Regional Seminar 2017 in Latin America

Plantas de la Mata Atlántica brasileña con potencial para ampliación de la seguridad alimentaria

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Where are we?

Curitiba, Parana, Brazil

Where are we?

Federal University of Parana

Laboratory of Plant Micropropagation

http://www.bio.ufpr.br/portal/lmv/

Atlantic forest biome

- **Brazil**, Argentina and Paraguay;
- Only 8% of its original extent.

World's most threatened biodiversity hotspots

Brazilian Atlantic Forest – food resources

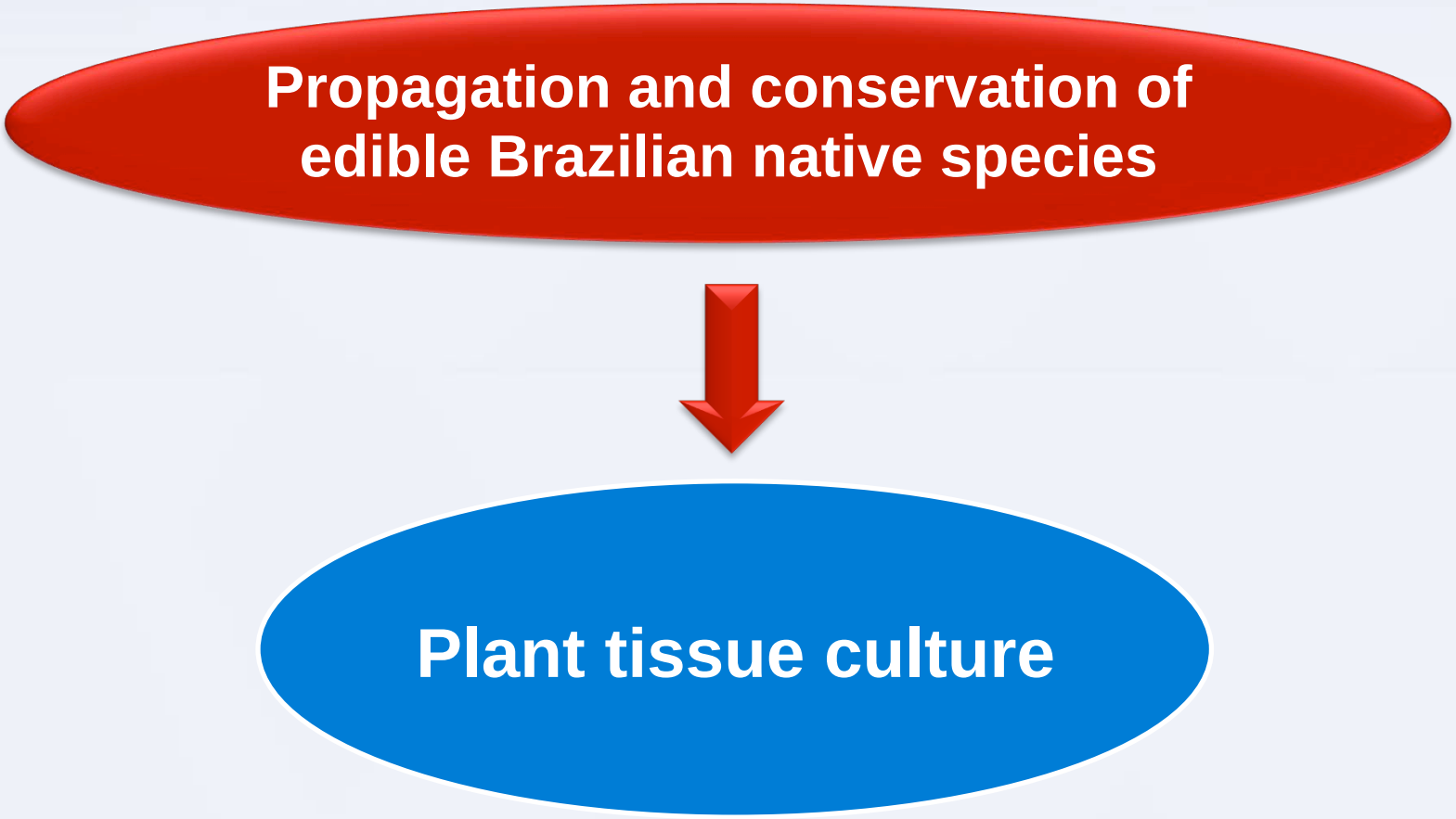
Brazilian initiatives – food resources

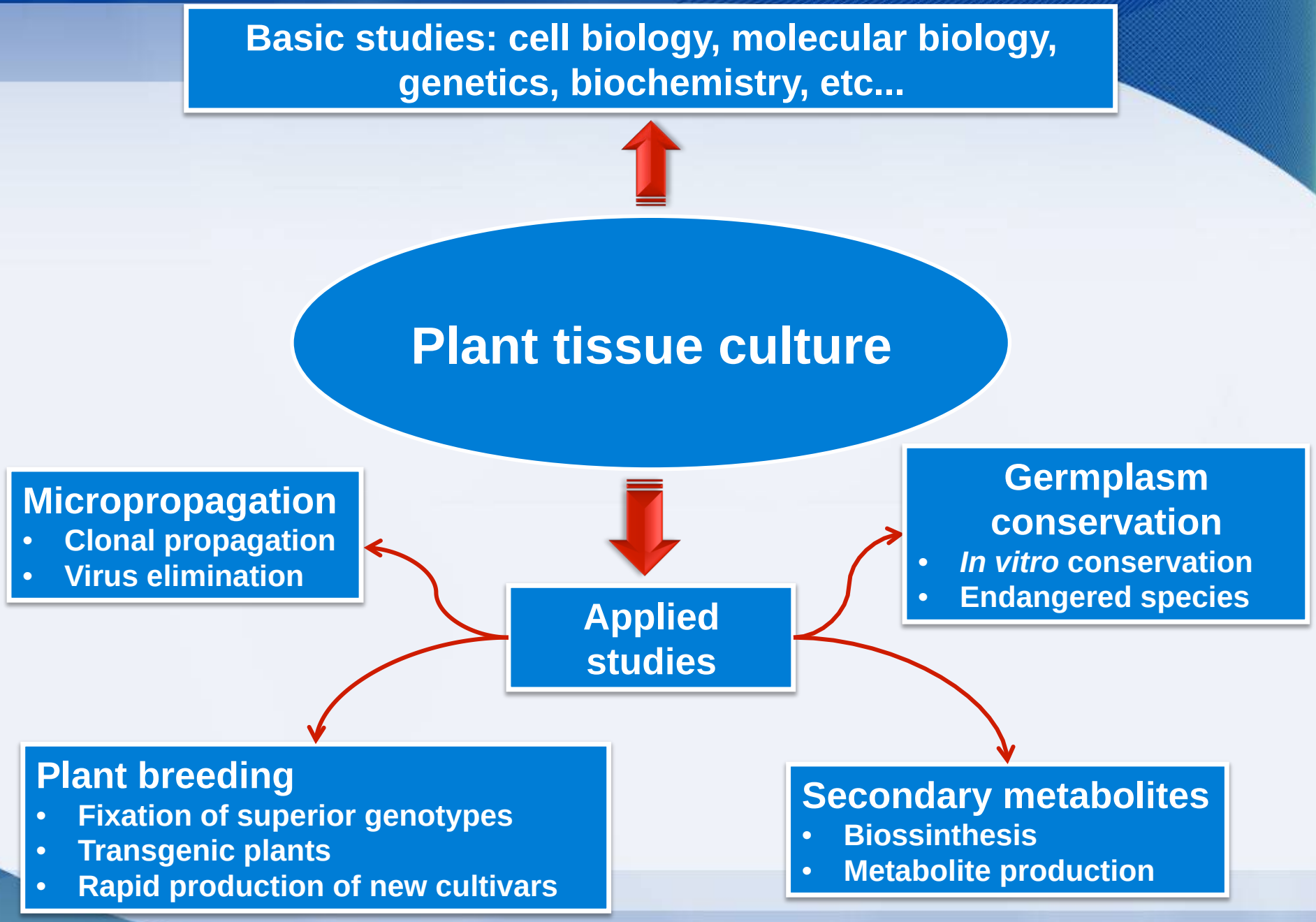
Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial

Plantas para o Futuro - Região Sul

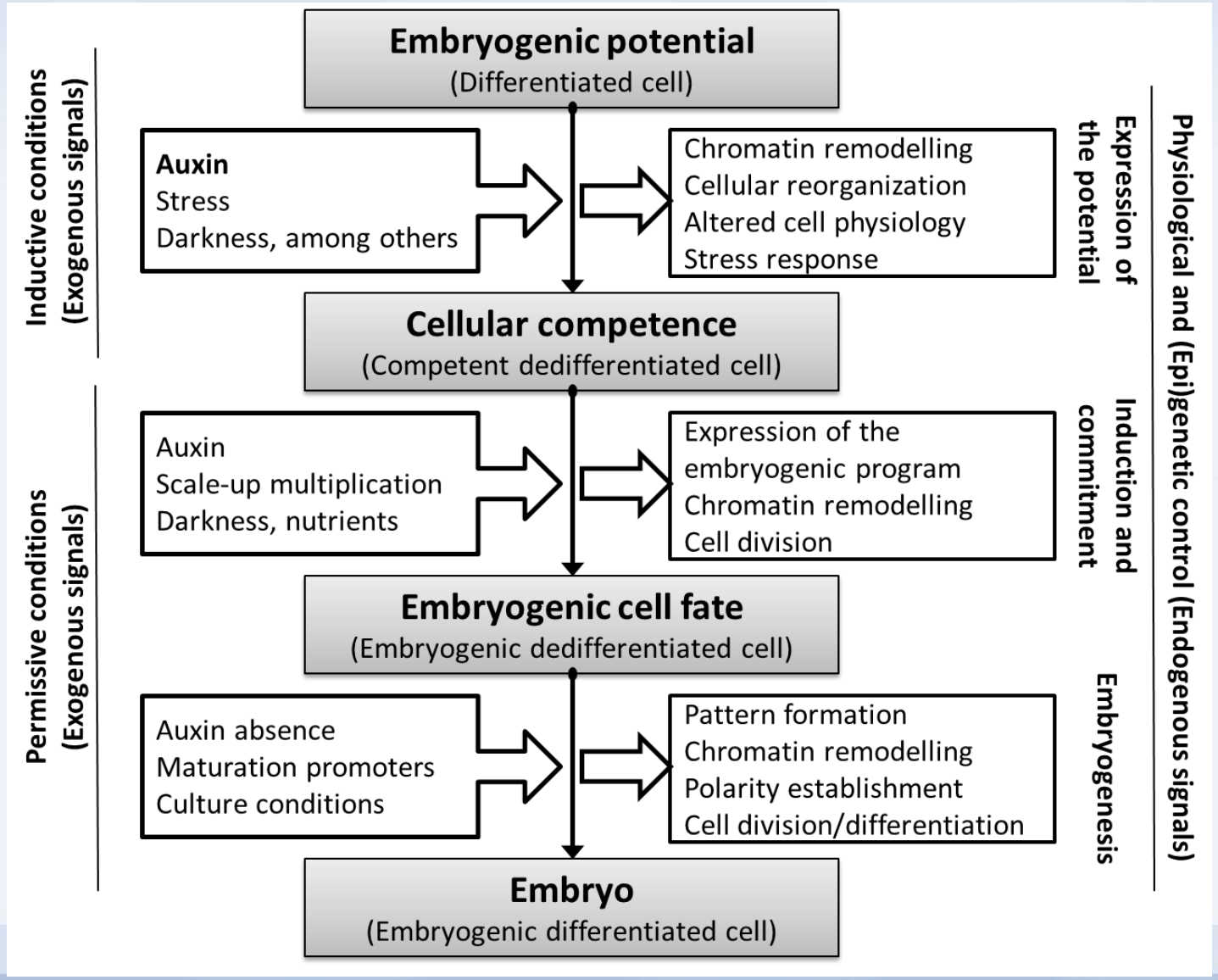
Coradin et al. (2011)

Brazilian initiatives – food resources



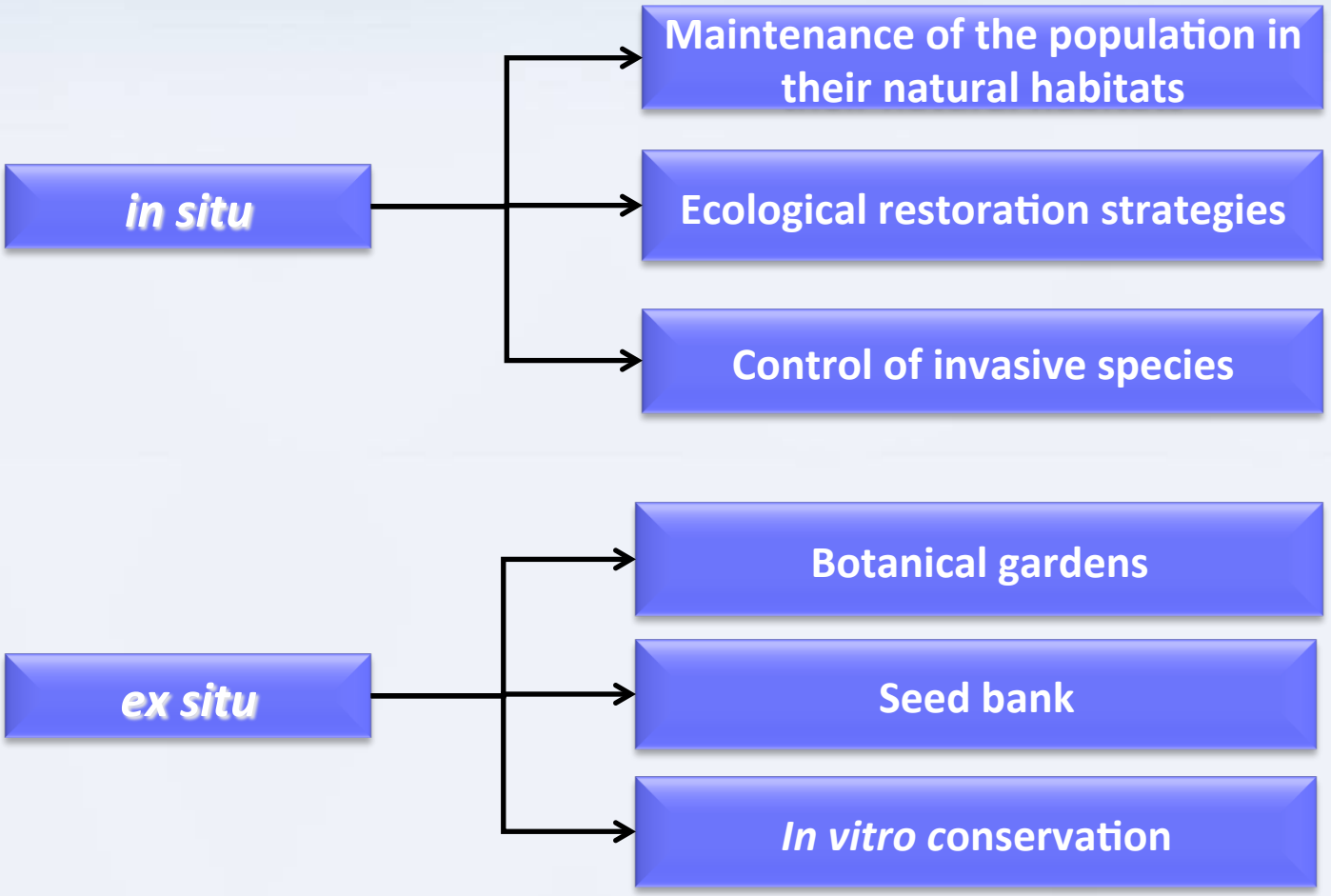

Basic studies: cell biology, molecular biology, genetics, biochemistry, etc...
Plant tissue culture
Micropropagation
• Clonal propagation
• Virus elimination
Germplasm conservation
• In vitro conservation
• Endangered species
Applied studies
Plant breeding
• Fixation of superior genotypes
• Transgenic plants
• Rapid production of new cultivars
Secondary metabolites
• Biossinthesis
• Metabolite production

Modulation of SE



Guerra et al. (2016)

Conservation strategies



Cryopreservation

Advantages:

- **Applicable for recalcitrant species;**
- **Prevents contamination from the subculture;**
- **Prevents loss of embryogenic competence;**
- **Long-term conservation.**

Models of study

Acca sellowiana

Araucaria angustifolia

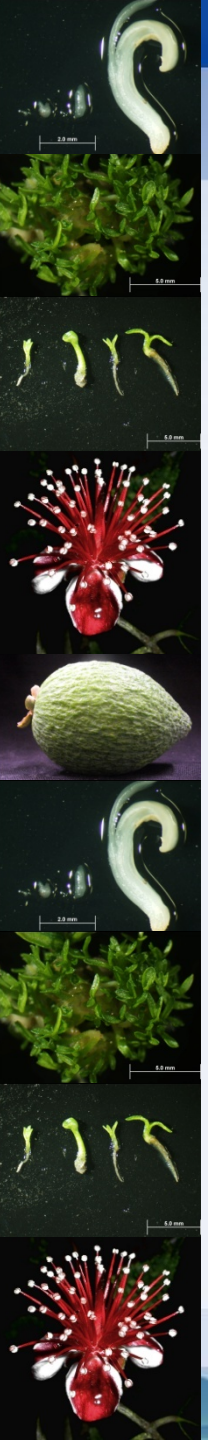
Acca sellowiana (O.Berg) Burret

- Native to South Brazil and North Uruguay.


Direct somatic embryogenesis in *A. sellowiana*


Somatic embryo maturation


Conversion of somatic embryos



Mother Plant

Zygotic embryos

2,4-D Darkness

Induction of Competence/ determination

Embryogenic culture

Scale-up multiplication

Multiplication cycle

A

2,4-D

-Citocinine
-Light
-Osmotic agents

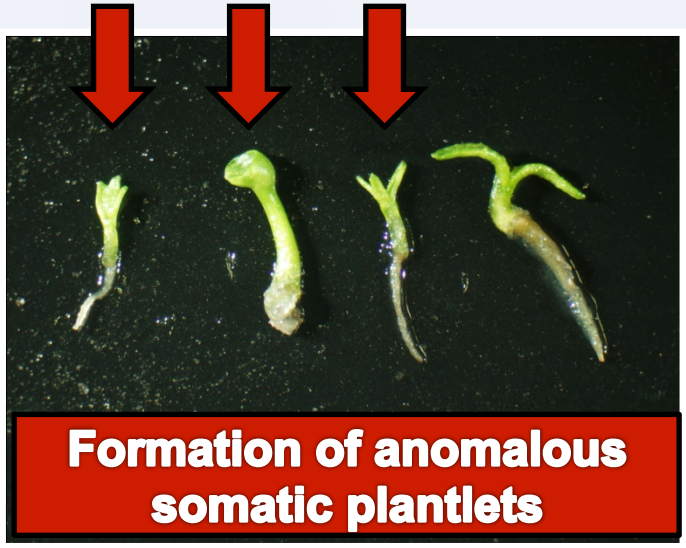
Maturation cycle

B

Inhibition of multiplication

Somatic Embryo maturation

Somatic embryos conversion



Formation of anomalous somatic plantlets


2.0 mm

Plant Science 210 (2013) 224–231

Contents lists available at SciVerse ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Comparative proteomic analysis of off-type and normal phenotype somatic plantlets derived from somatic embryos of Feijoa (*Acca sellowiana* (O. Berg) Burret)

Hugo Pacheco de Freitas Fraga[a,1], Sarah Zanon Agapito-Tenfen[a,b,1], Clarissa Alves Caprestano[a], Rubens Onofre Nodari[a], Miguel Pedro Guerra[a,*]

[a] Graduate Program in Plant Genetic Resources, Plant Developmental Physiology and Genetics Laboratory, Federal University of Santa Catarina, Rodovia Admar Gonzaga, 1346, ZC: 88040-900 Florianópolis, Santa Catarina, Brazil
[b] Genøk, Center for Biosafety, The Science Park, P.O. Box 6418, 9294 Tromsø, Norway

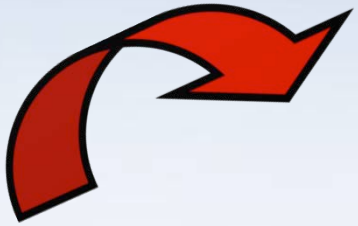
Material and methods

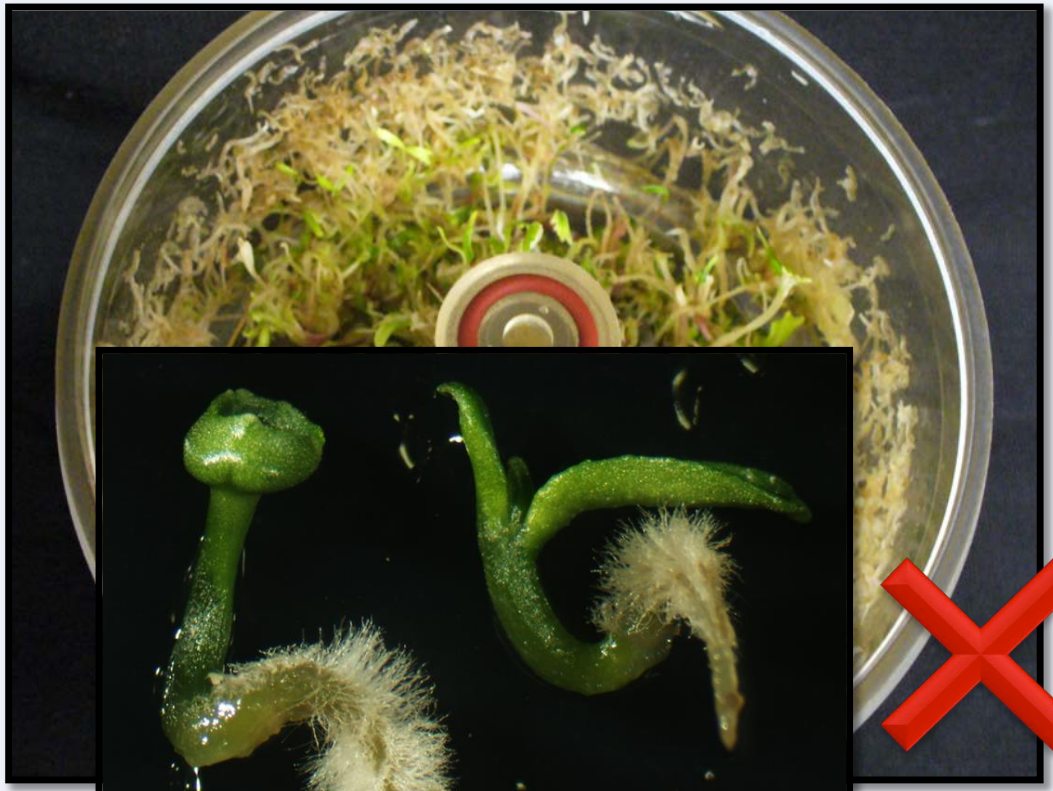
Plant material:

SE induction → **Plantlets formation**

Material and methods

Sample collection:





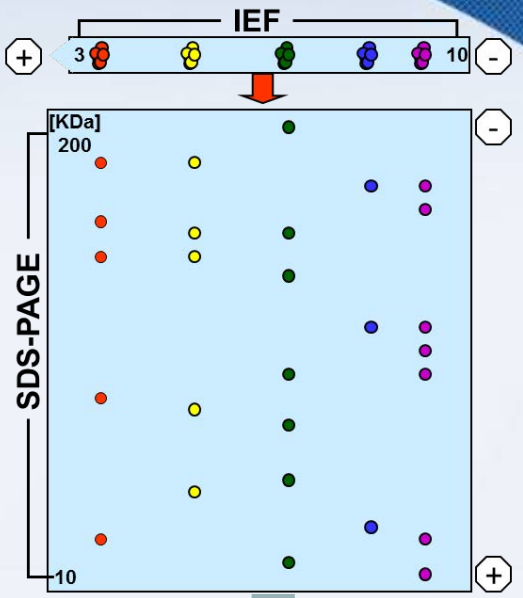
So ys convers

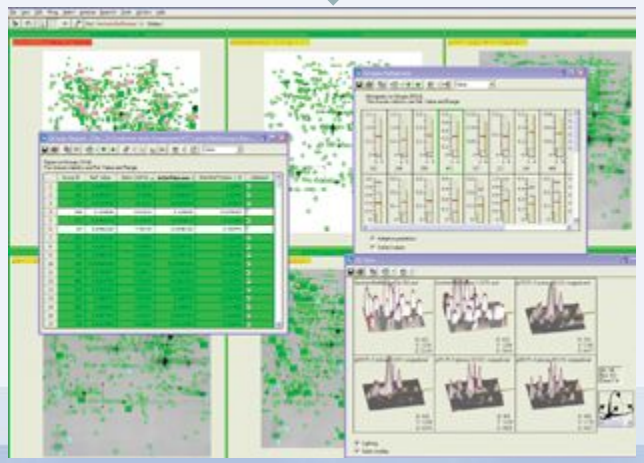
Material and methods

Proteomics procedures:

Protein extraction

Protein quantification





Gel analysis

Protein identification

Results

Table 2. MS/MS identification of the differentially expressed proteins in off-type somatic plantlets of *Acca sellowiana* at 10 and 20 days conversion in comparison to normal phenotype somatic plantlets.

Protein name[a]	Spot No.[b]	MW (kDa) theor./exp.	pI theor./exp.	NCBI accession No.	No. of peptides matched	Mascot score	Fold of variation[c]	Cellular component GO term[d]	Biological process GO term[d]
10 days after somatic embryos conversion									
vicilin-like storage protein [*Araucaria angustifolia*]	1	53.4/10.5	7.74/5.00	gi 21913852	12	543	ON	Protein bodies or vacuoles	nutrient reservoir activity
glyceraldehyde-3-phosphate dehydrogenase C1 (GAPDH) [Pyrus x bretschneideri]	154	36.9/41	8.24/6.10	gi 381393064	14	472	ON	Cytoplasm	glycolysis metabolic process
luminal-binding protein 5 [*Vitis vinifera*]	157	73.5/78.5	5.10/5.02	gi 225426230	5	269	OFF	Endoplasmic reticulum	Signal transducer activity
20 days after somatic embryo conversion									
3-phosphoglycerate kinase (PGK) [*Zea mays*]	182	31.6/44.5	5.01/6.23	gi 28172915	6	312	ON	Cytoplasm	glycolysis metabolic process
heat shock cognate 70 kDa protein-like [*Glycine max*]	694	70.8/75	5.10/5.14	gi 356569000	9	441	OFF	Endoplasmic reticulum	Signal transducer activity

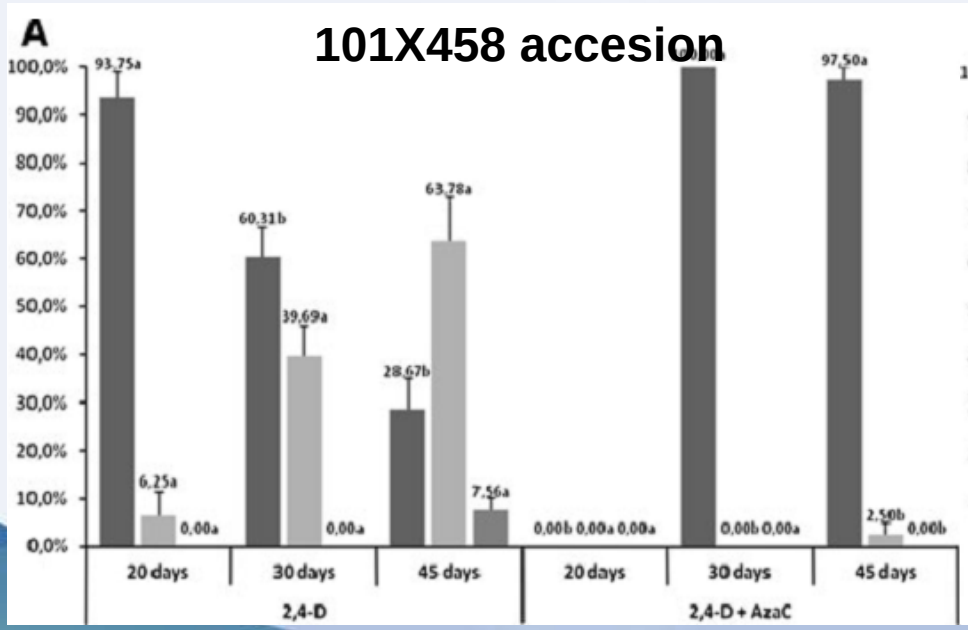
Plant Cell Rep
DOI 10.1007/s00299-012-1327-8

ORIGINAL PAPER

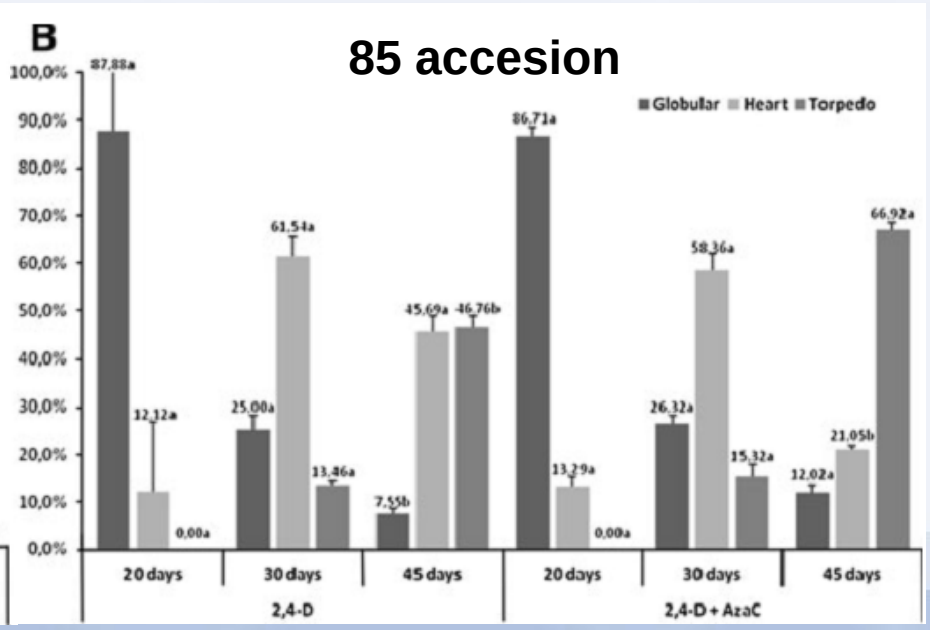
5-Azacytidine combined with 2,4-D improves somatic embryogenesis of *Acca sellowiana* (O. Berg) Burret by means of changes in global DNA methylation levels

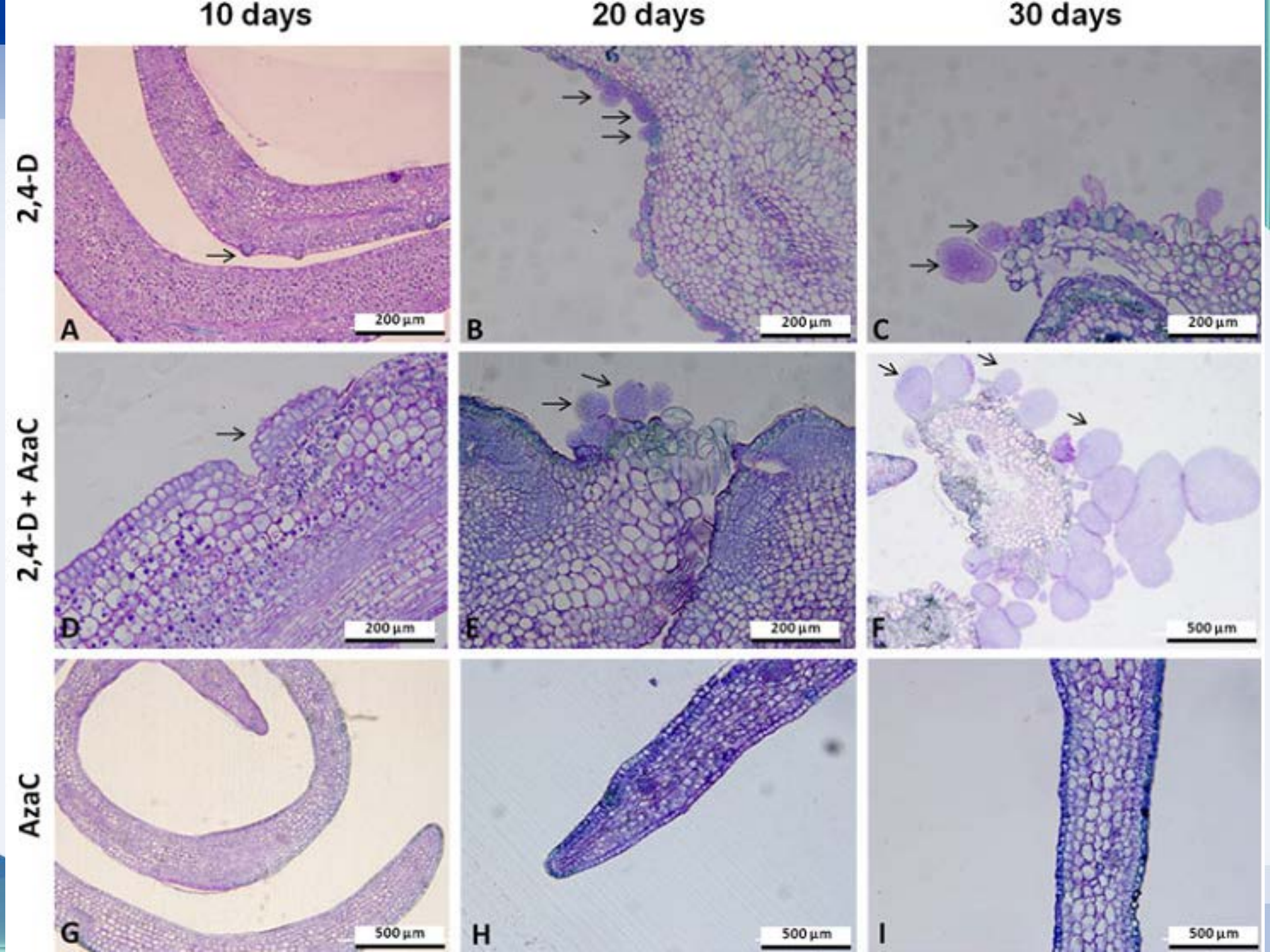
Hugo P. F. Fraga · Leila N. Vieira · Clarissa A. Caprestano · Douglas A. Steinmacher · Gustavo A. Micke · Daniel A. Spudeit · Rosete Pescador · Miguel P. Guerra

101X458 accesion

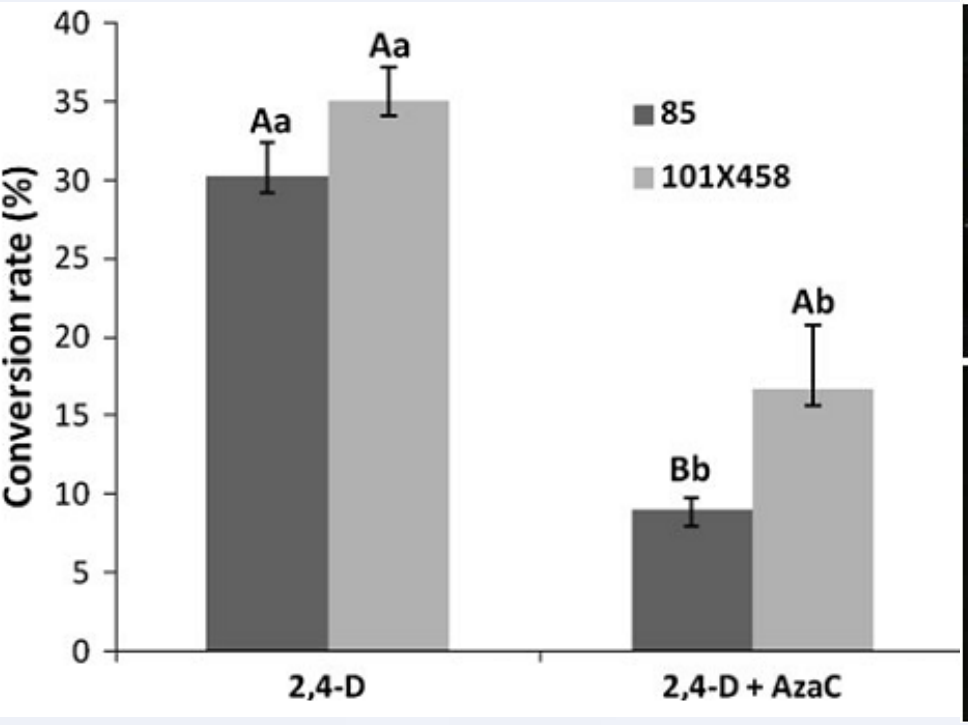


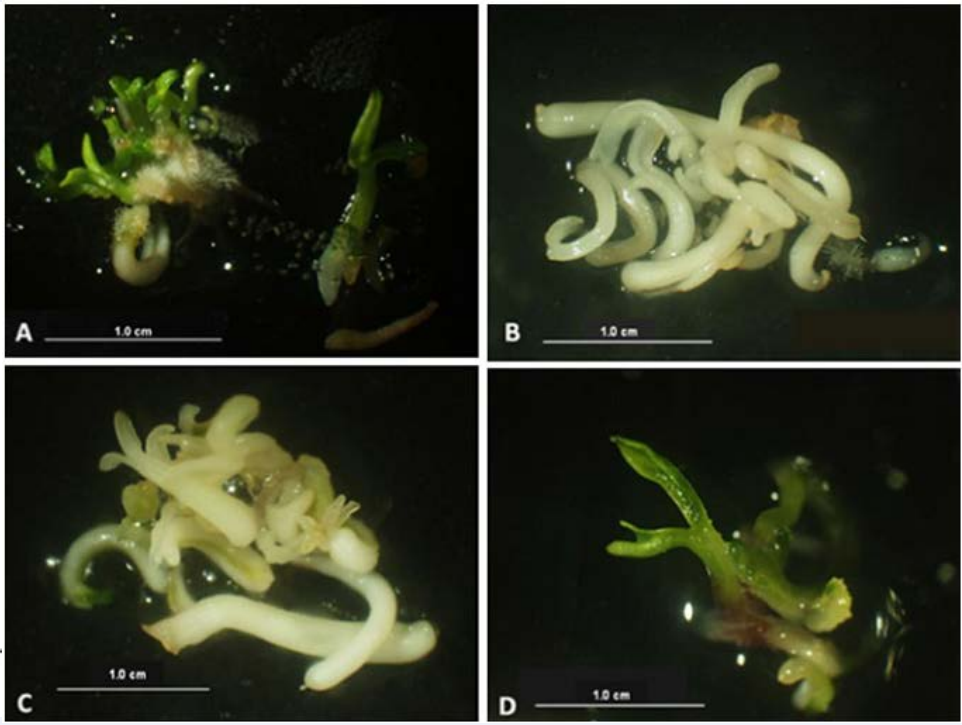
85 accesion



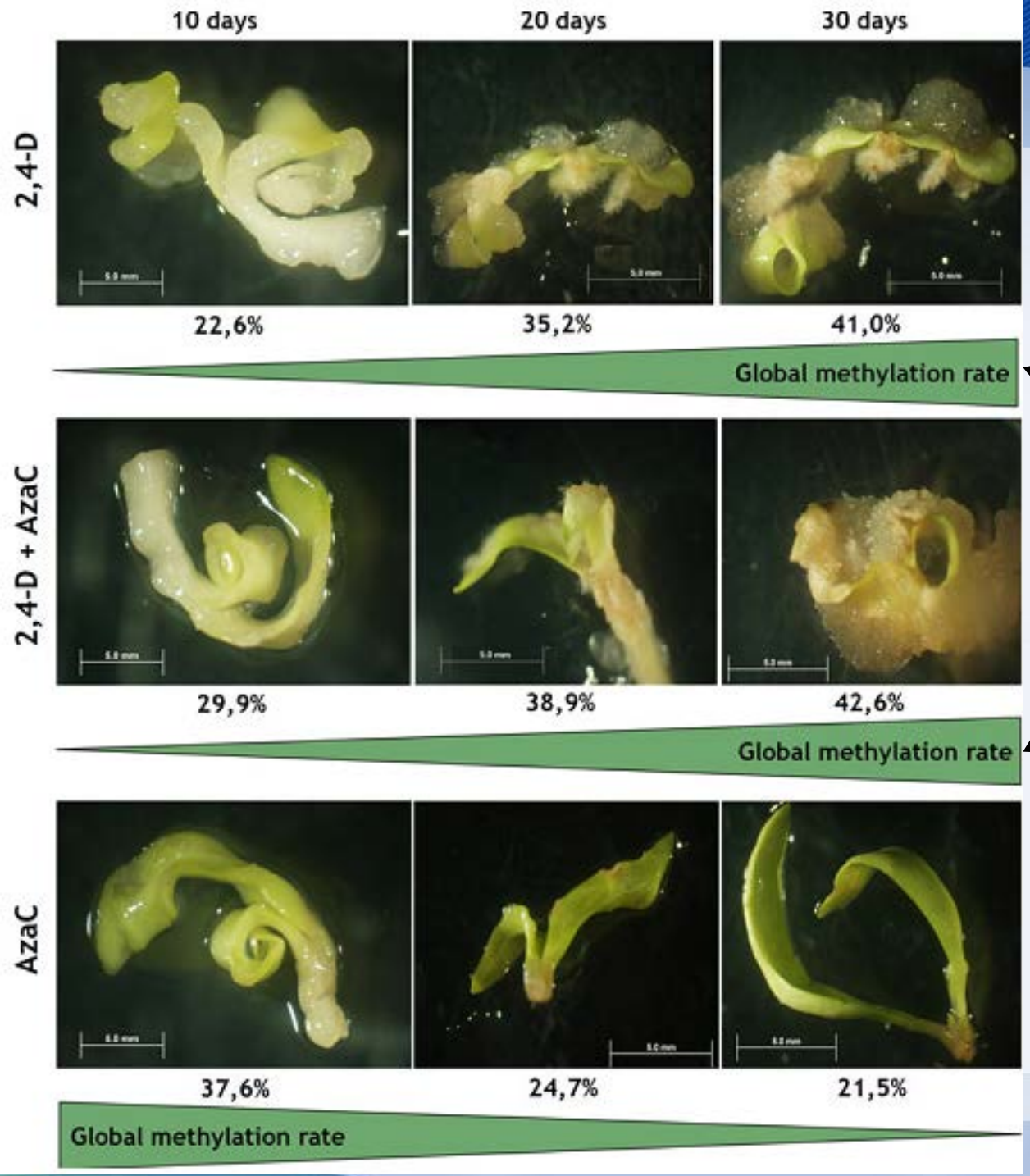

10 days
20 days
30 days
2,4-D
2,4-D + AzaC
AzaC
A
B
C
D
E
F
G
H
I
200 µm
500 µm

Morphological disorders during SE conversion of *A. sellowiana*





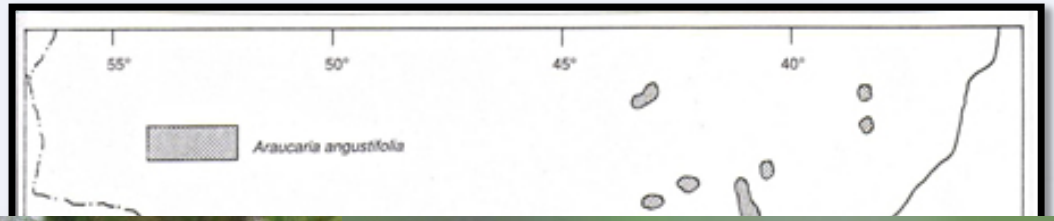
Fraga et al. (2012)



The presence of 2,4-D clearly affects the global DNA methylation rates.

Araucaria angustifolia (Bertol.) Kuntze

- **Atlantic forest → PR, RS, SC, RJ, MG e SP ↑500 ~ 1800 m.**
- **Economic relevance – wood, resin and seeds;**
- **Ecological relevance.**



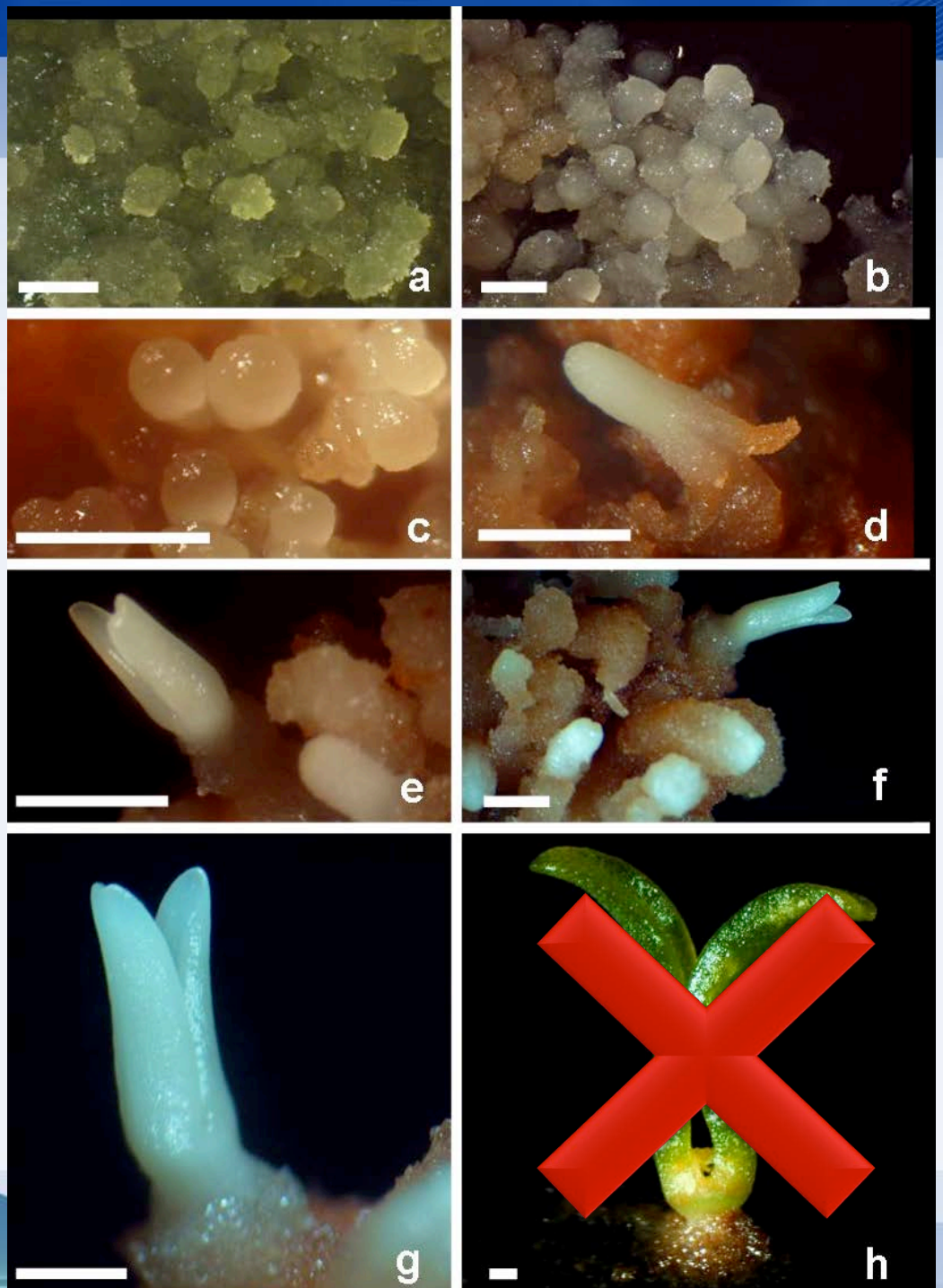
Araucaria angustifolia (Bertol.) Kuntze

Conservation status

Extinct | **Threatened** | **Least concern**

EX | EW | CR | EN | VU | NT | LC

Critically Endangered (CR) IUCN



SOMATIC EMBRYOGENESIS IN *A. angustifolia*

Steiner & Guerra (2005)

Trees
DOI 10.1007/s00468-015-1244-x

ORIGINAL ARTICLE

Time-lapse cell tracking reveals morphohistological features in somatic embryogenesis of *Araucaria angustifolia* (Bert) O. Kuntze

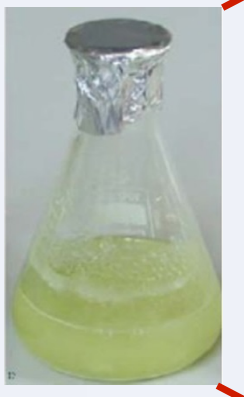
Hugo P. F. Fraga[1] · Leila N. Vieira[1] · Catarina C. Puttkammer[1] · Eliana M. Oliveira[2] · Miguel P. Guerra[1]

Received: 12 April 2015 / Revised: 19 June 2015 / Accepted: 22 June 2015

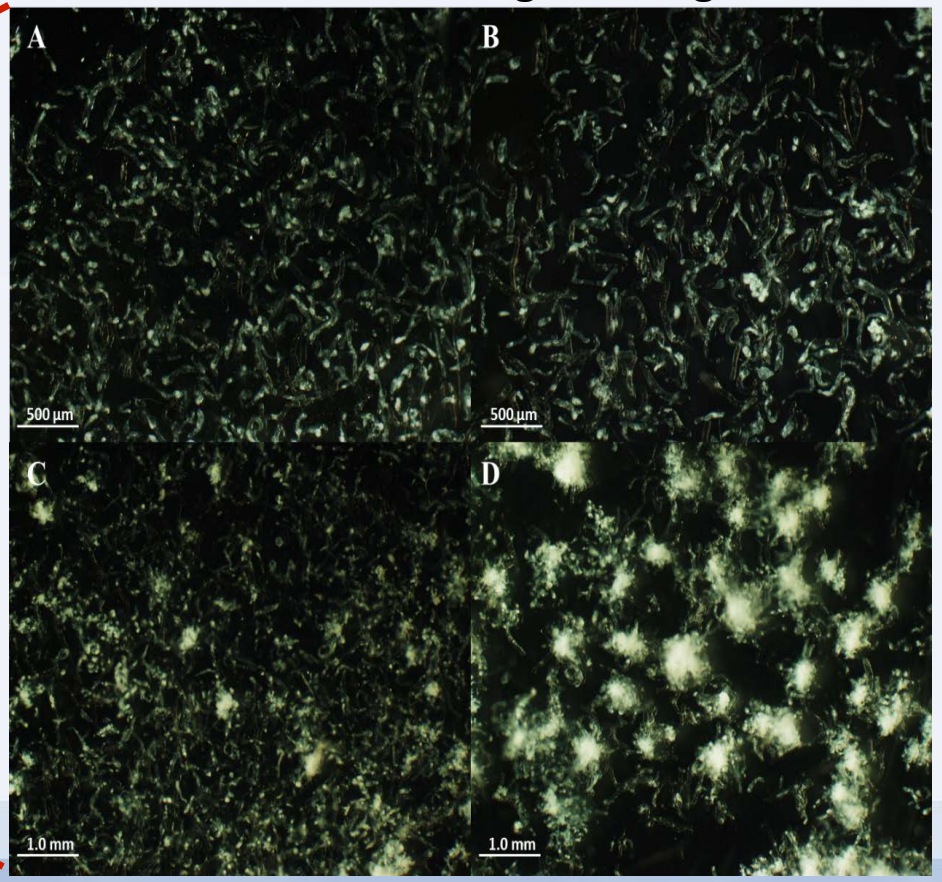
Material and methods

- Cell fractionation and *cell tracking*
- LM and TEM of different cell types

Imobilization
BM + agarose 6 g L^{-1}

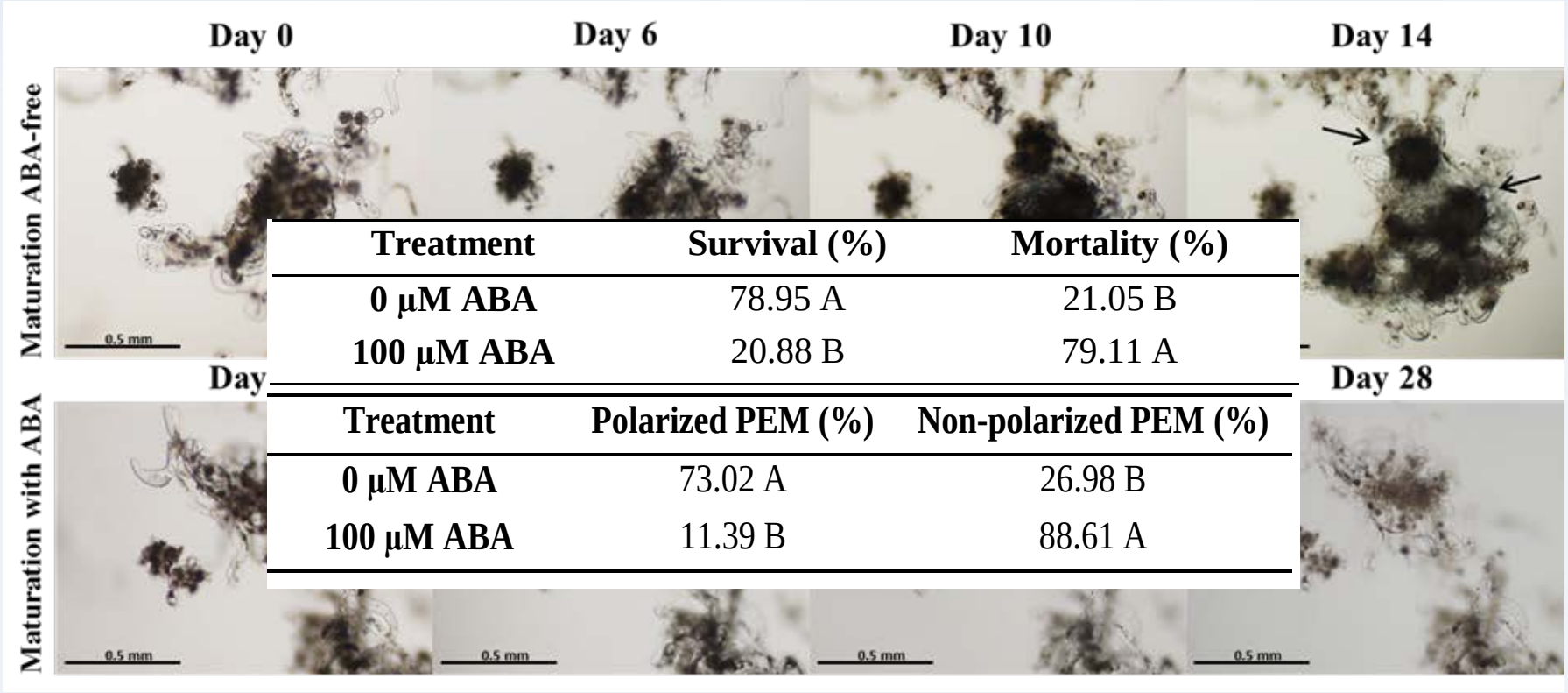


EC in
multiplication



Fraga et al. (2015)

▶ Results



Treatment	Survival (%)	Mortality (%)
0 µM ABA	78.95 A	21.05 B
100 µM ABA	20.88 B	79.11 A

Treatment	Polarized PEM (%)	Non-polarized PEM (%)
0 µM ABA	73.02 A	26.98 B
100 µM ABA	11.39 B	88.61 A

Fig 5. *Cell tracking* during EC maturation

Plant Cell Tiss Organ Cult
DOI 10.1007/s11240-015-0895-z

ORIGINAL ARTICLE

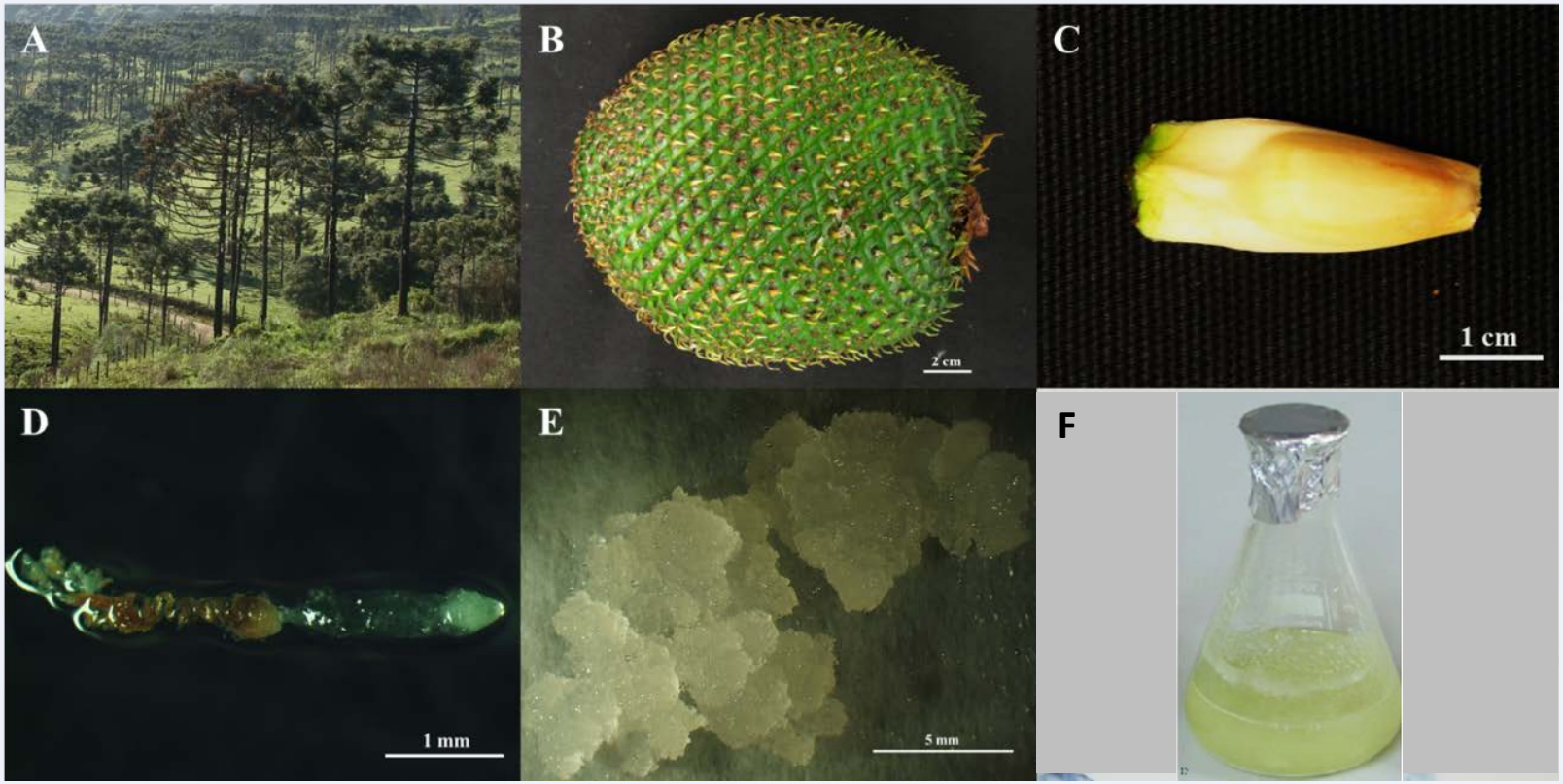
High-efficiency cryopreservation of *Araucaria angustifolia* (Bertol.) Kuntze embryogenic cultures: ultrastructural characterization and morpho-physiological features

Hugo P. F. Fraga[1] · Leila N. Vieira[1] · Catarina C. Puttkammer[1] · Jamily M. da Silva[1] · Karina G. dos Anjos[1] · Eliana M. Oliveira[2] · Miguel P. Guerra[1]

Received: 4 July 2015 / Accepted: 20 October 2015

Material and methods

EC induction



Material and methods

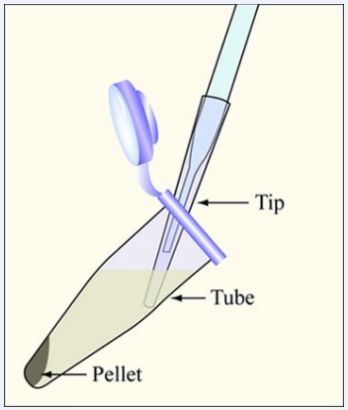
Cryopreservation experiments

1,8 mL EC

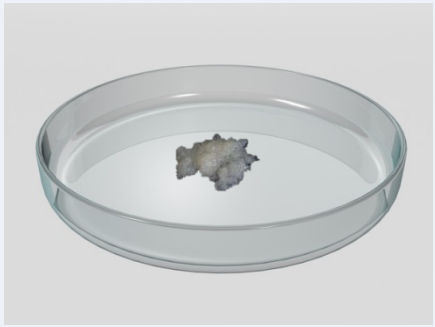
-80°C for 4h

LN fro 24h

40°C for ≈2 min



2 X centrifugation



Regrowth analysis
Days 30 and 60 culture

Mustafa et al. (2011)

▶ Results

EC regrowth after cryopreservation

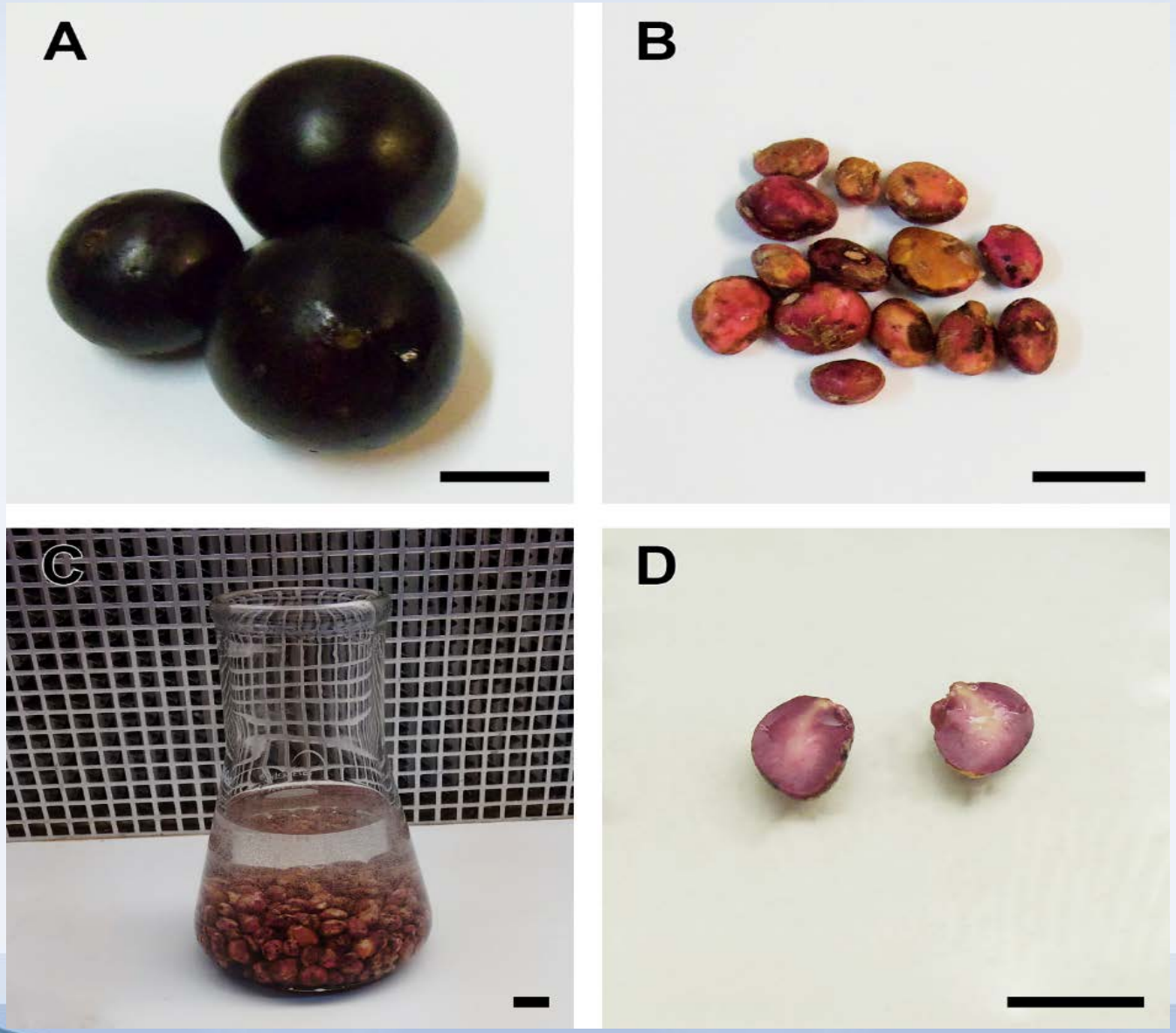
Incubation time		Cell lines regrowth rate (%)	
		Cr01	Cr02
30 min	Day 30	16.67 B	0 B
	Day 60	100 A	27.77 B
60 min	Day 30	50 A	0 B
	Day 60	100 A	5.56 B
120 min	Day 30	38.89 AB	0 B
	Day 60	100 A	11.12 B
240 min	Day 30	44.45 AB	88.89 A
	Day 60	100 A	100 A

Table 1 EC of *A. angustifolia* subjected to different incubation times (30, 60, 120 and 240 min) after thawing and culture for 30 and 60 days.

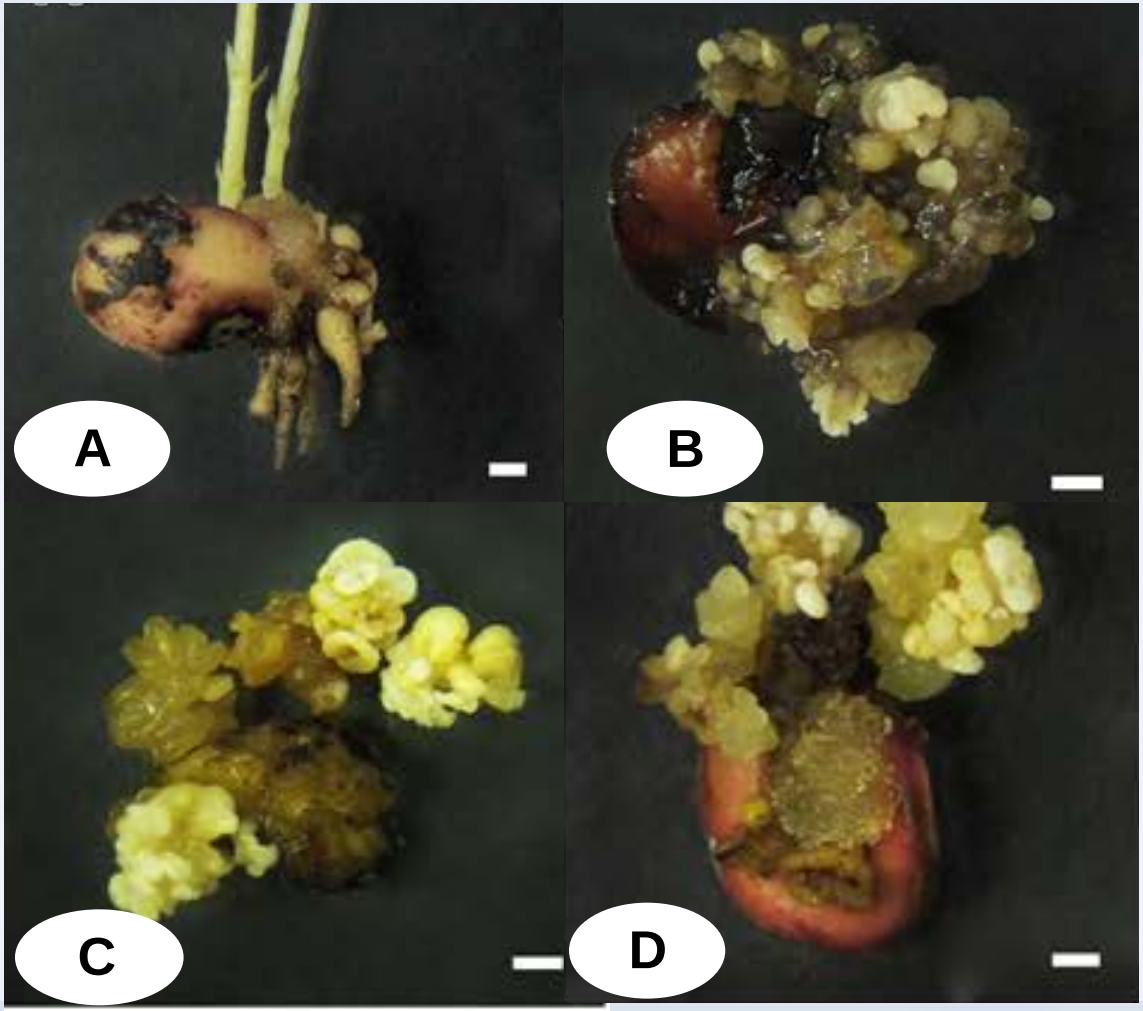
Jabuticaba – *Plinia cauliflora*

- Underutilized species;
- High-value nutritional properties;
- High-value organoleptic features;

Jabuticaba – *Plinia cauliflora*



Jabuticaba – *Plinia cauliflora*




Thank you all!
Muchas gracias!

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Website: http://www.bio.ufpr.br/portal/lmv/