum* Cav.)

Bruno Casimiro (PhD student)
Development of bioreactor systems for plant-derived metabolite production in tamarillo (*Solanum betaceum* Cav.)

Mariana Neves (PhD student)
The role of the plant hormone ethylene in tamarillo (*Solanum betaceum* Cav.) in vitro micropropagation: biochemical and molecular analyses

Ricardo Ferraz (PhD student)
Exploring the rRNA methyltransferases signalling pathway during *Solanum betaceum* and *Arabidopsis thaliana* somatic embryogenesis

Tamarillo somatic embryogenesis

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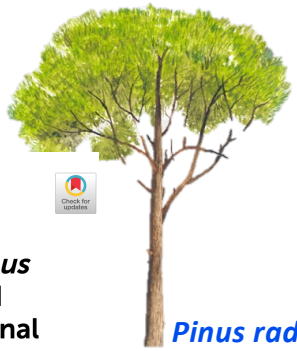
Ongoing works on somatic embryogenesis

Pinus halepensis

ORIGINAL RESEARCH article
Front. Plant Sci., 12 April 2021 | https://doi.org/10.3389/fpls.2021.631239

Proteome-Wide Analysis of Heat-Stress in *Pinus radiata* Somatic Embryos Reveals a Combined Response of Sugar Metabolism and Translational Regulation Mechanisms

Ander Castander-Olarieta[1], Cátia Pereira[1,2], Itziar A. Montalbán[1†], Vera M. Mendes[3], Sandra Correia[2], Sonia Suárez-Álvarez[1], Bruno Manadas[3], Jorge Canhoto[2] and Paloma Moncaleán[1*†]



Pinus radiata

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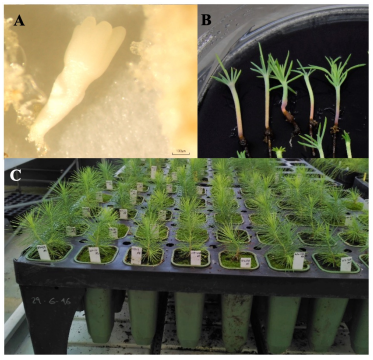
Ongoing works on somatic embryogenesis

Cátia Pereira



plants MDPI

Article

Heat Stress in *Pinus halepensis* Somatic Embryogenesis Induction: Effect in DNA Methylation and Differential Expression of Stress-Related Genes

Cátia Pereira [1,2], Ander Castander-Olarieta [2], Ester Sales [3], Itziar A. Montalbán [2], Jorge Canhoto [1,*] and Paloma Moncaleán [2,*]

forests MDPI

Article

Embryonal Masses Induced at High Temperatures in Aleppo Pine: Cytokinin Profile and Cytological Characterization

Cátia Pereira [1,2], Ander Castander-Olarieta [2], Itziar A. Montalbán [2], Aleš Pěnčík [3], Ivan Petřík [3], Iva Pavlović [3], Eliana De Medeiros Oliveira [4], Hugo Pacheco de Freitas Fraga [5], Miguel Pedro Guerra [6], Ondrej Novák [3], Miroslav Strnad [3], Jorge Canhoto [1,*] and Paloma Moncaleán [2,*]

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Ongoing works on somatic embryogenesis

Cloning adult trees of *Arbutus unedo* L. through somatic embryogenesis
Martins J; Correia S, Pinto G & Canhoto J

Plant Cell, Tissue and Organ Culture (PCTOC)
Journal of Plant Biotechnology


João Martins

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Collaborations

CNR-IVALSA TREES AND TIMBER INSTITUTE
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FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR
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UNIVERSITY OF MARYLAND
UNIVERSIDADE FEDERAL DE SANTA CATARINA
cesam universidade de aveiro centro de estudos do ambiente e do mar
CSIC
cebal Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo
CNC CENTER FOR NEUROSCIENCE AND CELL BIOLOGY UNIVERSITY OF COIMBRA, PORTUGAL
itqb UNIVERSIDADE NOVA DE LISBOA
ESAC ESCOLA SUPERIOR AGRÁRIA POLITÉCNICO DE COIMBRA
NEIKER MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE
INRA SCIENCE & IMPACT
iBET Instituto de Biologia Experimental e Tecnológica
INSTITUTO GULBENKIAN DE CIÊNCIA
CiB centro de informação de biotecnologia
COTHN CENTRO OPERATIVO E TECNOLÓGICO HORTOFRUTÍCOLA NACIONAL
EDUCACIÓN PÚBLICA Y GRATUITA
UNIVERSIDAD NACIONAL DE LA PLATA

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We, the people

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Thank you

Jorge Canhoto

Centre for Functional Ecology,
Department of Life Sciences,
University of Coimbra

Summer Course – Plant Biotechnology

July 11th 2022

CENTRE FOR FUNCTIONAL ECOLOGY

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