4th International Conference of the IUFRO Working Party 2.09.02

Somatic Embryogenesis and Other Vegetative Propagation Technologies

BOOK OF ABSTRACTS

Development and application of vegetative propagation technologies in plantation forestry to cope with a changing climate and environment.

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4th International Conference of the IUFRO Working Party 2.09.02

Somatic Embryogenesis and Other Vegetative Propagation Technologies

Development and application of vegetative propagation technologies in plantation forestry to cope with a changing climate and environment

- September 19-23, 2016. La Plata, Buenos Aires, Argentina -

4th International Conference of the IUFRO Working Party 2.09.02

Development and application of vegetative propagation technologies in plantation forestry to cope with a changing climate and environment

Hosting Organizations

Supporting Organizations

Natural Resources Canada

Ressources nature Canada

Organizing Committee

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Fourth International Conference of the IUFRO Working Party 2.09.02 "Somatic Embryogenesis and Other Vegetative Propagation Technologies"

La Plata (Argentina) September 2016

Development and application of vegetative propagation technologies in plantation forestry to cope with a changing climate and environment

CONFERENCE PROGRAM

September 19 – MONDAY

14:00-16:00 – Workshop "innovations and challenges in vegetative propagation of trees". Faculty of Agricultural and Forest Sciences. Public access.

16:00-18:00 – Council of Economic Sciences: Registration & Poster Setup

18:00-20:00 – Council of Economic Sciences: Opening Reception by Hosting Authorities & Welcome Cocktail

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September 20 – TUESDAY

8:00-8:30 – Council of Economic Sciences: Registration & Poster Setup

8:30-9:10 – Opening Messages of the Local Organizing Committee, chaired by **Sandra Sharry**, President of the Local Organizing Committee of the Fourth International Conference of the IUFRO Unit 2.09.02.

9:10-9:50 – **Jean-François Trontin**, coordinator of the IUFRO 2.09.02 Unit and President of the Organizing Committee

- Presentation of the Fourth International Conference of the Working Party on "Development and application of vegetative propagation technologies in plantation forestry to cope with a changing climate and environment"

- Presentation of IUFRO and IUFRO 2.09.02 Working Party

- Presentation of the day's program & Introduction to Sessions 1 and 2

Session 1: Strategies for integration of vegetative propagation into breeding programs in the context of global warming and associated stresses

Chairman: **M. Raj Ahuja**

9:50-10:30 – Opening Lecture**: Sebastián Galarco,** Forestry Direction-Ministry of Agro-industry-Buenos Aires Province

10:30-10:50 – **Luciano Marcos Roussy**: "Habitat enhancement: the importance of vegetative propagation, especially for trees, in Landscape Engineering", by Roussy LM, Sceglio PO, Vera Bahima J

10:50-11:10 – **María Elena Aguilar Vega**: "Contributions of somatic embryogenesis and other in vitro propagation techniques to the genetic improvement of tropical woody species: Coffee, Teak and Gmelina", by Aguilar Vega ME, Ortiz Vargas JL, Kim YW, Moon HK

11:10-11:40 – Coffee Break

11.40-12:00 – **Stefaan Werbrouck**: "In vitro Biotechnology of *Melia volkensii*, a high potential forestry tree from eastern África", by Werbrouck S, Magomere T, Omondi S

12:00-12:20 – **Fernando Niella**: "Integrating low cost vegetative propagation techniques with a domestication and conservation strategy for multipurpose native species of Misiones, Argentina", by Niella F, Rocha SP

12:20-12:40 – **Miguel Pedro Guerra**: "Somatic embryogenesis model systems in selected Brazilian native trees", by Guerra MP

12:40-13:00 – **Juan Schapovaloff**: "Operational transfer of genetic improvement in loblolly pine at Arauco Argentina S.A.", by Schapovaloff J

13:00-13:40 – Key Invited Speaker: **Olivier Monteuuis** - "Vegetatively propagating forest trees"

Session 2: Towards multivarietal/clonal forestry: environmental factors affecting vegetative propagation of trees

Chairwomen: **Marie-Anne Lelu-Walter & Tuija Aronen**

13:40-14:00 – **Yong-Wook Kim**: "Somatic embryogenesis and mass propagation of clonal plants in *Larix kaempferi* (Japanese larch)", by Kim YW, Kim JA, Kim TD, Lee NN.

14:00-15:20 – Lunch

15:20-15:40 – **Marguerite Quoirin**: "Somatic embryogenesis in palm tree species: effect of growth regulators and anatomical aspects", by Quoirin M, Padilha JHD, Bonetti KAP, Ribeiro AZ, Amano É, Steinmacher DA

15:40-16:00 – **Isabel Arrillaga**: "Increasing resilience in forest tree species: a possible additional advantage for somatic embryogenesis technology", by Arrillaga I, Morcillo M, Cano M, Sales E, Peris JB, Segura J, Orlando L, Alborch A, Cano V, Corredoira E, Martínez MT, Cernadas MJ, Montenegro R, Vieitez FJ, Nisa M, Ramírez N, Hernández I, Ruiz-Galea M, González-Cabrero N, Celestino C, Montalbán I, Alegre J, Ballester A, Moncaleán P, San-José MC, Toribio M

16:00-16:20 – **Kateřina Eliášová**: "The effect of UV-B radiation on the development of Norway spruce somatic embryos", by Vondráková Z, *Eliášová K, Gemperlová L, Pešek B, Trávníčková A, Malbeck J, Fischerová L, Vágner M, Cvikrová M

16:20-18:20 – POSTER INTRODUCTION SESSION – Introducers: **Jean-François Trontin & Sandra Sharry**

18:20-20:00 – City Tour

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September 21 – WEDNESDAY

8:00-8:25 – Council of Economic Sciences: Poster Viewing

8:25-8:30 – **Jean-François Trontin** - Presentation of the day's program & Introduction to Session 3

8:30-9:10 – Key Invited Speaker: **Marie-Anne Lelu-Walter** – "Achievements in somatic embryo development in some conifers: what did we learn? Where are we going?"

Session 2: Towards multivarietal/clonal forestry: environmental factors affecting vegetative propagation of trees (continuation)

Chairwomen: **Marie-Anne Lelu-Walter & Tuija Aronen**

9:10-9:30 – **Iselen Trujillo**: "Somatic Embryogenesis of *in vitro Musa* clones", by Trujillo I

9:30-9:50 – **Kanagaraj Suganthi**: "In vitro propagation of mangroves for greenbelt development to mitigate climate change", by Suganthi K, Chinnappan RS, Govindaraju M, Kandasamy K

9:50-10:10 – **Gustavo J. Oberschelp**: "The design of a species-specific basal media for *Eucalyptus* considering the mineral nutrition status of young plant tissues", by Oberschelp J, Gonçalves AN

10:10-10:30 – **María Ángeles Basiglio Cordal**: "First advances in somatic embryogenesis of *Phytolacca tetramera*, an endangered shrub endemic species of the Province of Buenos Aires, Argentina", by Basiglio Cordal MA, Panarisi M, Dobler N, Sharry S

10:30-10:50 – **Valeria Rudoy:** TECNOPLANT: "From *Vaccinium* spp plants to blueberry extract tablets" by Rudoy V.

10:50-11:10 – **Patricia Boeri**: "Somatic embryogenesis of *Prosopis alpataco*", by Boeri P, Barrio D, Sharry.

11:10-11:40 – Coffee Break

Session 3: (Epi)genomics of embryo or other vegetative propagule development

Chairman: **Scott Merkle;** Chairwoman: **Isabel Arrillaga**

11.40-12:00 – **Jorge Canhoto**: "Genes are important to understand somatic embryogenesis but someone has to work: the proteins", by Correia S, Canhoto, JM

12:00-12:20 – **Celia Miguel**: "Identification of regulatory miRNA-target nodes across embryo development in *Pinus pinaster*", by Rodrigues AS, Chaves I, Costa B, Lin Y-C, Lopes S, Correia S, Bohn A, Miguel CM

12:20-12:40 – **Sandra Correia**: "Strategies for the early detection of embryogenic competent cells in tamarillo somatic embryogenesis", by Correia S, Caeiro A, Alhinho A, Augusto D, Veríssimo P, Canhoto JM

12:40-13:00 – **Conchi Sánchez**: "Auxin-mediated expression of a GH3 gene during adventitious rooting in chestnut in relation to ontogenic state in chestnut", by Vielba JM, Varas E, Rico S, Covelo P, Vidal N, Sánchez C

13:00-13:20 – **Nuria González Cabrero**: "Effect of culture conditions on DNA methylation in stone pine embryogenic lines", by González-Cabrero N, Guevara MA, de María N, Vélez MD, Díaz L, Toribio M, Cervera MT, Celestino C

13:20-13:40 – **Bruno Navarro**: "Cell-to-cell trafficking patterns of Brazilian Pine (*Araucaria angustifolia* Bertol. Kuntze) cell lines with contrasting embryogenic potential", by Navarro BV, Elbl PM, dos Santos ALW, de Oliveira LF, Demarco D, Buckeridge MS, Floh EIS

13:40-14:00 – **Anna Maria Wójcik**: "Auxin-related miRNA molecules involved in the induction of somatic embryogenesis", by Wójcik AM, Gaj MD

14:00-15:20 – Lunch

Session 3: (Epi)genomics of embryo or other vegetative propagule development (continuation)

Chairman: **Scott Merkle**; Chairwoman: **Isabel Arrillaga**

15:20-16:00 – **Jean-François Trontin**: "Somatic embryogenesis as an enabling technology for reverse genetics: achievements and prospects for breeding maritime pine (*Pinus pinaster* Ait.)", by Trontin J-F, Ávila C, Debille S, Teyssier C, Canlet F, Rueda-López M, Canales J, De la Torre F, El-Azaz J, Pascual B, Cañas R, Boizot N, Le Metté C, Lesage-Descauses M-C, Abarca D, Carneros E, Rupps A, Hassani SB, Zoglauer K, Arrillaga I, Mendoza-Poudereux I, Cano M, Segura J, Miguel C, De Vega-Bartol J, Tonelli M, Rodrigues A, Label P, Le Provost G, Plomion C, da Silva Perez D, Harvengt L, Díaz-Sala C, Cánovas FM, Lelu-Walter M-A

16:00-19:00 – POSTER SESSION

17:00-19:00 – Satellite Meeting organized by CIEFAP: "Current status and prospects of vegetative propagation technologies in Argentinean Patagonia". Council of Economic Sciences.

19:00-20:00 – Free Time

20:00-22:30 – Gala Dinner – Central Hall, Building of Presidency, La Plata University. Argentine Asado / Tango & Salsa

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September 22 – THURSDAY

8:00-8:25 – Council of Economic Sciences: POSTER SESSION

8:25-8:30 – **Jean-François Trontin -** Presentation of the day's program & Introduction to Sessions 4 & 5

8:30-9:10 – Key Invited Speaker: **Scott Merkle** – "Integration of selection, breeding, somatic embryogenesis and cryostorage to conserve and restore threatened North American forest trees", by Merkle SA, Ahn C, Tull AR, Montello PM, Dassow JE., Gladfelter HJ

Session 4: Preservation and adaptation of wild and selected genetic resources to environmental and socio-economic changes

Chairman: **Jorge Canhoto**

9:10-9:30 – **Itziar Aurora Montalbán**: "*Pinus radiata* and *Pinus halepensis* somatic embryogenesis: Can we modulate the success of the process provoking abiotic stress at the initial stages?", by Montalbán IA, García-Mendiguren O, Pereira C, Correia S, Canhoto J, Moncaleán P

9:30-9:50 – **Vanesa Cano Lázaro**: "Transformation of *Quercus suber* and *Quercus ilex* somatic embryos with a gene encoding a thaumatin-like protein", by Cano V, Corredoira, Martínez T, Ballester A, Toribio M, San José MC

9:50-10:10 – **João F. Da Silva Martins**: "Shoot proliferation of chestnut (*Castanea sativa* Mill.) and in vitro protective effect of endophytes against Phytophthora cinnamomi", by Martins JF, Canhoto JM

10:10-10:30 – **Nelly Aggangan**: "Mycorrhization improved growth and survival of somatic embryogenesis derived *Kalopanax septemlobus* and *Liliodendron tulipifera* microplants", by Aggangan NS, Moon H-K, Kim YW, SH Han S-H

10:30-10:50 – **Hernán Mattes Fernández**: "Induction of embryogenic cultures from mature seeds of a fragile population of *Nothofagus obliqua* (Nothofagaceae)", by Mattes Fernández H, Galván D, Guerra M, Ferrada M, Dezzotti A

10:50-11:10 – **Patricio Rojas Vergara**: "Cloning drought tolerant *Eucalyptus globulus* in the region of Bio-Bio, Chile", by Rojas Vergara P, Gutierrez B, Molina Brand MP, Koch L, Reyes M A

11:10-11:40 – Coffee Break

Session 5: Lessons from in vivo growth of vegetative propagules, especially in various pedoclimatic conditions

Chairmen: **Olivier Monteuuis & Tsuyoshi Maruyama**

11.40-12:00 – **Tsuyoshi Maruyama**: "Somatic embryogenesis and plant regeneration in Japanese pines and cypresses", by Maruyama TE, Hosoi Y

12:00-12:20 – **Saila Varis**: "Somatic embryogenesis in *Picea abies* using primordial shoot explants, and the germination of somatic embryos under different LED light systems", by Varis S, Lappalainen F, Tikkinen M, Aronen T

12:20-12:40 – **Karl-Anders Högberg**: "Selection effects of somatic embryogenesis in Norway spruce", by Högberg K-A

12:40-13:00 – **Jens I. Find**: "Results from the first full rotation of growth in clonal field trials of nordmanns fir (*Abies nordmanniana*)", by Find JI

13:00-13:20 – **Tuija Aronen**: "Field evaluation of Scots pine (*Pinus sylvestris* L.) emblings", by Aronen T, Harju A, Piri T, Hantula J

13:20-13:40 – **Mikko Tikkinen**: "Registration process of Norway spruce embryogenic cell lines for commercial forest regeneration in Finland", by Tikkinen M, Varis S, Aronen T

13:40-14:00 – **Corina Graciano**: "Sprout vigor of poplar cuttings from stoolbeds fertilized with nitrogen or phosphorus", by Graciano C, Rodríguez ME, Faustino LI

14:00-15:20 – Lunch

Session 5: Lessons from in vivo growth of vegetative propagules, especially in various pedoclimatic conditions (continuation)

Chairmen: **Olivier Monteuuis & Tsuyoshi Maruyama**

15:20-15:40 – **David Breton**: Industrial implementation of somatic embryogenesis for the production of *Coffea canephora* plantlets", by Breton D, Garcia Martinez C, Ducos JP, De Faria Maraschin S, Navarro LC, Broun P

15:40-16:00 – **Catherine Reeves**: "A hybrid tissue culture protocol that combines conifer somatic embryogenesis with organogenesis as an alternative propagation platform for specialist applications", by Reeves C, Hargreaves C, Lelu-Walter M-A, Trontin J-F, Moncaleán P, Montalbán I

16:00-17:00 – IUFRO 2.09.02 BUSINESS MEETING

17:00-22:30 – Visit to: ASTRONOMY OBSERVATORY "PLANETARIO" and Cocktail

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September 23 – FRIDAY

8:00-8:25 – Council of Economic Sciences: Poster Viewing

8:25-8:30 – **Jean-François Trontin** - Presentation of the day's program & Introduction to Session 6

8:30-9:10 – Key Invited Speaker: **Cathy Hargreaves** – "The development and application of conifer tissue culture and somatic embryogenesis protocols in New Zealand: The *Pinus radiata* D. Don story", by Hargreaves CH

Session 6: Reducing socio-economic and environmental costs of plantation forestry

Chairwoman: **Cathy Hargreaves**

9:10-9:30 – **Pramod Gupta**: "Progress on scale-up somatic embryogenesis and manufacture seed technology of conifer species at Weyerhaeuser", by Gupta P

9:30-9:50 – **Ricardo Penchel**: “Challenges of bioreactor for large-scale eucalypt clonal propagation”, by Penchel RM, Gatti KC, Xavier A, Otoni WC, Mingossi FB

9:50-10:10 – **Guillermo Rafael Salvatierra**: “Biofabrica Misiones S.A.: biotechnology accessible to producers”, by Salvatierra GR, Rodriguez VM Kubiak de Salvatierra D, Cabral JA

10:10-10:30 – **Aurélien Masson**: “Mass production of self-rooted *Hevea brasiliensis* industrial clones by tissue culture and nursery methods”, by Masson A, Monteuuis O

10:30-11:10 – Closing Lecture: **M. Raj Ahuja** – “Current status of forest tree biotechnology in a changing climate”, by Ahuja MR

11:10-11:40 – Coffee Break

11.40-12:20 – GENERAL DISCUSSION AND CLOSING SESSION

12:20-14:00 – Visit to: Nursery “Charles Darwin”. Province of Buenos Aires

14:00-15:20 – Lunch - Typical Argentinean Food - Folklore dance - Beer Taste

15:20-18:00 – Visit to The Parque Pereyra Iraola and Children Republic (República de los Niños)

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	MONDAY September 19 Arrival day	TUESDAY September 20 Council of Economic Sciences	WEDNESDAY September 21 Council of Economic Sciences	THURSDAY September 22 Council of Economic Sciences	FRIDAY September 23 Council of Economic Sciences
		SESSION 1: Strategies for integration of vegetative propagation into breeding programmes	SESSION 2: Continuation	SESSION 4: Preservation and adaptation of wild and selected genetic resources	SESSION 6: Reducing socio-economic and environmental costs of plantation forestry
8:00-8:25		Registration & Poster Setup	Poster Viewing	POSTER SESSION	Poster Viewing
8:25-8:30			Session Introduction (JF Trontin)	Sessions Introduction (JF Trontin)	Sessions Introduction (JF Trontin)
8:30-8:50		Opening Messages of the Local Organizing Committee	Key Invited Speaker Marie-Anne Lelu-Walter	Key Invited Speaker Scott Merkle	Key Invited Speaker Cathy Hargreaves
8:50-9:10					
9:10-9:30		IUFRO 2.09.02 Unit meeting & Session introduction Jean-François Trontin	Chairwomen: MA Lelu-Walter T Aronen Iselen Trujillo	Chairman: J Canhoto Itziar Montalbán	Chairwoman: C Hargreaves Pramod Gupta
9:30-9:50			Kanagaraj Suganthi	Vanesa Cano Lázaro	Ricardo Penchel
9:50-10:10		Opening Lecture Sebastián Galarco	Gustavo J. Oberschelp	João F. Da Silva Martins	Guillermo Rafael Salvatierra
10:10-10:30			María Angeles Basiglio Cordal	Nelly Aggangan	Aurélien Masson
10:30-10:50		Chairman: MR Ahuja Luciano Roussy	Valeria Rudoy	Hernán Mattes Fernández	Closing Lecture M. Raj Ahuja
10:50-11:10		María Elena Aguilar Vega	Patricia Boerl	Patricio Rojas Vergara	
11:10-11:40		Coffee Break			
			SESSION 3: (Epi)genomics of embryo or other vegetative propagule development	SESSION 5: Lessons from *in vivo* growth of vegetative propagules	
11:40-12:00		Chairman: MR Ahuja Stefaan Werbrouck	Chairman: S Merkle Chairwoman: I Arrillaga Jorge Cannoto	Chairmen: O Monteuuils T Maruyama Tsuyoshi Maruyama	General Discussion and Closing Session
12:00-12:20		Fernando Niella	Celia M. Miguel	Salla Varis	
12:20-12:40		Miguel P. Guerra	Sandra Correia	Karl-Anders Högberg	Visit to: Nursery "Charles Darwin" Province of Buenos Aires
12:40-13:00		Juan Schapovaloff	Conchi Sánchez	Jens I. Find	
13:00-13:20		Key Invited Speaker Oliver Monteuuis	Nuria González Cabrero	Tuija Aronen	
13:20-13:40			Bruno Navarro	Mikko Tikkinen	
13:40-14:00		Yong-Wook Kim	Anna María Wójcik	Corina Graciano	
14:00-14:20					
14:20-14:40	Workshop "Innovation and challenges in the forestry sector"	LUNCH			Typical Argentinean Food Folklore dance Beer Taste
14:40-15:00					
15:00-15:20					
		SESSION 2: Environmental factors affecting vegetative propagation of trees	SESSION 3: Continuation	SESSION 5: Continuation	
15:20-15:40	Faculty of Agricultural and Forest Sciences Public access	Chairwomen: MA Lelu-Walter T Aronen Marguerite Quoirin	Chairman: S Merkle Chairwoman: I Arrillaga Jean-François Trontin	Chairmen: O Monteuuils T Maruyama David Breton	Visit to: The Parque Pereyra Iraola And Children Republic (República de los Niños)
15:40-16:00		Isabel Arrillaga		Catherine Reeves	
16:00-16:20	Registration & Poster Setup Council of Economic Sciences	Katerina Eliášová	POSTER SESSION	IUFRO 2.09.02 Business Meeting	
16:20-16:40		POSTER INTRODUCTION SESSION			
16:40- 17:00					
17:00-17:20					
17:20-17:40			Satellite Meeting *Current status and prospects of vegetative propagation technologies in Argentinean Patagonia* Council of Economic Sciences		
17:40-18:00					
18:00-18:20					
18:20-19:00	Opening Reception by Hosting Authorities Council of Economic Sciences Welcome Cocktail	City Tour	Free time	Visit to: Astronomy Observatory "Planetario" Cocktail	
19:00-20:00			Free time		
20:00-22:30	Free Evening	Free Evening	Gala Dinner Central Hall Building of Presidency La Plata University Argentine Food Tango & Salsa		

About the Unit 2.09.02

The main objective of the IUFRO Working Party named "Somatic Embryogenesis and other Vegetative Propagation Technologies" is to foster the development and application of somatic embryogenesis (SE) and other vegetative propagation technologies in woody plants. Research areas of this unit include: the development and refinement of propagation systems for commercially and ecologically important tree species; the application of vegetative propagation in tree breeding and deployment in multi-varietal forestry balancing genetic gain and diversity; the use of vegetative propagation in genetic resource conservation, biotechnology, genomics, molecular biology, and insect and disease resistance; and the study of related disciplines such as cryopreservation, molecular genetics, and epigenetic effects.

Objectives of this conference

Global warming and other climatic-related biotic and abiotic changes (e.g., drought, frost, disease and pest/insect conditions) are currently central preoccupations in most research and breeding programs. Vegetative propagation of selected varieties will be a critical tool to maintain the productivity in plantation forestry, balancing genetic gain of genetically improved varieties with environmental and other socio-economic considerations. There are also strong synergies of vegetative propagation with enabling technologies (genetics, epigenetics, reverse genetics, cryopreservation, and genomics) to cope with climatic and environmental changes.

Conference Topics

Somatic embryogenesis (SE) and other vegetative propagation (VP) technologies offer new opportunities for tree breeding, vegetative deployment, and genetic resources conservation and restoration. It also provides indispensable tools for R&D in biotechnology, genomics and molecular biology. Important advances have been made in SE and other VP of tree species as well as in tree breeding in recent years. The aim of this conference is to bring vegetative propagation specialists, tree breeders, biotechnologists, and industrial practitioners together to exchange advances in scientific knowledge and technology and to promote collaboration among the disciples for the sustainable management of future forests and plantations. International collaboration appeared to be of prime importance in the context of both already active and expected long-term climate related changes. The conference topics include:

1. **Strategies for integration of vegetative propagation into breeding programs in the context of global warming and associated stresses**
   Adaptation & resilience of plantation forests, impact of marker-based (gene and genome) selection and heritable epigenetic processes on breeding practices, impact of vegetative propagation on variety deployment.

2. **Towards multivarietal/clonal forestry: environmental factors affecting vegetative propagation of trees**
   Development of somatic embryogenesis and other in vitro methods from juvenile explants or mature woody plants, production of cuttings, grafts, etc.

3. **(Epi)genomics of embryo or other vegetative propagule development**
   Omics, Functional studies of transcription factors and other candidate genes, systems biology, epigenetics, impact on protocol refinement.

4. **Preservation and adaptation of wild and selected genetic resources to environmental and socio-economic changes**
   Cryopreservation, embryo rescue, rejuvenation technologies, manipulation of biotic/abiotic resistance traits (including genetic transformation or other genome editing tools), maker-assisted management of clonal material, “breeding” with endophytes and mycorrhizas.

5. **Lessons from in vivo growth of vegetative propagules, especially in various pedoclimatic conditions**
   From lab to effective variety deployment, field evaluation of clones/varieties.

6. **Reducing socio-economic and environmental costs of plantation forestry** Automation, simulation tools for optimization of labor and costs, artificial seeds, addressing public concerns on forest biotechnologies.

Description of La Plata, the City of Diagonals

La Plata was born in 1882 as the capital of the province of Buenos Aires, Argentina. Governor Dardo Rocha officially founded it on 19 November of 1882. That day, the foundation stone was placed at the geographical centre of La Plata: the Moreno Square.
La Plata was planned from the political and avant-grade urban conception: the Hygienist Movement. The city was a perfect exponent of the ideas. La Plata is famous for its system of green public spaces and its diagonal avenues. The squares are placed every six blocks and they were connected along the pavements with tree lines, so the walker will always keep in touch with nature.

La Plata has many touristic attractions. The Cathedral is situated in front of Moreno Square and it has a New Gothic style with 37 French and German vitreaux. The very well-known Natural Sciences Museum is considered the fifth in importance in the whole world. The beautiful and picturesque "República de los Niños" (Children's Republic) which is the biggest place for children in South America, and the amazing Astronomic Observatory. The Observatory was one of the first inhabitants of the City Park and it was created in October 1882.

Another important scientific institution is the Forestry Station “Pereyra Iraola Park”. It belongs to Ministry of Agriculture Affairs, being premise of the FDD (Forestry Development Directorate) and is coordinated by the Department of Community Forestry Services. In these place we can found different working areas like production of propagation material (Eucalyptus, Salicaceae, ornamentals and urban trees); applied experimentation; events, courses, commercialization and donations.

In addition to tourist conditions, La Plata is a University city, which contains among other careers The Agricultural and Forestry Engineering, which University form part of the venue of the IUFRO's meeting. The University of La Plata was ranked in January of 2016 by the Cibermetry Laboratory which belongs to the Higher Council for Scientific Research of Spain, it is located in the position 489 of the world (over 25,000 registered institutions), 14 in Latin America (over 3,735 registered) and 2nd in Argentina (over 116 registered).

Welcome Message

Welcome to "La Plata 2016", the Fourth International Conference of the IUFRO 2.09.02 Unit: Somatic Embryogenesis (SE) and other Vegetative Propagation (VP) technologies!

The main goal of this IUFRO Working Party launched by Yill-Sung Park in 2008 is to foster the development and applications of SE and other VP (bio)technologies in both coniferous and hardwood tree species and more generally woody plants. Practical outcomes are highly expected for conservation, breeding/selection and efficient, cost-effective deployment of resilient, productive varieties in multi-varietal or even clonal plantation forestry. We strongly believe that our Working Party offers excellent opportunities to share experience and network with colleagues around the world. We have now over 700 members from 65 countries in our IUFRO 2.09.02 mailing list. One striking illustration of the growing collaborative IUFRO 2.09.02 network was the publication this year by the National Institute of Forest Science (NIFoS, Republic of Korea) of a reference book on Vegetative Propagation of Trees edited by Yill-Sung Park & Jan Bonga (Canadian Forest Service) as well as Heung-Kyu Moon (NIFoS). Thanks again to Yill-Sung, Jan and Heung-Kyu for such a productive initiative!

After three successful conferences in Republic of Korea (Suwon 2010, inaugural meeting organized by Heung-Kyu Moon), Czech Republic (Brno 2012 organized by Jana Krajňáková) and Spain (Vitoria-Gasteiz 2014 organized by Paloma Moncaleán), we are particularly pleased that we have this IUFRO 2.09.02 conference for the first time in South America where there is so much interest, from scientists to industrial practitioners, for tree multiplication and plantation forestry. With such a diversity of tree species growing in this world part, the Latin America scientific community has much to share in this IUFRO Unit as reflected by our scientific program!

This year's meeting is organized by Sandra Sharry in La Plata, the capital city of the Province of Buenos Aires (Argentina). La Plata is a model example for modern urban planning during the XIX century. Remarkably, La Plata is encouraging children to vigorously debate the inner workings of a democratic society as illustrated by the Children's Republic, a proportionally-sized children's theme park that represents the entire workings of a democratic city. The city is also close to Pereyra Iraola Park, a remarkable site with the highest biodiversity in the Province of Buenos Aires and declared as "Biosphere Reserve" in 2008 by UNESCO. Thank you very much Sandra, and enjoy your stay in La Plata, Argentina!

The conference is co-hosted and funded by the Faculty of Agriculture and Forestry Sciences (FCAyF, Facultad de Ciencias Agrarias y Forestales) of the National University of La Plata (UNLP, Universidad Nacional de La Plata, Argentina), the Center for Forest Research and Extension Andean Patagonian (CIEFAP, Centro de Investigación y Extensión Forestal Andino Patagónico, Argentina) and, for the third time, by the NIFoS, Korea Forest Service (Republic of Korea). The Ministry of Science, Technology and Productive Innovation (MINCyT, Ministerio de Ciencia, Tecnología e Innovación

Productiva, Argentina) and UNLP provided funding support to this conference and we have also various other sponsors providing helpful support. We are so fortunate that we have again such strong supporters of our IUFRO 2.09.02 activities!

The selected theme for La Plata 2016 is "*Development and application of vegetative propagation technologies in plantation forestry to cope with a changing climate and environment*". Global warming and other climatic-related biotic and abiotic changes are indeed central preoccupations in current research and breeding programs. VP of selected varieties are expected to be a critical tool to maintain the productivity in plantation forestry, balancing genetic gain of genetically improved varieties with environmental and other socio-economic considerations. Also one significant conclusion from our last meeting (Vitoria 2014) was that multivarietal/clonal forestry "revival" is highly expected in conjunction with ongoing development of genome-wide approaches for selecting individual trees. Similarly, there are strong synergies of VP, especially SE, with enabling technologies (e.g. cryopreservation, reverse genetics, genome editing …) for efficient preservation and adaptation of wild and selected genetic resources. So an important aim of this conference is to discuss the latest advances in scientific knowledge and technology from a growing number of species towards implementation of VP into breeding/conservation programs for increased resilience of forests and tree plantations. As usual, we have a collection of interconnected expertise in this conference from lab developments (tissue culture, physiology, molecular aspects …) to effective implementation in tree breeding and variety deployment. We also believe that a good balance of scientific program and attractive social activities was achieved. So we wish you a productive conference and happy networking!

On behalf of the Organizing Committee, I would like to thank all the contributors to La Plata 2016.

My special thanks first go to Dr. Sandra Sharry, the Dean of FCAyF-UNLP, Lic. Raúl Aníbal Perdomo, President of UNLP, Dr. José Daniel Lencinas, Director of the CIEFAP, and Dr. Nam Sung Hyun, Director General of NIFoS for graciously agreeing to co-host and financially support this conference. We are also particularly grateful to Minister Dr. Lino Barañao and Dr. Alejandro Mentaberry from MINCyT for providing direct funding support, and to Minister Ing. Leonardo Sarquís (Ministry of Agroindustry, Province of Buenos Aires) for providing organizational support.

Many thanks also to all our sponsors and supporters for their help in organizing the conference and to Dr. Michael Kleine in charge of the *IUFRO-SPDC's Scientist Assistance Program* who provided full support to one young scientist from an economically disadvantaged country to attend the conference. Without this collective effort, having this conference at these particularly hard economic times would simply not be possible.

Young scientists are a priority for our Unit and La Plata 2016 was the opportunity to organize our Second Biennial Student's Scientific Competition. On behalf of the Evaluation Committee chaired by Dr. Mariano Toribio, I would like to thank again the winner, João Filipe da Silva Martins (Portugal),

and all 5 excellent runners-up, Giovanna Campos Mamede Weiss de Carvalho (Brazil), Evelyn Raquel Duarte (Argentina), Taiane Pires de Freitas de Oliveira (Brazil), Kanagaraj Suganthi (India) and Anna Maria Wójcik (Poland) for contributing to the scientific program. All our encouragements and success in your respective project!

Of course I would like to express my warm thanks to the Local Organizing Committee, particularly Sandra who worked tirelessly for the conference (especially research of subsidies) despite her new responsibilities (April 2016) as Dean of the FCAyF-UNLP and a particularly difficult economic context in Argentina. I'm also most grateful to Ms. Maite Romero Alves and Ing. Claudia Kebat (FCAyF-UNLP), Ing. Sebastián Galarco (Ministerio de Agroindustria, Buenos Aires), and Ms. Elina Moreno (El Nuevo Agro) who all looked conscientiously after the local arrangements. The organization of La Plata 2016 was a challenging project! Thanks Maite, Elina, Claudia and Sebastián!

Finally, as new Coordinator in charge of the Unit since 2015, I would like to take this opportunity to warmly thank all my colleagues and friends of the Conference Organizing Committee (Sandra, Paloma, Jana, Heung-Kyu, Mariano and Yill-Sung). They provided kind and essential collaborative support based on their strong respective experience to meet this new challenge. In particular, the devotion of Drs. Mariano Toribio (IMIDRA, Spain) and Heung-Kyu Moon (NIFoS, Republic of Korea) to this Working Party as Deputy Coordinators since 2008 was again critical for the successful organization of La Plata. Thank you so much Mariano and Heung-Kyu!

This conference is also an important milestone for the coordination of this Unit with the retirement period starting for Yill-Sung, Mariano and Heung-Kyu. They can look back on their endeavours with pride! I have now the feeling to carry some kind of heritage and I'm most certainly committed to ensure some continuity with all the goodwill of the members of our IUFRO 2.09.02 Unit and also the resources provided by FCBA.

Here we are in La Plata with 114 attendees and 99 communications in our program and everything in good order to have a successful and productive conference! I'm however so sorry that Yill-Sung, Mariano, Heung-Kyu, and Paloma could not attend the conference this time for some personal reasons. You have all the kind consideration of our Unit and of course we would like very much to see you during our next conference as special guests!

"¡Una vez más, bienvenidos todos, y disfruten de esta nueva conferencia de la unidad de IUFRO sobre embriogénesis somática y otras tecnologías de propagación vegetativa!"

Jean-François Trontin

Coordinator, IUFRO 2.09.02 Unit

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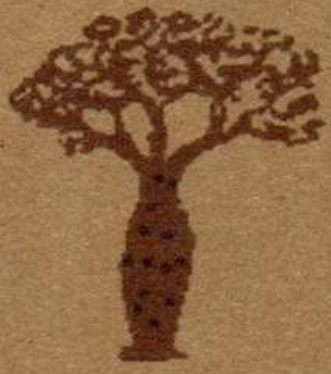
ABSTRACTS OF ORAL PRESENTATIONS

SESSION 1: **Strategies for integration of vegetative propagation into breeding programmes in the context of global warming and associated stresses.**



Ceiba chodatii (Hassl.) Ravenna (PALO BORRACHO)

It is a medium and big, bulky stem "paunchy" tree of 5-20 meters high. Alternate, deciduous leaves, with 5 leaflets serrated. Flat, greenish to greyish bark according to age, with vertical grooves and horizontal lines and thick woody conical stingers.

KEY INVITED SPEAKER, OPENING LECTURE

Status of forest resources in Argentina

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KEY INVITED SPEAKER

Vegetatively propagating forest trees

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By contrast with propagation by seeds in which each individual is genetically different from the other, asexual or vegetative propagation consists in duplicating, theoretically unlimitedly, genotypes while preserving through mitotic divisions their original genetic make-up, and consequently every of their individual characteristics. This is essential to ensure the transfer of traits which are under non-additive control, especially those that have a great economic impact. Moreover, vegetative propagation can be applied to any individual that does not produce fertile seeds, either because it has not entered the mature stage yet, or due to unfavorable environmental conditions. Its usefulness is obvious for research as well as for operational activities, depending on the ultimate objectives and on the most suitable strategies to meet the goals. Species characteristics and cost efficiency need also to be taken into account. *In vitro* micropropagation in axenic culture conditions can be distinguished from more conventional nursery techniques for vegetatively propagating forest tree species. The respective pros and cons of these two options, which can synergically complement each other, are considered, producing with the shortest delays and at the cheapest cost the needed quantity of improved quality planting stock remaining the priority.

Keywords: Forest Tree species, *In vitro* methods, Mass production, Nursery techniques, Planting stock, Vegetative propagation.

Habitat enhancement: the importance of vegetative propagation, especially for trees, in Landscape Engineering

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Landscape is a polysemous concept and therefore has multiple definitions. We adopt the definition proposed by the Landscape European Convention 2000: "landscape is an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors". There are three core concepts in this simple definition: territory, population and interaction. Landscape and landscaping regard problems arising from urban sprawling, production methods, environmental degradation, climate change and habitat loss as new challenges to be taken up. In this line, urban vegetation management -regarded as a green infrastructure- can offer solutions. Benassi (2015) recently stated that vegetation management faces challenges such as the outfitting of obsolete urban areas, linear parks and greenways, the urban greening (in buildings, streets, squares), the restoration of environmentally degraded areas through phytoremediation or bioremediation, the great urban forests as areas for carbon sequestration, the social landscaping as a culture of integration, and indoor vegetation for pollution reduction and aesthetic enhancement of close spaces. Another emerging issue is the need to combine production and healthy environment in green industrial sites, and intensive and extensive food and flower production in periurban and rural areas.

A green infrastructure project that is capable of implementing such proposals is calling for a broad concept of vegetation that includes the successful use of plants on the basis of different plant functional types for individual situations. It bears no relation to the ornamental function of plants in the urban space. In landscaping, from the first plant exchanges between botanical gardens until today, vegetative propagation of specific varieties or biotypes has been determined by an aesthetic approach. Consequently, species or varieties selected for their flowering (abundance and color), fruiting (absence or color), size ("nana or dwarf" varieties), shape and foliage color could be included in -mainly residential- landscaping following propagation at macro- or micro-scale. In landscaping, climate change and associated biotic or abiotic stresses will imply to reconsider the purposes of traditional propagation practices. In this regard, some of the challenges for the propagation of tree species and selected varieties are resistance to temporary flooding and temporary drought, fast growing and easy implantation, as well as successful propagation of local flora genotypes -which have not been explored yet- from adequate species for stabilizing slopes and watersheds and restoring soils. Vegetation management projects should include “social landscaping” based on popular propagation methods.

Considering that multiplying proper habitats for people in megacities is a key issue, especially through vegetation management, vegetative propagation of selected species and varieties may play a significant role in landscaping engineering.

Keywords: Landscaping, Urban planning, Landscape architecture, Urban vegetation, Green infrastructure.

Contributions of somatic embryogenesis and other *in vitro* propagation techniques to the genetic improvement of tropical woody species: Coffee, Teak and Gmelina

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Somatic embryogenesis is a technique that has been used in Central America for the multiplication of *Coffea arabica* F1 hybrids since its creation. The coffee breeding program for Central America - PROMECAFE (1992- 2006) – was implemented with the participation of CATIE, CIRAD and the coffee institutes in the region. Of the 98 hybrids obtained, three were recommended that met the objectives of the selection satisfactorily, i.e. new varieties are more vigorous than the traditional ones with higher value in productivity, precocity, tolerance to leaf rust and cup quality. Although somatic embryogenesis is the ideal technique for the multiplication of these materials; the process is intensive and of long duration, so the final cost per plant is very high in comparison with the plants obtained from seeds. These factors and others have limited the transfer of these hybrids to the producers who need to renew their plantations. To facilitate this process, CATIE established a two-stage multiplication strategy: 1- The regeneration of juvenile mother plants by somatic embryogenesis; and 2- The establishment of clonal gardens in the greenhouse for horticultural multiplication. This innovation allows the rapid multiplication of the hybrids and reduces the cost per plant produced in the greenhouse. Starting in 2017, the alliance with a horticultural company specializing in commercial scale vegetative propagation, will enable the multiplication of hybrids and the transfer of plants to producers at lower cost than if the plants come directly from the laboratory.

The silviculture of exotic, rapid growth species such as teak and Gmelina has gained recognition in Latin America for good yields in commercial plantations, producing high-value timber for international markets. Although these species are easily propagated using rooted cuttings, it is increasingly more common for forestry companies to request technical services from laboratories for rapid multiplication, disease cleansing or reinvigoration of their mother plants. CATIE, in collaboration with the National Institute of Forest Science (NIFoS) of the Republic of Korea, is working on improving the micro-propagation protocols for these species and promoting the use of high-quality clonal material in the region. The excellent in vitro response of teak has allowed some companies to rely on the laboratory for production of mother plants from superior clone and subsequent use in the establishment of clonal gardens for shoot production. Therefore, these in vitro techniques constitute a support tool to clonal silviculture programs.

Keywords: Somatic embryogenesis, Coffee hybrids, Micropropagation, Forest species.

In vitro Biotechnology of *Melia volkensii*, a high potential forestry tree from eastern Africa

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Melia volkensii belongs to the Meliaceae and is able to grow in semi-arid lands, yet producing high quality mahogany-like timber within 15-20 years. It is a key indigenous species for reforestation of Kenya, in regions highly affected by climate change. The tree is able to grow in homogeneous stands and is compatible with agroforestry systems. It is highly valued by farmers and timber merchants for its ready sales value as high quality termite resistant timber. Notably for this reason, natural populations of *M. volkensii* have been drastically reduced, and top quality specimens are becoming exceedingly hard to find. Seedling propagation is hampered as the fragile seeds are difficult to extract from their extremely hard nut. In vitro cloning of a set of elite trees will allow reforestation on a bigger scale than is now realized with seedlings. An overview is giving of the in vitro biotechnology of this tree, from micropropagation with promising new cytokinins and somatic embryogenesis to genetic transformation. Also the bottle-necks are treated, such as rooting and acclimatization.

Keywords: Melia, Reforestation, Mahogany, Vitro

Integrating low cost vegetative propagation techniques with a domestication and conservation strategy for multipurpose native species of Misiones, Argentina.

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The Atlantic Forest or *Selva Paranaense*, represent the most biodiverse regions of Argentina, currently covering an area of 1,183,791 hectares. However, selective logging of native timber species, the advance of the agricultural frontier and the overexploitation of natural resources have led to the degradation of the remaining forest, with the consequent loss of genetic variability. A direct consequence is the continued underutilization of the genetic potential of plant species and seeds are every day more scarce and difficult to harvest. Representative of this situation are the legume species tree as *Peltophorum dubium* (cañafistola), *Enterolobium contortosiliquum* (Timbó), and native fruit trees like *Eugenia involucrata* (Cerella or native cerezo), *Acca sellowiana* (native Guayabo) and *Rheedia brasiliensis* (Pacurí), all of them have been selected for our studies. The aim of our research was to facilitate and increase the availability of planting material and clonally multiply select genetic material, making accessible the genetic gain for local producers, and nurseries.

To achieve this goal, we have carried out a project to establish a network of trees and seed areas in the province of Misiones, and initiated the study of the genetic structure of two native species (*Peltophorum dubium* (cañafistola), *Enterolobium contortosiliquum* (Timbó)), as well as the development of clonal propagation techniques. So far, we have obtained four microsatellite markers for *Peltophorum dubium*, and eight for *Enterolobium contortosiliquum*, and started a short-term provenance and progenies test, for both species, to assess the genetic diversity and to contribute to its conservation and sustainable management.

We have also proposed to develop, a methodology for *in vitro* and *ex vitro* propagation. For the *in vitro* studies, different sources of explant, nutrient media, hormones, culture conditions and acclimation were studied. For *ex vitro* propagation, a mini-stumps/mini-cuttings methodology was addressed. Different container sizes, fertilization and the use of semi-hydroponics systems, and growing environments, were studied for ministumps management. For rooting of minicuttings, different inductive treatments (ministumps pre-treatments, application form and auxin concentrations) were considered. Through this study, it was generated: 1) an *in vitro* germination and establishment protocols for the proposed species, 2) an axillary multiplication methodology for *Peltophorum dubium, Enterolobium contortosiliquum, Eugenia involucrata* and *Acca sellowiana*; and 3) induction of organogenic and embryogenic tissue from explants of *Peltophorum dubium* and *Enterolobium contortosiliquum*. On the other hand, a ministumps operational management and rooting of minicuttings protocols for *Peltophorum dubium, Eugenia involucrata* and *Acca sellowiana* were developed.

Keywords: Multipurpose species, Micropropagation, Macropropagation.

Somatic embryogenesis model systems in selected Brazilian native trees

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Brazil is the richest biodiversity country in the world, and member of the group comprising 17 megadiverse countries. The megadiverse countries are a group of nations that contains more than 70% of the earth's biodiversity, identified in 1998 by Conservation International (CI), to promote the awareness for biodiversity conservation among the world nations. Biotechnologies comprise several appropriate tools in order to characterize, to manage and to use the plant genetic resources (PGR) components of the biodiversity. Among the biotechnologies somatic embryogenesis (SE) is the process by which somatic cells dedifferentiate to form embryos by reorganizing their epigenetic and biochemical configuration, and re-entering the cell cycle to create specialized tissues in morphological steps similar to zygotic embryogenesis. This *in vitro* regenerative route arises from a single totipotent somatic cell that has differentiated into an embryonic state. Additionally, it comprises a high performance *in vitro* regenerative system for both the mass clonal propagation of elite genotypes and/or the mass propagation of endangered plant germplasm. At the Laboratory of Developmental Physiology and Genetics of the Graduate Program in Plant Genetic Resources, Federal University of Santa Catarina, South Brazil, we have focused our efforts in the study of basic and applied aspects of somatic embryogenesis in native woody species representing different taxonomic groups: *Araucaria angustifolia* (Bert.) O. Ktze. (Araucariaceae), *Podocarpu slambertii* Klotzsch ex Endl (Podocarpaceae), *Acca sellowiana* (O. Berg) Burret (Myrtaceae), *Bactris gasipaes* Kunth (Arecaceae), and Bambusoideae. Our approach to SE seeks to contribute to the better understanding of plant cell totipotency, as well as to address its application in capturing genetic gains from elite genotypes, and conservation programs of endangered species.

Keywords: Brazilian trees, Somatic embryogenesis, Sustainable use, Conservation.

Operational transfer of genetic improvement in loblolly pine at Arauco Argentina S.A.

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Significant advances in genetic improvement programs of pines have been made in recent decades. Some key issues in loblolly pine (*Pinus taeda* L.) are controlled pollination techniques and cloning of genotypes from elite families through somatic embryogenesis. One major current challenge at Arauco Argentina S.A. (in the province of Misiones) is the development of innovative technologies to make easier and more efficient the transfer of genetic gains to commercial forest plantations. The management of mother somatic plants in gardens hedges for production of rooted cuttings, turn out to be a key technology for the production of improved varieties. In this presentation we will report on the efficiency at various steps during this process and discuss the actual operating profit in forestry business.

Keywords: *Pinus taeda*, Cuttings, Improvement.

ABSTRACTS OF ORAL PRESENTATIONS

SESSION 2: **Towards multivarietal/clonal forestry: environmental factors affecting vegetative propagation of trees.**

Schinopsis balansae Engler (QUEBRACHO COLORADO CHAQUEÑO)

Native from the north of Argentina, Bolivia, Brasil and Paraguay. It's a big portly tree of 24 meters high, cylindrical shank and thin treetop. With simple, deciduous, alternate or fasciculated, astringent leaves, leathery consistency. Greyish brown bark with deep furrows that delimit irregular plates. Small yellow flowers, gathered in panicles. Sámara woody fruit with a membranous wing, with bright red that turns reddish-brown when ripes.

KEY INVITED SPEAKER

Achievements in somatic embryo development in some conifers: what did we learn? Where are we going?

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Attempts to scale up somatic embryogenesis has been demonstrated in both broadleaved and conifers species (Lelu-Walter et al. 2013, Klimaszewska et al. 2016). In recent years, many researches focused on somatic embryos development, namely maturation, an important step for subsequently producing vigorous somatic trees. Somatic embryo maturation is a complex process triggered by many parameters such as environmental conditions that are also depending on the species. Optimized conditions resulted in recovery of cotyledonary somatic embryos that are morphologically similar to the zygotic counterpart. The optimal duration of maturation has hitherto been determined mainly on the basis of these morphological features and the ability of somatic embryos to germinate and convert into plantlets. However, this empirical approach does not provide any accurate information about the intrinsic quality of harvested cotyledonary somatic embryos as demonstrated by physiological, biochemical and molecular analyses (Morel et al. 2014a, 2014b, von Aderkas et al. 2015). Referring to different conifer model systems (larch, pine) we will present the achievements and some lessons that could be learnt for further refinement of cotyledonary somatic embryo development.

Keywords: Somatic embryo, Maturation, Quality, Zygotic embryo.

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Somatic embryogenesis and mass propagation of clonal plants in *Larix kaempferi* (Japanese larch)

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For successful embryogenic tissue initiation, immature zygotic embryos at different developmental stages were compared with their collection dates of seeds. Most of the embryogenic tissue induced from the zygotic embryos that were at the stages ranging from late cleavage polyembryony to the early proembryo, not globular or precotyledonary. As for the most optimal somatic embryo maturation, the advanced treatments were invented that consisted of 60 μM abscisic acid (ABA), 0.15 M maltose, 7.5% Polyethylen glycol (PEG) MW 8,000 and 0.8% gellan gum. The germination rates were high (68-71%) when germinated with the concentrations of 0.2 or 0.3% gellan gum. The germinants were transplanted directly to Larch soils (NongKyeong, Korea), however, they were survived with only 30% rate after 4 weeks. On the other hand, the survival rate was sharply increased to 95% or more when using Peat-plug (ihort, USA).

Keywords: Acclimatization, Conversion rate, Peat-plug, SE maturation

Somatic embryogenesis in palm tree species: effect of growth regulators and anatomical aspects

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Somatic embryogenesis of three species of palm trees was studied. Two of these species, *Acrocomia aculeata* and the hybrid *Elaeis guineensis* x *Elaeis oleifera* (oil palm), have their fruits and seeds used in Brazil for oil production. The last one, *Bactris gasipaes* (peach palm), is cultivated for heart palm production. The different stages of somatic embryogenesis from thin cell layers and/or zygotic embryos were compared in the three plants and the effect of growth regulators; putrescine and explant origin was studied during these stages. The anatomy of leaves of *in vitro* cultured *B. gasipaes* plants was compared with that of plants obtained from zygotic embryos and from *ex vitro* plants, in order to understand some abnormalities of plants derived from somatic embryos and improve final stages of somatic embryogenesis and acclimatization. The leaf cuticle, mesophyll and vascular tissues were characterized and root anatomy studied in plantlets from various origins.

Keywords: *Acrocomia aculeata*, Arecaceae, *Bactris gasipaes*, *Elaeis* hybrid, Histology

P. radiata and *P. halepensis* somatic embryogenesis: can we modulate the success of the process provoking abiotic stress at the initial stages?

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Somatic embryogenesis brings great advantages and applications to biotechnology, presenting a large-scale propagation system for ‘‘elite’’ clones with high multiplication potential. The development of somatic cells to somatic plantlets comprises three stages: induction of embryonal masses, maturation of embryogenic tissues and conversion into somatic plants. During the last years we have focused on the study of the effect of environmental conditions in the abovementioned phases in two different species, *Pinus radiata* (García-Mendiguren et al. 2016) and *Pinus halepensis* (Pereira et al. 2016). Radiata pine has a commercial potential worldwide and it has become one of the most widely planted exotic pine species in rainfall environments of the Southern hemisphere (Yan et al. 2006). On the other hand, Aleppo pine is of a great importance due to its adaptability to dry, calcareous and poor soils. In light of predictions of global warming, there is some interest about the physiological ability of *P. halepensis* to persevere in large afforestation in the future (Oliveras et al. 2003; Maestre and Cortina 2004). To this respect, in the future climate change scenario, the survival is still considered as one of the main bottleneck processes for forest productivity. In order to increase the productivity of *Pinus* species and their quality, our main objective has been the study of the effect of abiotic stress, changing temperature and water availability, in the SE success as well as to carry out an approach about proteins and phytohormones involved in this process. It was found that temperature and water availability applied during the initiation or maturation stage influence the rate of initiation and the number of embryos produced in both species studied although the effect is higher in radiata pine. The global objective of our project is the development of somatic plants with different adaptability to abiotic stress situations taking into account the epigenetic changes suffered by tissues along the embryogenesis process (Mahdavi-Darvari et al. 2014). To this respect, we have found different physiological parameters associated to the somatic plants characteristics at the end of embryogenic process.

Keywords: Aleppo pine, Embryonal masses, Radiata pine, Somatic embryos.

The effect of UV-B radiation on the development of Norway spruce somatic embryos

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Norway spruce (*Picea abies* (L.) Karst.) - native European conifer is widely planted both in the cool and the temperate regions and represents one of the economically most important coniferous species within Europe. Due to its adaptability it has been introduced around the world. Various environmental conditions bring about a wide range of abiotic stresses that affect the growth and the development of the plants and create their morphology. We focused our investigation to the effect of various doses of the UV-B irradiation on spruce somatic embryos (SE). The effects were evaluated biochemically and morphologically.

We have used a model system of Norway spruce somatic embryogenesis to follow the stress effect on SE in desiccation, when embryos mature biochemically. The adequate stress induced by the cultivation without any medium during desiccation is the prerequisite for a subsequent successful germination of SE. The most general plant response to UV-B radiation is the activation of polyamine and flavonoid biosynthetic pathways as polyphenolic compounds and polyamines possess free radical scavenging properties that can improve the plant stress tolerance. We examined the kinetics of the polyamines and the enzymes involved in the polyamine biosynthesis. Content of phenolic acids was assessed; polyphenolics were localized histochemically. The level of malondialdehyde (MDA) indicated the extent of lipid peroxidation. We found the changes in polyamine content and in ratio between putrescine, spermidine and spermine induced by irradiation by high UV-B doses. The detected increase of MDA content points to an increase in oxidative stress.

HPLC - MS analyses revealed the presence of eight phenolic acids in extracts of SE: two cinnamic acid derivatives - *p*-coumaric and ferulic acids and six benzoic acid derivatives - *p*-hydroxybenzoic, protocatechuic, vanillic, gallic, salicylic and anisic acid. Total content of phenolic acids in irradiated embryos increased by about 25% relative to control. The effect of irradiation was most clearly manifested in the accumulation of glycosides of benzoic acid derivatives. Polyphenolics accumulated in the epidermis and idioblasts of cotyledons and hypocotyls and in cells of the root cap of irradiated SE, contrary to the control SE, where polyphenolics occurred only in cells of the root cap. These findings, together with increased autofluorescence of flavonoids in the epidermal layer of irradiated SE, detected under confocal microscope, represent avoidance mechanisms important for protecting embryo tissue against UV-B.

Keywords: Norway spruce, Somatic embryogenesis, Abiotic stress, Phytohormones, Polyamines, Polyphenolic compounds

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Somatic embryogenesis of *in vitro Musa* clones

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Banana is a major crop in Venezuela as well as in most tropical countries, due to its high content of vitamins and minerals. Due to the high demand for healthy plants, and for genetically improved materials with resistance to diseases, biotechnology is a useful alternative to produce these plants. The objective of the present work is to induce somatic embryogenesis and plant regeneration from apical shoot of four *in vitro* clones of *Musa*: Titiaro (AA), Giant Pineo and Brasilero (AAA) and Tetraploide (AAAA), economically important cultivars in Venezuela. In order to induce somatic embryogenesis, MS culture medium was supplemented with Morel vitamins (10 ml/l), sucrose (30 g/l), cysteine (60 mg/l), 2,4-D (0.2 or 4 mg/l) and Dicamba (0,2 or 4 mg/l) and gelrite (2 g/l) was used as a solidifying agent. Somatic embryogenesis was carried out with vitroplants originated from two groups: a) vitroplants with 3 subcultures cultivated with 5 mg/l and b) vitroplants with 3 subcultures, two with 5 mg/l and one with 10 mg/l. The induction of embryogenic callus was achieved in the four clones from both experimental groups when 4 mg/l of 2,4-D was used. The multiplication of embryogenic callus was obtained with the same concentration of 2,4-D used during the induction phase. The induction and multiplication of embryogenic callus were performed in dark conditions. For differentiation of embryos, zeatin (15 mg/l) was added in the same basal medium. Ultrastructural studies of somatic embryogenesis are very important to understand and improve this morphogenetic process. Cultivated tissues were placed under light conditions (50 UE/m.seg) at 27 °C and histological evidences were checked after 6 months in culture. The embryogenic callus showed small isodiametric cells, with small vacuoles and thick walls. The origin of somatic embryos obtained from meristematic apices of selected *Musa* clones was analyzed through histological and morphological studies during the various development phases of the process. Histological sections of globular embryos showed a radial disposition of cells and the existence of an epidermal layer that completely surrounds the embryo. When cytokinin (Z or BA) was added, some embryos remained in globular stage with mild signs of enlargement but with no later development of invagination. Other embryos reached the invagination stage and some reached the enlargement stage with active photosynthetic tissues. The adaptation of vitroplants from somatic embryogenesis was successful in the four selected clones for this research.

Keywords: Somatic embryogenesis, *Musa*, Clone, Vitroplant

In vitro propagation of mangroves for greenbelt development to mitigate climate change

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Global warming contributes to sea level rise, changes in climate extremes, ocean acidification, species extinction and expansion of deserts in the subtropics. Mangroves are salt tolerant trees found mainly in the tropical and subtropical intertidal regions of the world. It protects coastal areas by maintaining marine food chain, preserve water quality, providing habitat for fish and birds, prevent erosion and maintain the health of coral reef. Currently it is under pressure due to various reasons such as increasing temperature, tidal mixing, coastal current, human activities like conversion to aquaculture or agriculture, release of effluents and sewage. Therefore, conservation of mangroves is urgent need to protect the world most productive mangrove forest ecosystem. The aim of the present study is to induce the callus development from root explants using different growth regulators. The mangroves species selected for the study are *Acanthus ilicifolius, Callophyllum inophyllum* and *Excoecaria agallocha.* These 3 species has enormous bioactive compounds with various medicinal properties. The fresh mangrove root were collected from Pichavaram mangrove forest, Tamil Nadu, India and scientifically identified. Under *in vitro* condition the root callus was raised in MS medium supplemented with different concentration and combination of growth hormones auxin and cytokine such as BA, kinetin, NAA, IAA, IBA and 2,4-D.The presence of phytohormones at different concentration and combination in MS medium showed better development of callus. While using NAA and IAA alone with medium were found to be less effective for induction of *in vitro* root callus. The maximum growth was obtained in combination of phytohormones such as 2,4-D+KIN and 2,4-D+BAP compare to all different combination for the induction of in vitro root callus in all the three different species *Acanthus ilicifolius, Callophylumi nophylum* , *Excoecaria agallocha*. The developments of *in vitro* root callus of 3 species were most effective at 2 different concentrations 0.3+0.3 mg/L and 0.3+0.5 mg/L. The roots and shoots were regenerated from the callus in the presence of auxin and cytokine. Afterwards the plantlets were transferred to the greenhouse for acclimatization and survival then subjected to the field. The *in vitro* grown plants were used for the development of greenbelt model around the coastlines and riverbanks for protection from typhoons, erosion, tidal surges, cyclones and geomorphic erosion. Mangrove greenbelt development provides greater protection to the ecosystem by stabilizing sediments and trap heavy metals and nutrient rich run-off, filter freshwater discharge from land for coral reef growth, enhance the biomass of coral reef fish species, nursery habitats between seagrass beds and patch reefs that increase young fish survival. Also, the mangrove has the ability to store more carbon faster, permanently and deep in the soil by their entangled root system. It will be helpful to mitigate climate change and environment.

Keywords: Mangroves, In vitro propagation, Greenbelt model, Biodiversity, Carbon sinks, Climate change.

The design of a species-specific basal media for *Eucalyptus* considering the mineral nutrition status of young plant tissues

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As sessile organisms, plants have evolved to cope with spatial variations of resources by adapting to a variety of environmental niches. The struggle for life in a limited-resources scenario drive their anatomy and physiology to a high level of specialization. Among these resources, mineral nutrients are critical to safeguard plant growth and development. In higher plants, minerals nutrients are first taken up in ionic form by roots through interception, diffusion and/or mass-flow, and then have to pass through the endodermis to be loaded into the xylem and finally reach shoots and leaves. The relevance of these uptake mechanisms differs in most of the *in vitro* propagation systems, where tissues are directly exposed to culture media and diffusion is the main driving force, highlighting the relevance of fine-tuned nutrient media. Classical approaches to achieve this are one-factor-at-a-time or multifactorial designs, where high quantities of propagation material is needed, being a challenging condition to meet for recalcitrant species.

From intensive agriculture and silviculture we have learn that different cultures, species, varieties and genotypes have different nutritional requirements. These requirements are known for the major cultivated species; however, the differences in sampling characteristics and environmental conditions hamper their practical application under *in vitro* conditions. Plant tissue analysis is a valuable aid in plant nutritional management. This is especially important in soilless cultures, where mineral nutrition relies on salt mixtures and this information is used to improve the nutrient solutions. Despite its usefulness, this tool is not routinely used in tissue culture owing to sample size requirements, another main drawback when dealing with recalcitrant species.

All these issues can be minimized by sampling *in vivo* young plant tissues from the aimed genotypes and using this information as a basis for basal media formulation. Additionally, chemical speciation programs to model nutrient solutions and new analytical technologies, which allow small samples analysis, should be considered to aid media development and optimization. This and similar approaches, has been successfully applied for *in vitro* propagation of several *Eucalyptus* species and can be useful when dealing with other hard-to-propagate species.

Keywords: Mineral nutrients, Tissue culture, Tissue analysis, Hard-to-propagate species.

First advances in somatic embryogenesis of *Phytolacca tetramera*, an endangered shrub endemic species of the Province of Buenos Aires, Argentina

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Phytolacca tetramera Hauman, commonly known as "Ombusillo", is a dioecious shrub species (geophyte bush up to 1.5 m in height) belonging to the family Phytollaccaceae. Ombusillo is endemic in the Province of Buenos Aires with distribution area starting near the city of La Plata and extending up to the region of Ensenada Samborombón. The species is forming a fundamental part of the flora characteristic of the Parque Costero Del Sur, area classified in 1984 by UNESCO as "World Biosphere Reserve". Ombusillo is a highly endangered species (close to extinction) following anthropic actions because it produces fungicides of high medicinal interest. Fractionation of the butanol extract of the berries, allowed the isolation of three active triterpenoid saponins monodesmosidics: phytolaccosides B [3-O-β-d-xylopiranosyl-phytolaccagenin], E [3-O-β-d-glucopyranosyl-(1→4) - β -d-xylopiranosyl-phytolaccagenin] and F [3-O-α-l-rhamnopyranosyl-(1→2) - β-d-glucopyranosyl-(1→2) - acid β-d-xylopyranosyl-phytolaccagenic]. Moreover, the methanol extract of Ombusillo berries was shown to have antifungal activity against opportunistic fungal pathogens (Escalante et al, 2002).

Extraction of these compounds from forest plants harvested in the natural distribution area became critical. To solve this problem, one alternative for species that are difficult to propagate through sexual reproduction is asexual, vegetative propagation which would generate a large number of individuals from selected elite genotypes.

Somatic embryogenesis relies on the development of embryos directly from a somatic cell (Tisserat et al, 1979). Somatic embryogenesis offers the technical requirements for automatization of the process towards cost-effective production of plants and ultimately the development of synthetic seed technologies. In order to set up a protocol for somatic embryogenesis initiation in *Phytolacca tetramera*, thin sections of young leaves from *in vitro* micropropagated plantlets were placed on a Murashige and Skoog basal medium supplemented with 2, 4-dichlorophenoxyacetic acid at different concentrations. Somatic embryos were obtained directly from the cutting edges of the leaf blade and embryogenic callus (indirect way) could be initiated from the midrib. This is the first report of somatic embryogenesis initiation in this endangered medicinal plant and more importantly in the *Phytolacca* genus. Our findings are of importance for ombusillo germplasm conservation and also for deploying medicinal plant varieties as an alternative to traditional crops.

Keywords: Somatic embryogenesis, Endemic species, Ombusillo, In vitro culture, Germplasm.

TECNOPLANT: From *Vaccinium* spp plants to blueberry extract tablets

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It is important to enhance the wide experience and career of Tecnoplant as a laboratory specialized in agrobiotechnology. For more than 20 years Tecnoplant has rendered services to prestigious local and international institutions, such as:

- **Varietal identification of species through molecular biology techniques**
- **Identification of absence-presence of viruses known for certain plant cultures.**
- **Molecular identification of Crown Gall bacterial disease in cultures**
- Extractions of **plant extract** powder, through the lyophilization process.

In 1994, Tecnoplant began to micropropagate and commercialize blueberry plants, developing these micropropagation operations in its own laboratory, to perform then the rustication process in its own nursery, located in Baradero, Province of Buenos Aires.

Since 1996, blueberry culture has begun to increase dizzyingly. The management of these cultures, first in charge of small producers who restructured a part of their orchards to blueberry production, began to be a part of more important producers, including investors who had never ventured into agriculture-related business.

In the year 2000, Tecnoplant obtained the license from the United States universities in order to exclusively micropropagate and commercialize last generation varieties in Argentina and Uruguay.

Nowadays, Tecnoplant keeps micropropagating different plant species, committing to the development of new phytomedicinal projects that may add value to the whole industrial chain.

Keywords:

Somatic embryogenesis of *Prosopis alpataco.*

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Somatic embryogenesis and whole plant regeneration was achieved in callus cultures derived from cotyledons of *Prosopis alpataco* Phil, a native shrubs of Patagonian Monte. An important part of its habitats are degraded due to the expansion of agricultural frontiers and the cutting bushes for firewood.

The aim of this work was to establish the conditions for *P. alpataco* plant regeneration via somatic embryogenesis.

Embryogenic calli were induced for 2 months on Murashige and Skoog (MS) medium supplemented with a mixture of organic components plus N-6 benzyladenine (BA) (1,5 mg/l) and 2,4-dichlorophenoxyacetic acid (2,4-D) (2,0 mg/l). Embryogenic calli transferred onto half-strength MS medium without plant growth regulators were mainly and initially producing globular embryos. Then embryos of different developmental stages emerged simultaneously from calli. When embryogenic calli were maintained under these conditions, secondary somatic embryos were generated and could complete their development until the formation of a pair of true leaves. The conversion rate to seedling was 10%. Somatic embryo development was examined by light microscopy in relation to different callus growth stages and cultural conditions.

Keywords: Somatic embryogenesis, Native, Patagonia

ABSTRACTS OF ORAL PRESENTATIONS

SESSION 3: **(Epi)genomics of embryo or other vegetative propagule development .**

Cedrela fissilis Vell. (CEDRO MISIONERO)

Native of Argentina, Brasil and Paraguay is a tree up to 30 meters high with greyish bark. Compound leaves, even pinnate whose leathery leaflets are bright in the beam and have pubescence on the back. With flowers of 9 mm long, woody fruit capsule which opens in five valves giving off a reddish brown winged seeds. It's a melliferous tree whose wood is highly prized for its esthetic value and high strength.

Genes are important to understand somatic embryogenesis but someone has to work: the proteins

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During the last decades several genes have been identified as playing key roles during somatic embryogenesis induction or somatic embryo development. The identification of these genes has given important contributions to the understanding of somatic embryo formation and the acquisition of cell totipotency. However, genes are only part of a more complex picture in which proteins play a pivotal role not only as regulators of gene expression but also as cellular effectors controlling signal transduction pathways as well as being directly involved on cellular mechanisms and physiological responses.

Plant regeneration through somatic embryogenesis is a multi-step event starting with somatic embryogenesis induction, followed by somatic embryo development, maturation and germination. The culture conditions to achieve each one of these steps are different as different are the players controlling such phases. Proteomic analysis in different species, both gymnosperms and angiosperms, has shown that strong differences occur in the protein profiles between embryogenic and non-embryogenic cell lines, as well as during somatic embryo development and maturation. New challenges are to understand to what extent these differences could be only the expression of different metabolic states not directly related to somatic embryogenesis induction or whether they rather reflect cell behavior controlling the commitment of a cell into an embryogenic pathway.

Proteomic and physiological studies carried out on tamarillo (*Solanum betaceum*) and in *Pinus radiata* have shown differences in the protein profiles of embryogenic and non-embryogenic callus (in the case of tamarillo) and between embryogenic explants induced under different conditions. As could be expected, most of the differences detected were in proteins related with cell metabolism. However, differences have been also found in pathogenesis-related proteins as well as in heat-shock proteins and stress-related proteins. The observation that proteins involved on the response to plant stresses, either biotic or abiotic, may have a role on somatic embryogenesis induction is particularly interesting, suggesting that the acquisition of totipotency by plant cells may be triggered by stress conditions and could be part of a more general mechanism, also occurring in organisms beyond plants, to assure the perpetuation of an individual or a cell when subjected to adverse environmental conditions.

To be effective, proteomic analysis must be complemented with metabolomic and physiological studies to understand the role of the proteins differently expressed in embryogenic and non-embryogenic explants. From this point of view, tamarillo is a nice model since embryogenic and non-embryogenic callus can be obtained from a same explant and in the same culture conditions (2,4-D and high sucrose levels), thus avoiding genomic and environmental interferences on the acquisition of embryogenic competence. Based on this system we have found that some RNA methyltransferases negatively affect somatic embryogenesis induction. Recent studies proved that a RNA methyltransferase isolated from tamarillo has rRNA methyltransferase activity. The way rRNA methylation may negatively affect somatic embryogenesis needs further analysis.

Keywords: Embryogenic callus, Proteomics, rRNA methyltransferase, *Solanum betaceum*, Tamarillo

Identification of regulatory miRNA-target nodes across embryo development in *Pinus pinaster*

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Small non-coding RNAs (sRNA) play major roles in gene expression regulation associated to plant growth and development, and response to abiotic and biotic stresses. However, still little is known about the sRNA transcriptome of gymnosperms and its putative role in the distinct characteristics exhibited by these species when compared to the angiosperms. Microarray analysis of *P. pinaster* zygotic embryogenesis highlighted several epigenetic regulation mechanisms and showed that functions related to sRNA pathways appeared differentially regulated across development with a prevalence of micro RNA (miRNA) functions in mid to late embryogenesis (de Vega-Bartol et al. 2013).

A set of sRNA libraries spanning the maritime pine zygotic embryo development and including other reproductive tissues were sequenced using Illumina technology. Presently, there is no available genome sequence of maritime pine but the recent release of its reference transcriptome and the first gymnosperm genome sequences provided valuable resources to the bioinformatics analysis. By using an in-house sRNA pipeline (https://github.com/forestbiotech-lab/sRNA-workflow) for the global analysis of the sequencing data obtained so far, we have identified over 30 families of conserved miRNA that correspond to a small fraction of the total sequenced reads. Additionally, over 10,000 putative novel miRNAs were identified, of which approximately 4,000 are in the range of 20-22nt.

Different experimental methodologies have been performed to validate the presence of specific mature sequences of conserved and novel miRNAs in the isolated sRNA transcriptome, their precursor sequences and expression profiles along embryo development. Moreover, miRNA target genes have been predicted against the reference transcriptome of *P. pinaster* (Canales et al. 2014) and are currently being validated by degradome sequencing.

Based on the analysis of the data, several miRNAs have emerged as potential regulators of pine embryogenesis. Functional studies are being performed in order to analyze their specific role in conifer embryo development.

Keywords: Conifer, Embryogenesis, Micro RNA, Micro RNA target, Non-coding transcriptome, Degradome

References: de Vega-Bartol *et al.* (2013) *BMC Plant Biol* 13: 123; Canales *et al.* (2014) *Plant Biotech J* 12: 286-299.

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Strategies for the early detection of embryogenic competent cells in tamarillo somatic embryogenesis

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Plant somatic embryogenesis (SE) is a developmental pathway in which a complex regulatory network is involved in the acquisition of embryogenic competence by somatic cells and the expression of that acquired competence by the development of somatic embryos. Similar to other plant cell cultures, SE implies a heterogeneous mixture of cells that differ in size, shape, morphogenetic fate, and can also express different markers and interactions with each other's. The identification of subpopulations of embryogenic cells within a single culture and the study of their characteristics could provide new clues for better predicting or inducing the embryogenic pattern of development.

In the last years we have been using the SE induction system of tamarillo (*Solanum betaceum*) to characterize the very early stages of somatic embryo formation. The rationale behind the use of tamarillo as a model is that it has some advantages over other embryogenic systems, particularly for molecular analyses and experimental embryology approaches, since both embryogenic and non- embryogenic tissues can be induced from the same explant, on auxin-rich media, and can be easily separated and grown as independent cell lines in the same culture conditions.

Based on this system, we have been working in more straightforward approaches, necessary to target the specific cells that undergo the pathway of embryogenic competence acquisition and to detect gene expression modifications occurring in them, namely: 1) close monitoring of embryogenic cell behavior as a function of endogenous auxin levels and distribution patterns; 2) functional characterization and localization of a putative SE's inhibitory protein (NEP-TC, Non-Embryogenic Protein from Tamarillo Callus, GenBank JQ766254); 3) fluorescence-activated cell sorting (FACS) of embryogenic and non-embryogenic protoplasts for transcriptome profiling.

The results so far obtained show significantly higher endogenous IAA content in embryogenic samples, with a tendency to increase as the dedifferentiation of the original explant evolves. The immunohistochemical analysis shows that the accumulation of IAA occurs in very localized niches of cells. As for NEP-TC, the distribution pattern is specific of non-embryogenic cells, and the confirmed activity of rRNA methyltransferase of this protein further indicates its regulatory role in the process of embryogenic competence acquisition.

The FACS analysis of protoplasts derived from cultured tamarillo embryogenic cell cultures allowed the identification of cellular subpopulations of embryogenic and non-embryogenic cells, based on the distribution of light-scattering intensities at different angles. After sorting, these subpopulations were further characterized in terms of their cytological features and gene expression to confirm their embryogenic identity and proceed for transcriptome profiling by RNAseq. These results will contribute for the identification of differentially expressed genes in very specific and localized cells during the early stages of embryogenic competence acquisition.

Keywords: Embryogenic cells, FACS, IAA, Immunolocalization

Auxin-mediated expression of a *GH3* gene during adventitious rooting in chestnut in relation to ontogenic state in chestnut

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In many forest species, the lack of rooting response when trees attain maturity hinders massive production of plants through asexual reproduction. It also limits the propagation of selected genotypes, as many characteristics can only be observed once the trees have matured. In this study, an experimental system comprising juvenile-like (Basal Shoots, BS) and mature (Crown, C) chestnut microshoots established in vitro from shoot emerging from the base of the trunk and from crown branches of the same 80-year-old tree was used. During tree development, juvenile characteristics may be retained during in ontogenetically young tissues located at the base of the tree, whereas maturation levels increases toward the top of the tree as a function of increased cell divisions. Therefore, BS and C chestnut microshoots displayed different morphogenetic capacity. The different rooting ability of BS and C shoots in response to the same IBA treatment is linked to their maturation stage, as they share the same genetic background.

In this study a new auxin inducible gene isolated from chestnut microshoots was found to encode a protein belonging to group II of the Gretchen Hagen 3 (GH3) family. The gene was therefore named *CsGH3-1*. Predicted protein sequence analysis revealed the presence of conserved domains involved in the conjugation of amino acids to indole-acetic-acid (IAA). Modelling of the protein and molecular docking of IAA, indole-3-butyric-acid (IBA), 1-napthaleneacetic acid (NAA) and benzothiazole-2-oxyacetic (BTOA) into the active site of CsGH3-1 indicated a high and similar binding affinity for the four substrates.

Expression analysis by qPCR indicated that *CsGH3-1* is regulated by wounding, darkness and auxin in chestnut microshoots, in an ontogenetic-dependent manner. Under IBA treatment, upregulation of *CsGH3-1* was higher in mature than in juvenile shoots and was negatively correlated with the ability of microshoots to form roots. High levels of auxin-induced expression of *CsGH3-1* were detected in mature shoots 24 h after the IBA treatment, whereas transcript levels decreased in rooting competent shoots with cell-type-specific expression. *CsGH3-1* transcripts were specifically localized in cells involved in the initiation of adventitious roots only in rooting competent shoots at the time when cells switch their fate to root initial cells. Thus, these data show a correlation between the specific localization of transcripts and rooting competence, and they also suggest a role for *CsGH3-1* in regulating auxin homeostasis during the onset of adventitious root formation.

Keywords: Adventitious roots, Auxin, Chestnut, GH3, Molecular docking, Ontogenic state.

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Effect of culture conditions on DNA methylation in stone pine embryogenic lines

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Somatic embryogenesis is a way to regenerate clonal plants through the formation of somatic seeds. As a cloning technique is a main tool in forest breeding that captures all the genetic potential of selected trees and produces uniform offsprings. Otherwise seed formation has been identified in Norway spruce as a key point to establish environmentally induced epigenetic marks. These marks conform an epigenetic memory in plants that at long-last affects adaptive traits. Therefore somatic embryogenesis could be also used to produce primed plants conditioned to cope with different kind of stresses.

DNA cytosine methylation is one of the main processes underlying these epigenetic marks. The stone pine (*Pinus pinea* L.) is a genetically uniform but highly phenotypically plastic species of ecological and economic interest. Variability in cytosine methylation among genotypes and populations of the species been demonstrated. The aim of this study was to determine the effect of culture environment, temperature and water availability, on the whole genome cytosine methylation profile of stone pine embryogenic lines.

Two embryogenic lines at the proliferation stage were analyzed with the Methylation-Sensitive Amplification Polymorphism (MSAP) technique. Embryo-suspensor masses were subcultured at 18, 23 or 28 °C on media with 4 or 10 g/l of Gelrite for six weeks. After that, samples were harvested for MSAP analysis.

A total of 202 markers from which 83 were classified as Methylation Insensitive (MI) and 119 as Methylation Sensitive (MS) were scored. The whole degree of methylation, 58.91% of the cytosines at CCGG motifs, is in line with the reported for the species. Significant differences for frequencies of methylation were recorded between genotypes and among treatments within one of the genotypes.

Out of the 83 MI markers, 77 were found to be Monomorphic Methylation Insensitive. The remaining 6 MSAPs (2.97% of the total number of MSAPs) were identified as Polymorphic Methylation Insensitive (PMI).

Within MS markers, 86 were identified as Monomorphic Methylation Sensitive and the remaining 33 were identified as Polymorphic Methylation Sensitive (PMS, 16.34% of the total number of MSAPs). Of these, 5 PMS showed a different pattern in relation to culture conditions during proliferation.

Preliminary studies with embryogenic masses under maturation conditions and cotyledonary embryos showed an increase in intensity of fragments, suggesting a higher degree of demethylation associated with the differentiation processes.

Keywords: Epigenetic memory, Forest biotechnology, MSAP, Pinus pinea, Somatic embryogenesis.

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Cell-to-cell trafficking patterns of Brazilian Pine (*Araucaria angustifolia* Bertol.Kuntze) cell lines with contrasting embryogenic potential

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Besides the biotechnological application, somatic embryogenesis also constitutes an important tool to study early stages of embryogenesis that are not possible to be performed *in vivo.* At the cellular level, the acquisition of embryogenic competence involves aspects related to cell communication and signaling, such as the correct environmental signal receiving and its subsequent cellular internalization, and the regulation of cytoplasmic trafficking. Transcriptomic and proteomic studies of Brazilian Pine (*Araucaria angustifolia*) embryogenesis, a native conifer currently classified as a critically endangered species, have been demonstrated that there is a differential expression of genes and proteins related to cell-to-cell communication, especially in the vesicular transport and regulation of plasmodesmata (Pds) regions. In this context, we carried out a transmission electron microscopy (TEM) analyzes of embryogenic cell cultures used for Brazilian Pine transcriptomic/proteomic studies (responsive [SE1] and blocked [SE6] to embryo formation cell lines) in order to find new evidences about the importance of cell-to-cell trafficking to the embryo formation. Eight cell-to-cell communication and transport-related proteins were identified with a significantly different abundance in SE1 and SE6. *Beta-adaptin-like protein c, Vesicle-associated membrane protein 722* and *Ap-1 complex subunit gamma-1,* proteins related to the endocyticclathrin-mediated via, were more abundant in SE1, while *Gtp binding protein* was observed exclusively in this cell line. Images obtained by TEM showed a large presence of Golgi bodies, vesicles, lipid bodies and mitochondria in SE1 embryogenic cells, suggesting an intense metabolic activity. Otherwise, Pds regulation-related proteins were identified in SE6 cell line, such as *Glucan endo-beta-glucosidase-like* (overexpressed protein) and *Beta-glucan-binding protein* (exclusively observed in SE6) that degrade callose in the Pds edges, used to maintain the open and close control in this area. The presence of callose was confirmed by TEM using PATAg (periodic acid-thiocarbohydrazide-Ag proteinate test) as contrast for polysaccharides. Beside this, numerous amyloplasts with starch grains and an increase in the Pds regions were also observed in this cell line. In addition, *Dynamin-related protein 3b* and *Endomembrane family protein 70* were more abundant in SE6, indicating that for this cell line the vesicular transport was restricted to intracellular organelles. These results imply that the increase in SE1 cell-to-cell trafficking centered on transport is important to obtain information for embryogenic differentiation, while SE6 displayed cells with poor intercommunication selectivity and the accumulation of substances (e.g. starch), might indicating that energy storage is more predominant in this cell line. Therefore, it is crucial to understand the mechanisms that regulate cell-to-cell information trafficking, in order to apply this knowledge in the somatic embryogenesis tool.

Keywords: Vesicular transport, Plasmodesmata, Cellular communication, Conifer

Auxin-related miRNA molecules involved in the induction of somatic embryogenesis.

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Somatic embryogenesis (SE) involves development of the somatic embryos from already differentiated plant cells. SE has been widely used in biotechnology as a powerful way of plant regeneration but the genetic mechanism that governs the induction of the embryogenic pathway in somatic cells remains unclear. Among genetic regulators of SE, beside transcription factors (TFs), microRNAs (miRNAs) that control *TF* genes in developmental processes, are recently considered.

The aim of my PhD project is to extend the knowledge on genetic control of SE in terms of identification of the miRNAs and their targets involved in SE induction. Auxin (mostly 2,4-D) is widely used to induce embryogenic response under *in vitro* culture in various plant species including a model plant, *Arabidopsis* and woody trees. The study is focused on miRNAs (miR160, miR166 and miR393) and their targets related to auxin signaling pathway. Beside *Arabidopsis*, the analyzed miRNAs were found in genomes of the trees e.g. in *Malus domestica* (Ma *et al*., 2014). Evidence of similar functions of miRNAs in *Arabidopsis* and woody trees has been provided (Wang et al., 2011).

miR160 molecules regulate expression of the *ARF* (*AUXIN RESPONSIVE FACTOR*) genes (*ARF10*, *ARF16*, and *ARF17*) encoding TFs involved in the control of auxin-responsive genes. *ARFs* targeted by miR160 were found to control SE in *Dimocarpus longan* (Lin *et al*., 2015), *Gossypium hirsutum* (Yang *et al*., 2013), *Citrus sinensis* (Wu *et al.*, 2011), *Liriodendron tulipifera* (Li *et al*., 2012) and *Larix leptoleptis* (Zhang *et al.*, 2010). Our recent results indicate that drastically decreased level of the mature miR160 observed during SE in *Arabidopsis* is associated to the increased expression level of the *ARF*s targeted by miR160. In line with this results, up-regulation of *ARF10* and *ARF16* was observed in embryogenic culture derived from the insertional lines with decreased level of miR160. Further support for the involvement of miR160 in SE provided the analysis of the SE culture derived from *mARF16* line resistant to miR160. In this culture, a distinct up-regulation of *LEC2 (LEAFY COTYLEDON2)*, a key regulator of auxin-dependent mechanism of SE induction, was found.

miR166 was found to indirectly control *LEC2* expression *in vivo* by targeting the *PHB (PHABULOSA)* and *PHV (PHAVOLUTA)* encoding TFs that positively regulate *LEC2* (Mallory *et al.* 2004). The role of miR166 and *PHB, PHV* was shown in SE of tree species including *L. leptoleptis* (Zhang *et al.*, 2010), *L. tulipifera* (Li *et al*., 2012) and *D. longan* (Lin *et al.*, 2015). Our analysis in SE in *Arabidopsis* show a drastic decrease of mature miR166 content that is coupled with the increased level of *LEC2* transcripts. In further support for the miR166-mediated regulation of *LEC2*, a distinct up-regulation of *PHB* and *PHV* transcripts was indicated to accompany SE induction. Thus, in embryogenic transition, down-regulation of miR166 that results in the up-regulation of *PHB* and *PHV* seems to stimulate *LEC2* transcription. In line with this hypothesis, a distinct up regulation of *PHB* and *PHV* as well as *LEC2* was found in SE culture of the *STTM165/166* line with the silenced expression of *MIR166*, and a closely related *MIR165*.

miR393 negatively regulates the expression of *TAAR (TIR1/AFB AUXIN RECEPTORS)* genes including*TIR1* and closely related *AFB* genes (*AFB1*, *AFB2* and *AFB3)* encoding auxin receptors. *TIR1* was reported to regulate SE in *G. hirsutum* (Yang *et al*., 2013). Our study showed distinct increase in the accumulation of miR393 was coupled with a notable down-regulation of *TIR1* and *AFB2* targets in SE of *Arabidopsis*. In conclusion, miR393 was postulated to control SE induction via the modification of the tissue sensitivity to auxin (Wójcik and Gaj, 2016).

Collectively, the obtained results imply that miR160, miR166 and miR393 involved in auxinsignalling contribute to the embryogenic switch induced *in vitro* in somatic cells of *Arabidopsis*. Given that the genetic similarity between the developmental programs in different plants might be expected the knowledge on miRNA-mediated mechanism of SE induction in *Arabidopsis* may be useful for the improvement of the vegetative propagation technologies in crop plants including trees.

Keywords: Auxin, miRNA, Receptors, ARF, *Arabidopsis*

Somatic embryogenesis as an enabling technology for reverse genetics: achievements and prospects for breeding maritime pine (*Pinus pinaster* Ait.)

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As a major pine species with great ecological and socio-economic interests in Southern Europe, various approaches are concurrently developed in *Pinus pinaster* towards enhanced selection efficiency and deployment of improved, better-adapted varieties. Strong synergies are expected between traditional breeding, DNA-based selection (especially genomic selection) and somatic embryogenesis (SE) as a scalable vegetative propagation method of tested varieties for implementing multivarietal forestry. SE has been shown for more than 15 years to be an effective support for stable *Agrobacterium*-mediated genetic modification of selected genotypes (FCBA, INRA and iBET developments). Validating marker associations with specific properties before transfer into breeding selection models is still challenging. We developed reverse genetic studies (French and multinational/European initiatives) aiming at establishing direct associations between gene expression (including transcription factor genes) and adaptively significant phenotypes through overexpression or loss-of-function strategies such as RNAi. Data accumulated for various genes involved in wood formation, carbon and nitrogen metabolisms, stress resistance, embryogenesis and plant development. Based on this collective effort, we will highlight some major achievements, discuss weaknesses of current technology and new opportunities.

Keywords: Genetic modification, *Agrobacterium*, Transgene, Transcriptomics, Proteomics, Off-target effect

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ABSTRACTS OF ORAL PRESENTATIONS

SESSION 4: **Preservation and adaptation of wild and selected genetic resources to environmental and socio-economic changes.**

Jacaranda mimosifolia D. Don (JACARANDÁ)

Native tree of the north of Argentina, shared with Uruguay and Paraguay. With extended cup and bipinnate compound leaves, tardily deciduous foliage. The Blue-violet flowers turns in fruit capsule of 8 centimeters long in which small winged seeds are deposited. Sensitive to frost is used for public trees for their showy flowering.

KEY INVITED SPEAKER

Integration of selection, breeding, somatic embryogenesis and cryostorage to conserve and restore threatened North American forest trees

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The consequences of global climate change, as well as globalization of trade, include rapid increases in the number and scale of menaces to North American forest trees from insect pests and pathogens, loss of habitat and other stresses. These pressures make critical the application of biotechnological approaches to supplement conventional programs aimed at conserving germplasm of threatened forest species and restoring forests. Conservation of genetic material and selection and breeding of resistant or tolerant genotypes could be greatly enhanced by employing in vitro propagation systems. Somatic embryogenesis (SE), in particular, is well-suited for this purpose, due to the high multiplication rates and the amenability of embryogenic cultures to cryostorage. In collaboration with the American Chestnut Cooperators Foundation (ACCF), we have used SE to conserve large surviving American (LSA) chestnut trees and to propagate germplasm derived from crosses between the LSAs, so that clones can be tested for chestnut blight resistance. We have also collaborated with The American Chestnut Foundation (TACF) to implement clonal testing of conventionally bred material from their hybrid backcross breeding program for resistance to chestnut blight. Open-pollinated and control-pollinated BC3F3 seeds from BC3F2 seed orchard parents were used to initiate embryogenic cultures. The first BC3F3 somatic seedlings have already been deployed in clonal field tests. We are working with breeders at NC State University and the Forest Restoration Alliance to conserve and restore eastern and Carolina hemlocks, which are under threat of extinction by hemlock woolly adelgid (HWA), by using SE to clonally propagate putatively HWA-resistant eastern hemlocks and hybrids between Carolina hemlock and HWA-resistant Asian hemlock species. As part of the effort to conserve and restore ash populations that are being devastated by the emerald ash borer (EAB), we have developed embryogenic cultures of both green ash and white ash. In collaboration with scientists at Ohio State University, we have initiated embryogenic cultures from seeds collected from multiple "lingering" white ash trees in Michigan, which are potentially resistant to EAB, since they have survived infestations that have killed surrounding ash trees. While Atlantic white cedar is not threated by exotic pests or pathogens, loss of habitat due to overcutting and conversion of AWC sites to other uses has made restoration a priority. Recently, we produced the first AWC embryogenic cultures and somatic seedlings using a novel culture protocol. Chestnut, hemlock, ash and AWC embryogenic cultures have been successfully cryostored and recovered using a standard protocol. Integration of selection and breeding programs with in vitro culture tools like SE and cryostorage create a powerful combination to aid in conserving germplasm and restoring threatened trees to the forest.

Keywords: Somatic embryogenesis, Chestnut, Ash, Hemlock, Atlantic white cedar, Cryopreservation.

Increasing resilience in forest tree species: a possible additional advantage for somatic embryogenesis technology

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We present an overview of our project, supported by the Spanish government (AGL2013-47400-C4- R), and addressed to improve resilience in forest tree species using somatic embryogenesis (SE) technology as a basic tool. The project is being developed by four teams with large experience in developing SE regeneration protocols, both in hardwoods and softwoods. The species included in the study are forest species of economic and ecological interest in Europe.

Plants will have to cope with the predicted conditions of climate change, suffering increased biotic and abiotic stresses. In this context, we have suggested that both traditional breeding and the stimulation induced defense/adaptation responses could be used to increase resilience. SE biotechnology can shorten traditional breeding methods by cloning elite and resistant individuals that may maintain both attributes in the clonal offspring. In addition, genes of resistance can be introduced by direct genetic modification in somatic embryos of selected genotypes producing transgenic resistant plants. Changes in the plant genome under biotic or abiotic stress conditions have been reported, mainly of epigenetic nature that can be transferred to the offspring at the time of embryo formation. Overall, this is called transgenerational epigenetic stress memory.

Quercus suber (cork oak) and *Quercus ilex* (holm oak) are threatened by the syndrome "la seca" caused, among other factors, by oomycetes such as *Phytophthora cinnamomi*. This biotic stress is being approached taking advantage of the natural variability of both species and using SE developed protocols to clone resistant genotypes. Also we are obtaining oak plants by transforming SE with genes encoding pathogenesis-related proteins isolated from chestnut. Finally, we are inducing epigenetic memory (priming) by culturing developing somatic embryos with several elicitors to putatively induce a memory of resistance in the regenerated plants.

Previous work in Norway spruce demonstrated that temperature during the process of somatic embryo formation determined the phenology of the regenerated plants. Based on these results, temperature and water stresses during somatic embryo development in *Pinus pinea*, *Pinus pinaster*, *Pinus halepensis* and *Pinus radiata* are being checked for induced changes at DNA methylation levels, phenological and physiological parameters and/or for the ability to produce temperature and drought tolerant plants.

Finally, several current limitations of the SE regeneration and conservation protocols of these species are also being considered. Among them, low frequencies of induction in stone pine, maturation in maritime and stone pines, difficulties with the acclimatization of holm oak plants; and the development of an improved conservation method for somatic embryos of radiata pine are under study. To optimize holm oak embryogenic system, induction of SE in explants from axillary shoot proliferation cultures, and the histological characterization of the embryogenic process are also being investigated.

Keywords: Epigenetic breeding, Forest Biotechnology, Genetic transformation, *Phytophthora* spp, *Pinus* spp, *Quercus* spp, Somatic embryogenesis.

Acknowledgements: Research supported by the Spanish MICINN and European Union FEDER Funds (AGL2013-47400-C4-R).

Transformation of *Quercus suber* and *Quercus ilex* somatic embryos with a gene encoding a thaumatin-like protein

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Cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* L.) are widely distributed tree species in the Mediterranean ecosystem, where they are economically important mainly due to the production of cork and acorns, respectively. Over the last few decades, cork oak and holm oak populations have been decimated by a syndrome denominated oak decline, mainly caused by *Phytophthora cinnamomi*, *Diplodia mutila* and *Biscogniauxia mediterranea.*

Genetic transformation could enable production of plants tolerant to oak decline through overexpression of pathogenesis-related proteins (PR). PR proteins comprise a group of diverse proteins whose accumulation is triggered by pathogen attack, abiotic stress, hypersensitive response and systemic acquired resistance. In European chestnut, a thaumatin-like protein of 23-KD, termed *CsTL1* and purified from mature cotyledons, displayed *in vitro* antifungal properties (García-Casado et al. 2000). The objectives of the present study were to obtain cork oak and holm oak somatic embryos (SEs) that overexpress the chestnut thaumatin-like protein (*CsTL1*).

CsTL1 gene was cloned into the pK7WG2D plasmid. This plasmid also contains the neomycin phosphotransferase (*nptII*) as a selective gene and the green fluorescent protein (*egfp*) as a reporter gene.

In cork oak, small clumps of 2-3 SEs at globular and/or torpedo stages that were obtained from three cork oak embryogenic lines (ALM6, ALM80 and TGR3) were used as target explants. In holm oak proembryogenic masses or globular somatic embryos isolated from one holm oak embryogenic line (Q8) were used as target explants. In both species, SEs were co-cultured for 5 days with *Agrobacterium tumefaciens* strain EHA105 harboring the pK7WG2D-CsTL1 binary vector. Then, somatic embryos were cultured on selective medium containing kanamycin (125 mg/l in cork oak or 100 mg/l in holm oak) and carbenicillin (300 mg/l). After culture of the embryos for 14 weeks on selection medium, the transformation efficiency was determined on the basis of the fluorescence of surviving explants.

In cork oak, the transformation efficiency was clearly genotype dependent, as TGR3 yielded higher transformation frequencies (12.66%) than ALM80 (4.5%) and ALM6 (1.5%). In holm oak the transformation efficiency was 4%. Selected embryos will be multiplied and subjected to molecular analysis to determine the presence of genes involved in all lines established.

Keywords: Cork oak, Holm oak, "La seca", Pathogenesis-related proteins, *Phytophthora cinnamomi*, Somatic embryogenesis.

References: García-Casado et al. (2000) Physiol Plant 110: 172-180.

Acknowledgements: This work was supported by MINECO (AGL2013-47400-C4-3-R).

Shoot proliferation of chestnut (*Castanea sativa* Mill.) and *in vitro* protective effect of endophytes against *Phytophthora cinnamomi*

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Chestnut (*Castanea sativa*) is a widely cultivated forest deciduous tree that produces an edible nut, especially appreciated in southern Europe. Plant growth and fruit production have been greatly affected by several plagues and diseases, such as ink disease caused by the fungus *Phytophthora cinnamomi* that grows in the soil leading to crown and root rot. Because chestnut is severely affected by this pathogen, which usually leads to the dead of host plants, it is crucial to develop control strategies at different levels, but especially by improving host resistance, in order to mitigate the disease and minimize economic losses. Thus, the main objective of this work is to access the tolerance of selected clones of chestnut to this pathogen, and the protective effect of symbionts isolated from natural growing trees. For this purpose, roots and twigs of selected clones have been plated on PDA medium, and several symbionts were isolated and identified. Fungal DNA was extracted with a Macherey-Nagel kit and specific primers (ITS1 and ITS4) were used to amplify the ITS region of rDNA through PCR. The obtained amplicons were then searched on GenBank, and most of the isolates were identified as Ascomycota. To predict the behavior of the isolates when exposed to the pathogen, amylase, cellulase, lipase, pectinase and protease activities have been assessed, with cellulase activity being the most evident among the tested isolates. The antagonism effect of the endophytes was tested when co-cultured *in vitro* with two different strains of *P. cinnamomi* isolated from chestnut trees showing symptoms of the disease. Five isolates have been identified based on the ability to reduce the growth of the pathogen, either by causing a dead-lock or by substitution, and its protective effect against *P. cinnamomi* was tested. Thus, selected clones of chestnut have been established *in vitro* and multiplied through shoot proliferation on WPM medium supplemented with 0.2 mg/L zeatin and 3% sucrose. Shoots were successfully rooted (98%) on Knop medium with 2 mg/L IBA. Some plants were also rooted by dipping *in vitro* on jiffy pots and acclimatized for further analysis. Plants from the different clones produced by this method have been cultured *in vitro* with the selected endophytes and a highly virulent strain of *P. cinnamomi*. The mycelium growth of both pathogen and endophyte and the appearance of visible symptoms on the plant have been registered during a month, and compared to a non-infected control group. Without endophytes, the pathogen severely affected the plants, causing death in less than two weeks. Although some of the endophytes tested showed to minimize the symptoms, they were unable to avoid plant death. Nevertheless, two of the symbionts (*Trichoderma harzianum* and *Diaporthe* sp.) successfully protected the plants and no symptoms of the disease were observed. One of the isolates has already been identified to confer protection against pathogens, including *P. cinnamomi*. The results obtained on this work may open the way to develop a strategy that minimizes the effects of ink disease, based on the protective effect of natural symbiont microorganisms.

Keywords: Coculture, Endophytes, PCR, *P. cinnamomi*, Shoot proliferation, Symbiont.

Mycorrhization improved growth and survival of somatic embryogenesis derived *Kalopanax septemlobus* and *Liliodendron tulipifera* microplants

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In order to establish some cultural practices that can improve growth and survival of somatic embryogenesis-derived microplants during acclimatization period, two concurrent experiments were conducted. *Kalopanax septemlobus* and *Liliodendron tulifipera* microplants were uninoculated or inoculated with arbuscular mycorrhizal fungi (AMF) during transfer from aseptic culture to individual container filled with either sterile or non- sterile peat perlite vermiculite medium. *Kalopanax* produced from somatic embryogenesis were inoculated with unidentified species of *Glomus* and *Acaulospora* (AMM6) collected in Bonghwa closed mine tailings, Korea and *Glomus etunicatum* from the Philippines. On the other hand, *Liliodendron* microplants were inoculated with G. *etunicatum, G. macrocarpum* and *Gigaspora margarita*. After one month incubation in acclimatization room, the seedlings were transferred in a glasshouse. At this stage, inoculated plants were greener, with broader leaves and well developed root system and had higher (90-95%) survival than the uninoculated ones. At harvest, inoculated *Kalopanax* and *Liliodendron* were 50% and 4x, respectively, heavier than the uninoculated counterpart. Plant growth was better in sterile than in non-sterile medium throughout the five months observation period. *G. etunicatum* consistently promoted the highest plant height, diameter, leaf length, leaf width, and dry weight and nutrient uptake in both sterile and non-sterile medium. On the other hand, control plants consistently had the lowest growth and nutrient uptake. *G. macrocarpum* and *G. margarita* produced higher percent root colonization than *G. etunicatum* but these were less effectiveness in promoting plant growth and nutrient uptake. In conclusion, better growth and survival was obtained in sterile medium than in non-sterile one. AMF inoculation is important in improving growth, survival and nutrient status of SE-derived *K. septemlobus* and *L. tulipifera*. *G. etunicatum* promoted the highest leaf, stem, root and total plant dry weight in both sterile and non-sterile medium. Further experimentations are needed to verify these initial findings under nursery and field conditions.

Keywords: Arbuscular mycorrhizal fungi, *Glomus, Gigaspora,* Sterile and non-sterile growing media

Induction of embryogenic cultures from mature seeds of a fragile population of *Nothofagus obliqua* (Nothofagaceae)

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The degradation of natural forests is the main cause of biodiversity loss, emissions of greenhouse gases and deterioration of the global production capacity. Somatic embryogenesis (SE) is currently considered as the most appropriate biotechnology to propagate trees and can therefore contribute to the conservation, restoration and development of forest ecosystems. In this work the *in vitro* induction of embryogenic cultures from a fragile population of *Nothofagus obliqua* from the Lagunas EpuLauquen Reserve (Neuquén) is described. Seeds were harvested and conserved between 4 and 6 °C. Seeds without seed coat were used as initial explants and disinfected with 40% H_2O_2 for 30 min. Embryos with endosperm were then extracted and inoculated on culture media. For somatic embryos induction a basal medium based on either Murashige and Skoog (MS) formulation or broadleaved Tree medium (BTM), with or without glutamine and casein hydrolysate, and supplemented with 2-isopentyl adenine (IA), 2,4-dichloro phenoxyacetic acid (2,4-D) or 6- benzyl amino purine (BAP) was used. Embryogenic calluses were identified after macro- and microscopic observations (staining with Evans blue at 2% and 1% acetic carmine). Cultures were incubated in complete darkness, at 24 +/- 2 °C and 50% relative humidity for 5 months. The proportion of viable embryos varied between 75 and 80%. The BTM supplemented with 2,4-D (4.5 to 45.2 µM) and BAP (2.2 to 7.0 µM) always produced embryogenic cultures. This is promising results towards the development of an SE protocol for the propagation of a fragile *Nothofagus obliqua* tree population.

Keywords: Native tree conservation, Vegetative propagation, Broadleaved tree medium

Cloning drought tolerant *Eucalyptus globulus* in the region of Bio-Bio, Chile

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Three breeding populations were included as part of a strategy to improve drought tolerance of *E. globulus*: (A) open pollinated progeny with genetic ranking by volume of a clonal seed orchard, (B) a population of 107 plus trees generated by mass selection in drought conditions and (C) control pollinated progeny by intraspecific crosses between the A X B populations using the "one stop pollination" system. Micro propagation and rooting were experienced by somatic embryogenesis and organogenesis to transfer drought resistant for operational multiplication of genetic material in nurseries. *"In vitro"* protocols were developed to induce morphogenic responses and genetic transformation in adventitious buds and seeds of *E. globulus*. Traceability of superior genotypes were also made on the basis of molecular genetic markers and physiologically evaluated in greenhouse under water stress conditions to characterize their potential for drought resistance. It was established protocols that allow an appropriate transformation via *Agrobacterium tumefaciens* and regeneration system from indirect somatic embryogenesis in explants of *E. globulus* seed. It was possible to establish protocols through two pathways which allow an appropriate transformation via *Agrobacterium tumefaciens* and regeneration system of two highly productive clones of *E. globulus*. These morphogenic pathways were obtained indirectly from callus, one of these was from indirect somatic embryogenesis in explants seeds, and the other pathway was by indirect organogenesis of adventitious buds in explants One of the important objectives of the project was to evaluate the ability of rhizogenic clones of *E. globulus* and identify drought tolerant genotypes with greater rooting ability to propagate conventional operationally by rooting cuttings, mini cuttings and hydroponics system. The results confirm that *E. globulus* is a species that has a low rate of adventitious rooting stem cuttings and are part of the traditional values reported for this species. The limited material available for evaluation (18 clones) does not identify individuals with a high average percentage of rooting. However, there are clones that in some particular assays exhibited high rhizogenic response (up to 92%). Indeed, even though the average rooting reached 13.4%, with average values ranging between 0 and 30.7%, where great variability was observed on trials, identifying specific experiments clones that reach high percentages of rooting. Among them are clones 87, 48, 62, 82 and 77 to obtain values ranging between 60 and 93%. Such percentages are compatible with operational cloning of these individuals. Consistent in the rooting of clones in all trials evaluated behavior, a situation that is also documented in the literature and constitutes an additional obstacle to mass propagate the species by rooting cuttings is observed.

Keywords: *E. globulus*, Somatic embryogenesis, Organogenesis, Cuttings, Minicuttings, Hydroponics

ABSTRACTS OF ORAL PRESENTATIONS

SESSION 5: **Lessons from in vivo growth of vegetative propagules, especially in various pedoclimatic conditions.**

Salix humboldtiana Willd. var. Humboldtiana (SAUCE CRIOLLO)

A native of Argentina, Brasil, Chile, Paraguay and Uruguay, 10-12 meters high. Its bark is dark and rough, finely toothed leaves lanceolate of 15 centimeters long that turn yellow before falling. It is the only willow that retains the same color on both sides. Fruit capsule with small cottony seeds. Frost resistant, can be reproduced by cuttings.

Somatic embryogenesis and plant regeneration in Japanese pines and cypresses

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Japanese pines (*Pinus thunbergii, P. densiflora, P. armandii* var. *amamiana*) and cypresses (*Chamaecyparis obtusa, C. pisifera*) are important in Japan for reforestation and landscaping. However, these species are affected by various biological problems and need urgent measures for their propagation. Somatic embryogenesis is the most promising technique for mass propagation of clones, and for plant regeneration in genetic transformation protocols for basic studies and tree improvement programs. In this presentation, the current status in protocol development for somatic embryogenesis in Japanese pines and cypresses is described with special emphasis on germination and plant conversion rates after embryo maturation process.

Somatic embryogenesis was initiated from excised megagametophytes containing immature zygotic embryos. Embryogenic cultures were maintained and proliferated in a medium supplemented with 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine, sucrose, and glutamine. The somatic embryo maturation experiments were performed in darkness at 25°C. Embryogenic tissues were cultured on maturation media containing maltose, activated charcoal, abscisic acid, and polyethylene glycol (PEG). The addition of PEG to the medium dramatically stimulated the embryo maturation, with enhanced yield in mature embryos as PEG concentration is increased. Although the cotyledonary embryo production varied according to the species, supplementation of medium with 100-150 g l^{-1} PEG was found to be suitable for high-quality embryo production in Japanese pines and cypresses.

Mature somatic embryos germinated and then converted into plantlets after their transfer to the plant growth regulator-free medium. However, for the pine species, desiccation of somatic embryos after PEG-maturation was found to be essential for achieving both high germination and high conversion rates. Although the improved protocol represents a promising perspective for an efficient mass propagation of these species, more efforts are required to establish an optimal protocol for the commercial production of somatic plants with high field performance.

Keywords: *Chamaecyparis* spp., Germination, *Pinus* spp., Plant regeneration, Polyethylene glycol, Somatic embryogenesis.

Somatic embryogenesis in *Picea abies* using primordial shoot explants, and the germination of somatic embryos under different LED light systems

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In the case of Norway spruce, development of cost-efficient vegetative propagation techniques is important, since there is a lack of high-quality, bred forest regeneration materials due to irregular flowering of the species, as well as pest and pathogen problems hindering seed production in seed orchards. The flowering of ornamental forms of Norway spruce is especially rare, and the induction of embryogenic cell lines from adult trees with known characteristics would be beneficial for production of gardening and landscaping material for northern conditions as well as for special forest regeneration material.

SE induction protocol successfully applied in primordial shoots of white spruce was applied for field-grown Norway spruces of SE origin. Shoots were collected in May and April 2015 and April 2016 from trees 3- and 4-year-old at the time. In 2015 shoots were collected from 25 genotypes origin of Finnish Norway spruce breeding program, and from one ornamental crossing. In 2016 12 new genotypes were introduced and one genotype from previous year was restudied. We got positive response both year, totally 18 parts of shoots initiated new embryogenic cell lines. Mature somatic embryos were produced from year 2015 lines, from year 2016 lines the embryogenicity was verified with acetocarmine staining; maturation experiments being performed

We tested adjustable LED light system which uses Pulse Width Modulation (PWM) technique to control the brightness of LEDs. PWM controlling is based on the pulses of power which are sent to the LEDs, i.e. switching lights on and off in short intervals. The length of the interval defines the brightness and spectrum of the lights. In most of the commercial LED light systems the power is constantly on and intensity and spectrum are fixed. We compared adjustable LEDs using PWM controlling technique for four different wavelength to fixed spectrum lights at the stage of embryo germination and acclimatization.

Keywords: Somatic embryogenesis, Primordial shoots, Norway spruce, LED lights, Pulse Width Modulation

Selection effects of somatic embryogenesis in Norway spruce

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A substantial loss of genotypes is a typical result when propagating Norway spruce via somatic embryogenesis (SE). Does this selection for propagation ability affect important traits? To investigate this question, a project has been launched where SE plants and seedlings of the same families are compared.

Embryos from 50 half-sib families of Norway spruce were put on initiation medium in 2011, followed by proliferation and maturation of somatic embryos during 2012. Embryos germinated and plantlets were acclimatized in 2013, and cultivated until autumn 2014. Seedlings from the same families were grown in parallel and both plant types were planted in field trials in spring 2015. Prior to winter hardening, cuttings were excised from both SE plants and seedlings and put in rooting environment in late summer 2014.

When SE reached the acclimation step, 26% of the embryos were successfully propagated and one family was lost. As expected, the number of clones per family varied considerably, as well as the number of plants per clone. Seedlings were taller than SE plants at the end of plant production. Furthermore, SE plants had larger stem diameter in relation to plant height in two families. The cutting propagation resulted in low rooting percentages, lowest when SE plants were donors.

In spring 2016, time for flushing were assessed in one field trial and the behavior of SE plants and seedlings were compared.

Keywords: Propagation losses, Plant production, Cutting propagation, Field trials, Time for flushing

Results from the first full rotation of growth in clonal field trials of nordmanns fir (*Abies nordmanniana*)

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Nordmanns fir (*Abies nordmanniana*) is grown in Danish forestry as an ornamental for the production of Christmas trees. The production only covers 10 % of the forest area, but economically it is the most important tree species in Danish forestry, where it amounts to approx. 90 % of the total income. The Danish production is 12 million trees per year and the European market is 70 million trees each year.

There is great economical interest from conventional growers in uniform plant material selected on basis of form and growth. The presentation reports from test of larger scale production. Larger scale production of Christmas trees of nordmanns fir, based on organic principles, is at present not a realistic possibility. The area of organic production only amount for 1.5 % of the total production area in Denmark. The reason is mainly due to two problems: 1) a general reduced growth and faint coloration due to reduced application of nitrogen in fertilizers accepted in organic farming 2) severe damage on needles and shoots by the aphid *dreyfusia nordmanniana.* In conventional production, the aphid is mitigated by application of pesticides, which are not allowed in organic production. It is expected that plants selected for traits such as insect resistance and improved nutrient uptake may allow for organic production in larger scale.

The presentation will report on results from the first clonal field trial that was established in 2007, and will furthermore report on preliminary results from larger field trials with 500 clones established in 2014 and in 2015. Field trials established at different locations to test for genotype and environmental effects on specific traits, and on the possibility of clonal selection for insect- and fungi resistance and for improved efficiency in nitrogen uptake.

Keywords: Somatic embryogenesis, Clonal field trials, Selection, Traits

Field evaluation of Scots pine (*Pinus sylvestris* L.) emblings

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Scots pine (*Pinus sylvestris* L.) is considered to be a difficult species for vegetative propagation. Somatic embryogenesis of the species has, however, been developed to result in routine plant regeneration, even though initiation frequencies remain low when compared with other conifers. There is not much published information available on field performance of Scots pine emblings, and the first results on a seven-year Finnish field experiment are presented, as well as insights on on-going experiments.

The Finnish Forest Research Institute established a field experiment with Scots pine emblings in 2009 at Punkaharju (61°48'N, 29°17'E, 90m a.s.l.), and the experiment has been followed yearly to observe performance of the emblings. In this experiment, 13 embryogenic lines (10 emblings per line) originating in four donor trees were included, together with seedling controls, as single-tree plots. Both the embryogenic lines and seedlings were of open-pollinated seed origin, produced as described by Aronen and co-workers (2009), and planted as 2-year-olds. In addition, the Punkaharju experiment was planned to study the effect of somatic embryo quality on later field growth of the emblings. Therefore, in five replications, six lines were represented as emblings derived from somatic embryos of different quality; i.e. from embryos classified either as "good", "intermediate", or "inferior" in the beginning of their germination period according to Aronen and co-workers (2009).

As presented by Aronen (2016), following six years' growth in the field, 95% of the Scots pine emblings and 97% of the control seedlings were alive. The emblings have a normal growth habit, and show genotypic differences. When compared with seedlings of the same genetic background, their height is – depending on the line - either comparable or inferior. At the time of planting, the seedlings were bigger than most of the emblings, and this difference remained. If the yearly growth is examined, it is seen that for all the families there are both years in which the growth of the emblings and the seedlings differ and years when it does not. The quality of the original somatic embryos, however, did not affect the height or diameter growth of the emblings, when examined following 6 years at field.

Observations on the above-described field experiment will continue. Other on-going research with Scots pine emblings is focused on testing resistance / tolerance of the selected lines to various strains of *Heterobasidion annosum sensu stricto*, taking place both under field and greenhouse conditions.

Keywords: *Pinus sylvestris*, Somatic embryogenesis, Field performance of emblings

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Registration process of Norway spruce embryogenic cell lines for commercial forest regeneration in Finland

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Norway spruce (*Picea abies*) is the most cultivated species of coniferous trees in Finland. The constant demand of propagation material with high breeding value has driven the Finnish tree propagators and forest tree nurseries to search stable source for high quality propagation material. Production of somatic embryo (SE) plants of Norway spruce is one of the most promising techniques to achieve this.

In order to market clonal material, such as somatic embryo plants i.e. emblings, for forest regeneration in Finland, we have to go through a registration process according to our national legislation that is based on EU legislation.

Luke has now started this process in cooperation with Finnish Food Safety Authority (Evira), aiming to register the first set of embryogenic cell lines of Norway spruce. When we don't yet have results from field experiments with our SE cell lines we first register selected cell lines from high value parent trees for Bulk propagation. After this we can produce at least 4 M plants / family for the market.

Simultaneously with the registration process plants for field testing are produced from the selected cell lines. The clonal field testing is carried out by using rooted shoot cuttings from SE donors that are planted in several test sites, located mainly in the predicted utilization zone of the propagation material. After the test results it is possible to register combinations of clones with proven breeding values. In this phase it is mandatory to identify the registered clones so that they can be verified later on, e.g. using microsatellite markers.

A case study with the first two sets of Norway spruce SE lines to be registered as forest regeneration material in Finland will be presented, including also the practical point of view – i.e. laboratory management of circa 500 lines belonging to 12 selected families.

Keywords: Picea abies, Somatic embryogenesis, Registration, Field testing.

Sprout vigor of poplar cuttings from stoolbeds fertilized with nitrogen or phosphorus

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Salicaceae stoolbeds produce propagating material for clonal plantations. With the annual shoots harvest, nutrients are exported from the system and in consequence, in successive production cycles, soil fertility can be reduced. Fertilization enables to compensate for extraction of nutrients. However, there is little information if mineral fertilization affects the vigor of the cutting in its early sprout. The aim of this study was to evaluate if fertilization with N or P in stoolbeds of two *Populus deltoides* clones -`Australiano 129/60´ and "150-82"- affects the early vigor of the sprout of cuttings. Commercial stoolbeds were divided in plots that received one of the following treatments: fertilization with N, fertilization with P and unfertilized. Root and sprout production during the first months after planting was measured in cutting produced in each treatment. Fertilization with N increased the number of roots in `Australiano 129/60´ but increased shoot: root ratio. Early vigor of "150-82" was not affected by fertilization. If cutting from fertilized stoolbeds were subject to soil water deficit or flooding, their growth was affected similar to those coming from unfertilized stoolbeds. Hence, fertilization of stoolbeds has no negative effect in the early vigor of the sprout neither in their tolerance to water stress of poplar cuttings.

Keywords: *Populus deltoides*, Nutrient deficit, Nutrient concentration, Nutrient uptake, Nutrient use efficiency

Industrial implementation of somatic embryogenesis for the production of *Coffea canephora* plantlets

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Somatic embryogenesis has been investigated on *Coffea* species since late 70s with the aim to become an alternative method to conventional vegetative propagation of selected varieties such as rooted cutting or grafting. During years 2000s, protocols were optimized and defined for *Coffea canephora* and a pilot production unit was set up in R&D Tours facilities to validate the technical feasibility on the large scale (10M somatic embryos. year^{-1}). The somatic embryos were then shipped to coffee producing countries to be sown, grown and acclimated before their distribution to the farmers.

The following and logical step was to transfer the technology and the somatic embryo production in the coffee producing countries. AGROMOD SA de CV, a renowned company specialized in vitroplant production and commercialization in Mexico demonstrated interest in acquiring the process developed in Nestlé R&D center. A dedicated laboratory was equipped to host embryogenic callus cultured on semi-solid and liquid medium, to allow then torpedo embryo development and finally early germination stage (i.e. green cotyledonary embryos displaying root development) using temporal immersion system (box-in-bag bioreactors). All protocols regarding laboratory process and acclimation *ex vitro* were gathered into one single handbook and shared with the company. Embryo production started on March 2012 with the plan to reach step by step 100% of production capacity in 4 years.

The embryo production in the laboratory was rapidly mastered and only some optimizations of protocols were required to better fit the requirements for *ex vitro* acclimation. Callogenesis was observed on semi solid medium for all of the selected clones chosen for the project. In liquid medium, cell lines could be established and used to produce torpedo embryos. Further development of torpedo embryos was obtained in bioreactors fed by automated temporal immersions of liquid medium. On the other hand, the plantlets acclimation *ex vitro* was the most difficult part of the process to achieve and important losses were reported at the beginning of the project. The technical issues were finally solved and now the process allows to reach an embryo-to-plantlet conversion rate of 50% and beyond, as initially expected. By end of 2016, Agromod will have produced a total amount of 10M of coffee plantlets. A great challenge was also to change the somatic plantlets perception among the coffee producers community, more familiar with seedlings and rooted cuttings.

Keywords: Plantlet production, Mass propagation, Somatic embryo, Technology transfer

A hybrid tissue culture protocol that combines conifer somatic embryogenesis with organogenesis as an alternative propagation platform for specialist applications

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Current somatic embryogenesis (SE) protocols for many pine species remain suboptimal for the purpose of reforestation. Some of the bottlenecks include losses of lines through the SE process due to lack of continued proliferation, poor embryo maturation or poor conversion of somatic embryos to *ex vitro* conditions. There is often significant variation among genotypes with respect to productivity and SE protocol improvements do not always benefit all genotypes.

There is often the desire to propagate specific cell lines, for example from top-ranked families. Selected lines may show desirable characteristics such as disease resistance or a high ability to be genetically engineered. There is also a need to propagate cell lines from species that are in general recalcitrant to somatic embryogenesis (e.g. *Pinus densiflora*). Cell lines from hybrid crosses, where there can be incompatibility issues between parent species, also present several challenges. In particular there are no protocols available for these new hybrid crosses which are often less responsive to all steps in the SE process.

An example of this recalcitrance could be a cell line which is highly transformable but only produces a limited number of good quality mature somatic embryos. This makes direct planting of somatic embryos to *ex vitro* conditions an unviable option. If the desired cell line will not respond positively to modification of the standard SE protocols or media for the species then the options for bulking up this material are limited. A hybrid tissue culture system combining germinated somatic embryos with organogenesis allows the flexibility to work with these desirable cell lines and allows the best aspects of the component propagation methods to be exploited.

In conclusion, we have a propagation protocol that takes advantage of the benefits of SE, which include cryopreservation to ensure juvenility while field testing takes place and the potential for genetic engineering. These features combined with organogenesis, which provides the advantages of increased early multiplication, uniformity of shoots and high rooting and conversion to planting stock/stoolbeds is a winning strategy for some conifer species.

This talk will highlight where Scion has used this hybrid system to circumvent issues around certain protocol stages, cell lines and conifer species.

Keywords: Somatic embryogenesis, Organogenesis, Conversion, Recalcitrance, Hybrid protocol

ABSTRACTS OF ORAL PRESENTATIONS

SESSION 6: **Reducing socio-economic and environmental costs of plantation forestry.**

Erythrina crista-galli L. var. longiflora - *M.A. Zapater & E.C. Lozano* (CEIBO)

It is a tree of the family Leguminosae endemic of Argentina. Its bark is rough, has a large root development. Compound, trifoliate and opaque, with small prickles leaves. The vivid red flowers that are grouped in terminal inflorescences were designated as National Flower of Argentina. Its fruit is a dark legume.

KEY INVITED SPEAKER

The development and application of conifer tissue culture and somatic embryogenesis protocols in New Zealand: the *Pinus radiata* D. Don story

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Pinus radiata D. Don. is the dominant forestry species in New Zealand, where it occupies almost 90% of the current area of planted forests. Breeding programs are advanced and seed production well organized. A 2009 New Zealand nursery survey found that of the 37.7 million *P. radiata* tree stocks produced, 70% originated from control-pollinated seed (cuttings 25%, seedlings 45%). The remainder (30%) were produced from open-pollinated seed. New Zealand also supplies the Australian radiata pine seed market.

Propagation protocols to increase the availability of elite-cross material have been developed and tissue culture strategies have been tested and used commercially since the mid 1980's. The tissue culture work initially focused on adventitious shoot production for cotyledons of non-germinated control-pollinated seed. The advent of somatic embryogenesis (SE) in combination with cryogenic storage quickly superseded the earlier organogenic approaches. New and emerging technologies in forest-tree breeding include genomic selection. The potential to use this technology in combination with SE is attractive. Somatic embryogenesis is the underpinning technology for genetically modified *P. radiata* and potentially the pathway to producing rejuvenated planted stock directly from mature trees.

More recently, research and application efforts have been focused on integrating organogenic tissue culture protocols with SE and on current nursery practices. This presentation will focus briefly on some of the early propagation research at Scion, how these technologies were developed and how they are employed today not only in research initiatives but commercially in New Zealand.

Keywords: *Pinus radiata*, Somatic embryogenesis, Organogenesis, Commercial forestry

Progress on scale-up somatic embryogenesis and manufacture seed technology of conifer species at Weyerhaeuser

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Mass clonal propagation via somatic embryogenesis has been used for large number of horticultural and forestry species. Embryogenic cultures have also been used for mutation and genetic transformation. A large number of papers have been published on somatic embryo development, maturation, cryopreservation, germination and automation for several conifer species. Several patents have been granted to companies in the forest industries and universities on conifer somatic embryogenesis, automation and delivery system. Weyerhaeuser NR Company also has several patents on this technology. The implementation of this technology has already begun at Weyerhaeuser and several other forestry companies. Companies are testing the clones in the field and have been storing corresponding embryonal suspensor mass (ESM) in cryostorage. However, plantlets production via somatic embryogenesis is still expensive. At Weyerhaeuser, somatic embryo productions in bioreactors and delivery via manufactures seeds have been developed for Douglas-fir and loblolly pine. However, full commercialization is still limited due to the challenges to produce somatic embryos with zygotic-like quality. In this presentation we will discuss somatic embryo quality improvement and manufactured seed delivery using Weyerhaeuser patented technology.

Keywords: Somatic embryogenesis, Patents, Cryostorage

Challenges of bioreactor for large-scale eucalypt clonal propagation

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Bioreactor is a potential enabling tool for large-scale production of clonal elite plants in forestry. Progress in liquid culture bioreactor technology for eucalypt species are ongoing in both private and university laboratories around the world. Despite the increasing number of studies, the development of improved equipment, new directions and trends in academic and private sectors, all require further and continuous analysis. This work aims to review some challenges in the biofactory field to investigate its potential as a full-scale industrial clonal propagation system. Concurrently, traditional, large-scale clonal propagation methods have been continuously improved, resulting in a reliable, efficient system for commercial nurseries. As such new technology is becoming a more efficient alternative system for scale-up and automation, with very high productivity in micro-cuttings, the successful adoption of bioreactors for industrial and commercial applications still relies on several aspects. At the research level, mechanization, automation and robotics are industry megatrends being considered in the scale-up liquid culture process. Automation of part of the process is key to overcome the hurdle imposed by the existing conventional, labor-intensive methods. Harvest and quality assessment of micro-cuttings produced in bioreactor are major aspects that need advanced technologies related to robotics and automation by computerized image analysis. The efficient and successful exploitation of commercial micropropagation of clonal elite eucalypt species in bioreactor must be more competitive than the current operational mini-cuttings technology in terms of quality plant survival in the nursery. This could be achieved by improving the environmental conditions for rooting and acclimatization as well as by establishing micro-cuttings in the nursery. A major limitation in the development of this technology is the demand for constant evolution of nursery infrastructure, especially in homogeneity of irrigation and humidity systems in greenhouses that are key issues for the acclimatization of micro-cuttings. Research priorities should be given towards the refinement of the physiological parameters associated to aeration, gas exchange, flow rate, carbon enrichment, and culture vessel headspace rather than to the overexploited media composition and bioreactor designs. Understanding the effects of consumption and depletion of the various components throughout culture would pave the way for optimizing the biomass growth conditions and the eventual production of quality plants. This could be a significant shortcut to develop a robust protocol that fits most of the clones rather than developing genotype-specific solutions. Another challenge faced by large-scale liquid cultures is microbial contamination. The development of photoautotrophic protocols is a real opportunity to minimize this limitation. For the industry, the critical analyses of some aspects related to strategic technology and business expectations are equally relevant. Corporate technology plans should consider the time and resources needed to develop the process by experienced professionals in order to reduce pitfalls.
In conclusion, the combination of a simple, functional bioreactor and new technologies (such as automation, photoautotrophic cultures, improved nursery infrastructure and delivery systems) stands out as one of the required solutions for the cost-effective and efficient propagation for modern tree improvement programs and commercial nursery.

Keywords: Micropropagation; Micro-cuttings; Mini-cuttings; Nursery; Megatrends

Biofabrica Misiones S.A.: biotechnology accessible to producers

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Biofactory Misiones is a corporation, where the majority shareholder is the provincial government of Misiones. It has the vision to be a leader in the efficient application of technologies of massive vegetative propagation, the mission to leave biotechnology accessible to the producer and aims to vegetatively propagate plants of high genetic quality, increase yield per unit area and contribute to the sustainability of the production process. The production quality is certified since 2010 by the IRAM ISO 9001: 2008: *Design, development, large scale propagation and commercialization of in vitro plants, clonal plantlets and asexual seeds ensuring and excellent genetic and phytosanitary plant quality*. So all plant material is propagated with sanitary and quality assurance from its molecular certification by allowing regional agribusiness cluster increased yields and income.

It is located in the Mercosur, in the Argentine Northeast. The region is remarkable for the large water availability, large biodiversity and huge biomass production. Its economy is based on primary activities, such as forestry, floriculture, agriculture and horticulture with native and exotic species. Considering the production units, Misiones has the lowest average area per producer in Argentina. According to various censuses, about 80% of agricultural plots have less than 50 hectares and represent 40% of the rural population. This implies that agricultural policies must be aimed at diversified activities in small areas of land and to the grouping into larger entities (e.g. Producer cooperatives).

It was designed by a team of biotechnologists from University "Marta Abreu" from Las Villas, of Cuba; who transferred micropropagation technology, which was applied and adapted to local conditions, climate, genetics, laboratory, greenhouse and field crop systems. Temporary Immersion Systems were subsequently incorporated, expanding the production scale to more than 5 million plants. This alternative allowed to increase the multiplication coefficients, improved the outbreaks quality and production costs. It can be defined as a laboratory specialized in the vegetative propagation of plants from *in vitro* cultures, a nursery with the mission to acclimate the plantlets in *ex vitro* conditions and give to generated material in laboratory greater multiplication coefficient. Has an area of Technology Transfer that provides technical support, validates products and guides the producer with the establishment and management of biotech products; and an area of Research, Development and Innovation, whose objective is the generation, implementation and adjustment of new products, technologies and production processes. From here strategic linkages with universities, companies and laboratories in Argentina and abroad are made to strengthen networking, generate products or optimize processes. A second stage allowed the production of agricultural and industrial inputs, based on the use of microorganisms, low environmental impact bioinputs (bio-insecticides, bio-fungicides and growth stimulators). So Biofactory products are generated, giving priority to species of regional interest such as eucalyptus, cassava, pineapple, banana, sugar cane, orchids, heliconias, stevia, aromatic and medicinal plants, among others, dispatching until 2015 more than 9,000,000 clonal plantlets, which means more than 3.2 million U$D or more of AR$ 53 million, transferred into small holders in the form of forest, ornamental or agro-biotechnology products.

So since 2006, BIOMISA could exert a social-productive function, driving small producers to assume a productive role, as opposed to subsistence, through development, technology management, human resources qualification, operational flexibility, transfer, communication and awareness to producers and society of the benefits of biotechnology.

Keywords: Biotechnology, Tissue culture, Micropropagation, Biofactory, Clonal plantlets, Bioinputs

Mass production of self-rooted *Hevea brasiliensis* industrial clones by tissue culture and nursery methods

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Industrial clonal plantations of *Hevea brasiliensis* have been established for several decades with grafted/budded plants as an alternative to the mass clonal propagation of mature selected industrial clones on their own root system. Huge and long term investments have been devoted to the development of tissue culture techniques to reach this goal including somatic embryogenesis, micropropagation and to lesser extent propagation by rooted cuttings in nursery conditions. The recent mass production of self-rooted *Hevea brasiliensis* industrial clones prompted us to review these propagation methods, analyzing their respective effectiveness and limitations to meet large scale requirements of planting stock.

Keywords: *Hevea brasiliensis*, Mass production, Tissue culture, Rooted cuttings, Bud grafting, Industrial clones

KEY INVITED SPEAKER, CLOSING LECTURE

Current status of forest tree biotechnology in a changing climate

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Woody plants have been cultured *in vitro* since the 1930s. After that time much progress has been made in the culture of tissues, organs, cells, and protoplasts in tree species. Tree biotechnology has been making strides in clonal propagation by organogenesis and somatic embryogenesis. These regeneration studies have paved the way for gene transfer in forest trees. Transgenics from a number of forest tree species carrying a variety of recombinant genes that code for herbicide tolerance, pest resistance, lignin modification, increased woody biomass, and flowering control have been produced by *Agrobacterium*-mediated and biolistic methods, and some of them are undergoing confined field trials. Although relatively stable clonal propagules have been produced by organogenesis and somatic embryogenesis, as well as by genetic transformation in trees, there were also unintended unstable genetic events. Therefore, it is important to monitor phenotypic and genetic stability of clonal material, not just under greenhouse conditions, but also under natural field conditions. Genetically modified poplars have been commercialized in China, and eucalypts and loblolly pine are expected to be released for commercial deployment in USA. Clonal forestry and transgenic forestry have to cope with rapid global climate changes in the future. Climate change is impacting species distributions and is a significant threat to biodiversity. Therefore, it is important to deploy Strategies for the survival and evolution of forest tree species facing rapid climate change. In this direction, assisted migration and biotechnological approaches offer prospects for adaptation of forest trees to climate.

Keywords: Tree biotechnology, Clonal propagation, Gene transfer, Transgenic trees, Climate change, Conservation/adaptation strategies

ABSTRACTS OF POSTER PRESENTATIONS

SESSION 1: **Strategies for integration of vegetative propagation into breeding programmes in the context of global warming and associated stresses.**

Austrocedrus chilensis (D. Don) Pic. Serm. & Bizzarri (CIPRÉS DE LA CORDILLERA)

Evergreen tree endemic to the subantarctic forests of Chile and Argentina that adapts to many types of soils. Its leaves are scale-like and bark is rough with longitudinal fissures that emerges flake. The fruits are ovoid cones with woody scales and their seeds have a membranous wing which favors dispersion. The wood is very colorful, with yellowish white sapwood and heartwood yellowish brown.

"DendroMax" – a cornerstone to integrate biotechnology into traditional German forestry

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International efforts have advanced protocols for the system of somatic embryogenesis (sE) in woody plants not only to be applied as a tool for clonal propagation but also to accelerate current breeding strategies. These are fundamental prerequisites for the project "DendroMax", which aims at an operative integration of sE of coniferous timber species into established breeding programs. Specifically for hybrid larch *Larix x eurolepis* we are aspiring the proximate implementation within the public enterprise for forestry in the state of Saxony (Staatsbetrieb Sachsenforst). This entails a chain of operations: Controlled crossings between selected parental trees for sE-initiation, maintenance, storage and characterization of sE-material, plantlet production, acclimatization and transfer to nurseries and evaluation in field trials (for ecological adaptation, superior yield and wood characteristics). The challenges hereby not only lie in the incorporation of the entire process of sE into routine breeding procedures under economically competitive conditions, but also in the fulfillment of national guidelines and prevailing laws, e.g. regarding the marketability of newly developed varieties, particularly with respect to breeding for a modern and flexible forestry. The collaboration is supported by the Federal Ministry of Food and Agriculture (BMEL) in Germany and will allow public access to know-how and *in vitro* breeding material likewise, addressing foresters of both, the private and the public operating sector. Currently, field trials with clonal hybrid larch are being assessed in Saxony and represent first systematic plantations of sE-derived plants of *Larix x eurolepis* in Germany.

Keywords: Hybrid larch, *Larix x eurolepis*, Somatic embryogenesis, Clonal propagation, Forestry breeding, Field trials

Cloning cork oak trees selected as tolerant to *Phytophthora cinnamomi*

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Vegetative propagation is a main tool in forest breeding that captures all the genetic potential of selected trees and produces uniform offsprings. This way of propagation allow the simultaneous capture of different traits and therefore the transfer to the improved populations of not only better growth performance and product quality, but also tolerance to pests and diseases.

The cork oak (*Quercus suber* L.) is a Mediterranean tree species of ecological and economic interest. This oak sustainably produces cork, a renewable product used as stopper of high quality wine bottles, but also in other industrial applications. Acorns to fed cattle and gastronomically appreciated mushrooms are other products of interest. This species is threatened by a decline syndrome called "la seca" that is causing high mortality among cork and holm oak trees in the "dehesa" and "montado", agroforestry systems of Spain and Portugal.

Among the causes of this syndrome one of the most important is the infection by the oomycete *Phytophthora cinnamomi* Rands. The production of varieties tolerant to this pathogen could be one of the ways to address this problem. Clonal heritability of tolerance to *Phytophthora* sp. has been demonstrated in several species. In the framework of a project in which one of the objectives was to determine whether the tolerance trait can be transferred to cork oak clonal progenies, adult trees are being cloned by somatic embryogenesis (SE).

Ten trees were selected as tolerant on the basis that they were standing in a low-lying zone in which the oomycete was detected, and that the mortality of surrounding trees was high. Samples from five trees growing in the same area but at higher altitude without mortality in the mass were collected as controls. Following procedures previously established by our team, SE induction was performed in leaves from those trees. Embryogenic lines were obtained from 8 putative tolerant trees and from 4 control trees. Frequencies of induction varied with genotype, ranging from 2 to 74%. Proliferation was carried out by secondary embryogenesis. A decline of single embryo production with time in culture was observed, although a significant interaction with genotype was also recorded. Germination of somatic embryos was also influenced by genotype with mean frequency of 47% ranging from 16 to 65%. In order to increase the production of plants from the selected trees, multiplication by organogenesis from shoots of germinated somatic embryos as rejuvenated explants was accomplished. Rates of multiplication were dependent on genotype. The mean number of buds per nodal explant after 6 week of culture ranged from 2.5 to 9.5 and the mean number of shoots longer than 15 mm suitable for rooting from 0.3 to 2.6. Microshoots rooted after a 24 h treatment with IBA at frequencies between 10 and 47% depending on genotype. An effect of the length of the IBA treatment on rooting frequencies was recorded, doubling when it was increased to 48 h. Somatic embryogenesis has been a suitable tool to clone selected cork oak trees. Work is in progress to test the tolerance of the regenerated plants.

Keywords: Breeding, Forest biotechnology, Disease tolerance, *Quercus suber*, Somatic embryogenesis.

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Integrating vegetative propagation into conifer improvement programs in Mesopotamia Region, Argentina.

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More than the 90% of planted areas in the Mesopotamia region of Argentina are conifers. *Pinus taeda* is one of most important species. However, in the last years, the areas planted with *Pinus elliottii* var. *elliottii* (PEE) × *Pinus caribaea* var. *hondurensis* (PCH) have increased often due to their better performance. Currently, INTA is carrying out tree improvement programs for both taxa. Since late ´90s the improvement program of *P. taeda* was intensified up to nowadays that it is in its second cycle of breeding. While, for PEE x PCH F_1 hybrid, since 2001 economic restrictions for seed acquisition led that the National Institute of Agricultural Technology, through an agreement with PINDO S.A., implemented a program for their production and improvement.

In a general way, tree improvement involves three related phases: conservation, selection and breeding, and propagation. Breeding and selection are the central activities and involve formulation of mating designs, evaluation of resultant progenies, estimation of genetic parameters, and selection of desirable genotypes for propagation and further breeding for the next generation. It is only after reaching the propagation phase that we can realize potential gains from the previous activities. For the latter phase, time is the major constraint. Sexual maturity directly affects the rapidity in which generations can be created and therefore, the rate at each genetic material can be deployed in a field test program. This is the reason because of vegetative Propagation is one of the most important tool and widely used in tree improvement to manage breeding population more effectively. It has major advantage over sexual reproduction as a means of mass production. All the genetic components of “donor” plant can be captured and duplicated.

At the present, tree improvement programs of INTA take advantages from different techniques of vegetative propagation, from one side, to produce propagules more rapidly and to transfer them to farmers. But also, we applied clonal propagation because of it can increase the total amount of genetic gains that can be derived from a tree improvement program at any generation by using non-additive genetic variation.

Keywords: Loblolly pine, PEE x PCH F_1 hybrid, Tree improvement program, Vegetative propagation

ABSTRACTS OF POSTER PRESENTATIONS

SESSION 2: **Towards multivarietal/clonal forestry: environmental factors affecting vegetative propagation of trees.**

Araucaria araucana (Molina) K. Koch (PEHUEN)

Native of Chile and Argentina, which today is distributed in very restricted areas of the Cordillera de los Andes. It's found in rocky and sandy soil with good drainage (usually of volcanic origin). An evergreen tree, up to 50 meters high, with a straight, cylindrical trunk, sometimes very thick (3 or more meters). Its branches are arranged in whorls, extreme hardness leaves are strongly imbricated and have a terminal mucrón. There are female and male feet, the seeds ("Sprockets") have low viability, are edible and high nutritional value. It is a protected species.

Serial minicutting effect on productivity and morphological characteristics of *Toona ciliata* ministumps

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The Red cedar (*Toona ciliata*) stands out in the Brazilian forest sector due to its relatively short production cycle, good timber productivity and high marketing value. Studies show that the vegetative propagation of this species is feasible for commercial production as well as rescue and multiplication of adult genotypes with interesting features. However, the physiological age of the plant matrix may impact growth factors of the vegetative propagules, as these may have unfavorable morphological characteristics for the handling in minigardens. Studies suggests the use of serial minicutting to promote the strengthening of these materials and the induction of juvenile characteristics. This study aims to evaluate the productivity of ministumps and morphology of shoots in Red cedar clones submitted to the serial minicutting. In a clonal minigarden implanted with stakes of adult matrices, rescued after harvest, sprout of two clones were collected (TC9 and TC15). From the sprout, minicuttings with 5 cm in length, containing a leaf and a couple of leaflets reduced by 50%, were made. The minicuttings were placed in tubes of 280 cm^3, with commercial substrate based on pine bark and slow release fertilizer. Rooting was carried out under intermittent mist for 30 days. At the end of this period, they were transferred to the greenhouse and on the day 195 after staking the cuttings were transferred to suspend seedbed, where they had the apex pruned to 8 cm from the root collar, for the formation of the first subculture (SUB1) ministumps. Sprouts of SUB1 were collected to form the second subculture (SUB2) and these provided sprouts for subculture 3 (SUB3). Monthly evaluations on the productivity were carried out as sprouts and minicuttings number by ministump. To evaluate the morphology, 4 ministumps of each clone were selected and evaluated at 6, 7 and 8 months after the implantation of each subculture. The number of leaves and leaflets leaf area and dry mass were quantified in the harvested sprouts. Changes in the ministumps productivity or the biometric characteristics of the sprouts, due to three subcultures in a serial minicutting, were not observed in any case. This work will be continued to assess whether more subcultures may result in the reinvigoration of these clones and reestablishment of juvenile characteristics.

Keywords: Red Cedar, Vegetative propagation, Clonal mini garden

Seedlings production of *Toona ciliata* by serial minicutting

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This study aimed to evaluate the rooting of minicuttings and seedlings quality produced by serial minicutting and field rescue cultivation from adult strains in the field of three clones *Toona ciliata*. The minigarden was established in suspended seedbed with clonal seedlings produced from sprouts rescued got from strains of three matrices that suffered shallow cut (field rescue); with seedlings from clonal minigarden's minicuttings previous established, marking the subculture1(SUB1); and multiplied seedlings by serial minicutting, from SUB1 rooted minicuttings, marking the subculture 2 (SUB2). Successive sprouts collect were made on clonal minigarden with 30 days interval. On 30 days after the staking, the minicuttings were evaluated about the rooting percentage, number of first and second order roots and total root length. Base of fragments were collected from minicuttings of TC9 and TC15 clones for anatomical characterization. On 105 days after staking, the seedlings were evaluated about surviving, height, diameter at lap height, leaf area, shoot dry mass, number and length of first roots, total root length, roots dry mass and Dickson quality index. There were differences in the behavior of genetic material by the treatments. There was no positive effect of the two series of crops in rooting cuttings and quality of seedlings of the evaluated *Toona ciliata* clones. There were no anatomical barriers to rooting clones of the different types of farming evaluated.

Keywords: Australian cedar, Vegetative propagation, Reinvigoration

Factors influencing the *in vitro* acclimatization of plantlets of *Pinus radiata* D. Don originated from somatic embryos germination

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Ex vitro acclimatization is a critically phase of *Pinus radiata* plantlets produced through somatic embryogenesis pathway. With the aim of optimize this stage, it was analyzed the effect of light intensity, sugar presence, ventilation and CO_2 during the last phase of plantlets growing in laboratory, after germination of somatic embryos.

For this, it was evaluated the response of germinated plants, with respect to the intensity of light (50/90 $\mu mols^{-1}m^{-2}$), sucrose (0 / 20 gL^{-1}), CO_2 (with / without supplement) and container ventilation (with / without adhesive membranes with micropores). Plant material was placed in polypropylene boxes filled with substrate bark/perlite/peat autoclaved and moistened with WV5 medium without gelling in growth chamber. After 45 days, the plants were transferred to greenhouse conditions, was evaluated the number of plants with activation of growth after 120 days since its departure from the laboratory. For data analysis, a factorial design was used with n = 16 plants per treatment.

Main results show that exist significant interaction ($\alpha = 0.05$) between CO_2, light intensity and ventilation, establishing that when there was not container ventilation and there was not CO_2 supplementation, the presence of plants with apical activation growth at greenhouse was reduced from 62.5% (50 $\mu mols^{-1}m^{-2}$) to 37,5% (90 $\mu mols^{-1}m^{-2}$). Presence of sucrose showed a minor significant effect ($\alpha = 0.1$) comparing plants supplemented with 20 gL^{-1} of sucrose (53% of plants with apical growth activation) and plants without supplementation (42% of plants with apical growth activation).

As conclusion, apical growth activation at greenhouse it was determined by light intensity, CO_2 supplementation, and container ventilation at the last stage of growth at laboratory. Sucrose presence improves this parameter independent of light/ventilation/CO_2 supplement condition.

Keywords: Acclimatization, CO_2, Light intensity, Ventilation, Sucrose

Minicuttings production and nutritional requirement of three *Toona ciliata* var. australis clones ministumps

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Good nutritional status of the stock plant is essential for the maintenance of vegetative vigor and continuous minicuttings production. Therefore, the supply of nutrients in optimal amounts during handling of ministumps influences the success of propagation. The aim of this study was to evaluate and compare the productivity of minigarden and nutrients used by shoots of three *Toona ciliata* var. australis clones, and present the total nutrient consumption in the production period. The minigarden with TC3, TC9 and TC15 clones was established in suspend seedbed containing commercial substrate based on pine bark decomposed, coconut fiber and filter cake (2: 1: 1 v/v), in a greenhouse plastic cover (150 mM) and 30% shading. To form the mini garden, the cutllings of each clone had the apex pruned to 8 cm from the root collar and placed in suspend seedbed (0.15 x 0.15 m) arranged in a randomized block design (RBD) with six replications, and seven plants for repeat (42 ministumps/clone). In each sprouts harvest survival, the number of sprouts and cuttings produced by ministump, and monitoring of nutrients content exported were quantified in 18 harvests. At the end of 18 samples was conducted nutritional analysis of ministumps. The consumption curves for 432 days and the total amount of nutrients used in the process are shown. The ministumps survival ranges from 96.9 to 100%, indicating that this species is tolerant to apex pruning and sprouts successive crops. The TC3 clone was more productive, with greater efficiency in the conversion of nutrients absorbed to produce sprouts and minicuttings. From the 9th minicuttings harvest is indicated the replacement of nutrients aiming to maintain constant minigarden productivity. The nutrients most exported by sprouts of TC3 clones, TC9 and TC15 were, respectively, nitrogen (1239, 1905.7 and 1410.7 mg/ministump), potassium (1209, 1923.9 and 1314.3 mg/ministump), and calcium (612.9; 980.6 and 753.9 mg/ministump).

Keywords: Clonal minigarden; Nutrients; Red cedar

Ramin (*Gonystylus bancanus* Miq. Kurz) micro propagation: the endangered tropical trees

Putri, Asri Insiana

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Ramin (*Gonystylus bancanus* Miq. Kurtz) natural population has been decreasing sharply due to over exploitation, and nowadays has been leading to extinction. The lack of propagation technique information is among the reasons why there has not been enough initiation to develop ramin plantation yet. This research was conducted to ramin micro propagation study by Center of Forest Biotechnology and Tree Improvement (CFBTI), Forestry Research and Development Agency, Ministry of Forest in cooperation to Asia Pulp and Paper (APP). The aim of this study was the effect of growth media on the shoot initiation and growth of ramin in vitro. The ramin explants were from auxiliary shoot seedling. Research design of this study was used Complete Randomized Designed with 5 treatments and 50 replications. Five compositions of growth media used Murashige-Skoog media with benzyl-amino-purine (BAP), 6-furfurylaminopurine (kinetin/K) and napthalene-acetic-acid (NAA). BAP 3 mgl^{-1} : K 0,15 mgl^{-1} : NAA 0,5 mgl^{-1} (H1); BAP 3 mgl^{-1} : K 0,15 mgl^{-1} (H2); BAP 3 mgl^{-1} : NAA 0,5 mgl^{-1} (H3); K 0,15 mgl^{-1} : NAA 0,5 mgl^{-1} (H4) and control (H5). The result showed that medium H1 (1.79 $\pm$ 0.0141) that has BAP, NAA and Kinetin combination was significantly highest in proliferation coefficient. During 6 months' time periods, the percentage of axenic cultures was not significantly different for all medium treatments and it was very low. H1 (47.48 $\pm$ 0.0683) was also the highest in percentage initiated explants but not significantly different with H2. H1 (15.56 $\pm$ 0.2830) were not significantly different with H3 (17.67 $\pm$ 0.1694) and H4 (14.27 $\pm$ 0.2210) for percentage roots initiated, these all medium contain NAA.

Keywords: micro propagation, ramin, auxiliary shoots, axenic culture

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In vitro shoot induction and multiplication from nodal segments of *Austrochthamalia teyucuarensis* H. A. Keller

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Austrochthamalia teyucuarensis H. A. Keller is a species endemic to the province of Misiones, belonging to the Apocinaceae family, which blooms from September and lasts until February, and its fruit is a follicle. While the seeds show high germination rate (90%) and has high potential as an ornamental species, the only known population of the species has about 100 specimens, and is in an area of high anthropic incidence, being critically endangered. For this reason, the development of suitable propagation techniques to enable restoration and conservation ex situ is required.

The aim of this paper is to propose an alternative germination under controlled conditions and multiplication from nodal segments, using the *in vitro* culture techniques. The seeds were harvested in the Teyú Cuaré Spot, San Ignacio, Misiones (27 ° 16 '43.9' 'S - 55 ° 33' 44.9 " W) in June. Disinfection of seeds consisted of two disinfections steps (all in laminar flow chamber): 1) Immerse them in a peroxide solution of hydrogen (H_2O_2) 10 volumes for 30 minutes then in 70% alcohol for one minute and then transferred them to a solution of sodium hypochlorite (NaClO) with 1.5% Tween 20® (two drops) for 20 minutes. 2) Soaking in H_2O_2 10 volumes for 16 hours, then 70% alcohol for one minute and then transferred them to a 1.5% NaClO with two drops of Tween 20 for 20 minutes. The culture medium used for germination were the MS (Murashige and Skoog, 1962) and SH (Shenk and Hildebrandt, 1972), in their original concentrations, added 6 $g.L^{-1}$ of agar, free of sucrose and growth regulators. Two seeds were grown in 50 ml tubes, with 10 ml culture medium, incubated for 30 days at laboratory light conditions (116 $\mu mol.m^{-2}. S^{-1}$, PAR, photoperiod 14 hours) and controlled temperature (27 ± 2°C). Nodal segments, obtained from the *in vitro* germinated seedlings, cultured on MS medium supplemented with BA (0, 2.2, 4.4 and 8.8 µM), and incubated under the same conditions stated above for 45 days.

The results showed that the germination percentage was 100% in both media and the morphogenic process began two days after cultivation. Furthermore, the disinfectants and the disinfection process used were effective in the elimination of pathogens (fungi and bacteria). As multiplication treatments with 2.2 and 8.8 µM showed the best results with 3 ± 0.10 and 3.17 ± 0.95 shoots per explant, respectively.

In conclusion, we can state that the disinfections and culture media evaluated were appropriate to achieve the establishment and *in vitro* germination of mature embryos *A. teyucuarensis* HA Keller, and that BA induces growth of multiple shoots, generating an alternative reproduction method for *ex situ* conservation.

Keywords: Endemic, Apocinaceae, Seed, Shoots

The *in vitro* propagation of *Luma apiculata* (DC.) Burret, a tool to assist large plantations programs for this species

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Luma apiculata belongs to the Myrtaceae family and it endemic from Chile. The species yields a fruit with an attractive aroma and it has been used as medicinal plant. Nowadays, the species remains underutilized; however, it has a large potential considering its fruit properties and uses. Propagation methods for *L. apiculata* have not been studied in deep and still the plant is still propagated mainly by seeds, but if large clonal plantations from selected individuals are projected in the future it will be necessary to develop not only high efficient asexual propagation methods but also non expensive. This work was aimed to develop an *in vitro* propagation technology from nodal explants of *L. apiculata.* Vegetative buds grown during the spring season were disinfected with different concentrations of sodium chlorine during different times. The explants were cultivated on MS medium supplemented with benzylaminopurine (1.0 mgL^{-1}). Four weeks after planting nodal segments were harvested from the developed plants and multiplied on different plant growth regulators at different concentrations. After selecting the best propagation medium, rooting was evaluated by testing the effect of different concentrations of indolacetic acid and indolbutiric acid in the basal medium. Two rounds of disinfection with 2% sodium chlorine for 20 minutes gave the highest was the most efficient procedure for in vitro establishment. Meanwhile, the addition of 0.5 mgL^{-1} of BAP into the multiplication medium produced the highest propagation rates. Rooting was obtained in all the auxin treatments but not significant differences were obtained when compared the basal with not plant growth regulators. *Ex vitro* survival was highly improved when 100% peat was used as substrate. Giving a treatment with 3.0 mgL^{-1} of IAA gave the highest explants survival during the ex vitro step. Also, the presence of the *in vitro* arisen calli reduced plant survival and development.

Keywords: *In vitro* propagation, *Luma apiculata*, Aseptic nodal segments, Myrtaceae, Sodium hypochlorite

Induction of embryogenic tissue from apical meristems of loblolly pine

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Family forestry is currently implemented on a commercial scale in the province of Misiones; however, the genetic gains are lower than those obtained from clonal forestry. In this sense, the particularity of somatic embryogenesis technique to generate rejuvenated tissue from adult trees meristems results in appropriate technology to advance toward *Pinus sp.* clonal forestry. Our research focused on the development of embryogenic lines from apical meristems of juvenile and adult tissues of *Pinus taeda*. Different studies were carried out, such as: 1) *In vitro* establishment, which contemplate the pre-treatment of the donor plant sprouting and growing apical meristems from plants raised in pots, 2 and 4 years old, and grafts 18 years of age of loblolly pine. 2) Embryogenic induction from cross sections of 5mm thickness, obtained from sterile shoot apices of seedlings of 2 and 4 years old and graft established in vitro. Basic medium WV5 (W) described by Coke, et al. (1996) and the average DCR (D) described by Gupta and Durzan (1985) were used. The concentrations and combinations of growth regulators for both the pre-treatment, to the stages of initiation and maintenance, were the one described by Malabadi and van Staden (2006). The inductive pretreatment studied were: a) with inductive pre-treatment, the sterilized explants were cultured in pre-inductive medium, at 3°C, in darkness, for three days previously to be sub-cultured in the inductive medium and b) without inductive pre-treatment, explants were sterilized grown directly on inductive medium, at room temperature in darkness. The completely randomized design was used with a factorial arrangement of treatments with five replications with 10 explants each replicate in every tests conducted at each stage of study. The variables evaluated were number of surviving explants (vigorous and sterile)/treatment; number of induced explants/treatment; induction type and place, as well as cytological and histological observations.

The results, demonstrated, that the combination of the stock plant isolation, for at least 15 days, with weekly application of fungicide and bactericide, and the in vitro establishment provided immersing in a mixture of fungicidal and bactericidal solution, and subsequent immersion in an HCl solution (sodium hypochlorite), is suitable for a frequency above 80 ± 12% of explants survival. Moreover, the pre-cultivation of explants, at ° C, prior to culture them on inductive culture medium in the dark at 27°C, was a necessary step for the induction of embryogenic tissue, and ontogeny of the apical shoot used as explants, affects cytology and histology of the generated embryogenic tissue.

Keywords: *Pinus taeda*, *In vitro*, Histology

How to maintain embryogenic capacity of embryogenic lines initiated from Douglas-fir immature embryos

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Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco) is a native conifer from the Pacific North-West of the US and Canada, and is one of the most important timber species used in the world with both high productivity in a range of climatic conditions and highly valuable wood properties (quality) as well as strong tolerance to diseases and insects. In Europe, Douglas-fir is a major species for reforestation with increasing demand for its wood. Therefore, adaptation of new varieties to climate change and associated stresses is one challenging question for ongoing breeding programs. Efficient selection and vegetative propagation of improved varieties appeared to be key issues to maintain productivity in plantation forestry (Lelu-Walter *et al.* 2013). However, as in many other conifers, early maturation is preventing clonal forestry through conventional multiplication methods in Douglas-fir (Bastien *et al.* 2013). Somatic embryogenesis from immature seeds, coupled with cryopreservation, is a promising retroactive clonal propagation system of selected trees, currently developed for an increasing number of conifer species (Klimaszewska *et al.* 2016). Excluding patents, there are only a few published studies on Douglas-fir somatic embryogenesis. One recurrent problem is the sustainable multiplication of initiated embryogenic material, i.e. embryonal masses (EMs). Currently, EMs obtained after initiation are subcultured every two weeks in clumps on proliferation medium. Yellowish, non- embryogenic callus (NEC), which is interspersed with EM, is frequently observed during this process.

In this work we used high-resolution optical microscopy for cytological observation of three embryogenic lines initiated in 2012 (from different genotypes: D1, D2, D3) that were previously classified according to their macro-morphology as either "pure", white-translucent EM as typically described in conifer species (D1, D2) or as a mixture of EMs and NEC (D3). Using confocal microscopy with FDA/PI staining it was shown that EMs from a "pure" line (D2) are apparently interspersed with viable, persistent non-embryogenic cells of unknown origin. In addition, dead cells were observed in embryo suspensors from embryogenic lines suggesting actively occurring apoptotic and autophagic programmed cell death (PCD) that is required for normal embryo development. To tentatively reduce the occurrence of non-embryogenic cells, it is necessary to frequently subculture the EMs (each week), to vigorously dissociate them in liquid medium before transferring onto filter paper as a thin cell layer (Reeves *et al.* submitted).

Keywords: Douglas-fir, Somatic embryogenesis, Multiplication, Embryonal masses, Morphology, Cytology

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Induction of somatic embryogenesis in explants of shoot cultures established from adult holm oak trees

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Holm oak (*Quercus ilex* L.) is one of the commonest tree species in the Mediterranean basin. Acorns provide a rich source of nutrients for foraging animals (Iberian pigs, sheep, goats, and horses). In addition, the black truffle (one of the most expensive edible fungi in the world) is generated from a symbiotic relationship between the holm oak roots and the fungus *Tuber melanosporum*. Heavy mortality and decline of *Q. ilex* has occurred across the south east of the Iberian Peninsula due to a syndrome denominated in Spanish as "La seca". Propagation of disease-tolerant holm oak trees is therefore very important. However, traditional propagation by seeds and vegetative cuttings of this species is known to be recalcitrant. Micropropagation and somatic embryogenesis (SE) may be valuable tools when conventional propagation is difficult to achieve. The goal of the present study was to develop an efficient protocol for induction of SE in explants obtained from mature holm oak trees.

Axillary shoot proliferation cultures, previously established from two holm oak trees (both 100-year-old elite trees), were used as sources of initial explants for induction of SE. Shoot apex explants (2-2.5 mm long) and leaf explants (the most apical expanding leaves) were cultured on Murashige and Skoog (1962) medium without plant growth regulators or supplemented with different concentrations of naphthaleneacetic acid (NAA; 1, 2, 4 mg/l), indole-acetic acid (1, 2, 4 mg/l) and indole-3-butyric acid (1, 2, 4 mg/l) in combination with 6-benzylaminopurine (BA; 0.5 mg/l).

SE was induced in the shoot apex as well as in leaf explants of the two genotypes evaluated, although the rate of embryogenesis was influenced by the genotype, auxin and explant type. Shoot apex explants were more responsive to embryogenesis than leaf explants. The best results were obtained with apex explants of clone Q3 cultured on medium supplemented with NAA 4 plus BA 0.5 mg/l. The advantages of using shoot proliferation cultures as sources of explants for SE induction include the lack of need to sterilize the explants, all year round production of material at the same developmental stage and better control of the growing conditions of the stock material.

For proliferation of somatic embryos, proembryogenic masses were cultured on Schenk and Hildebrandt (1972) medium, yielding new cycles of somatic embryos every 5-6 weeks. Cotyledonary-stage embryos were isolated, placed in empty Petri dishes and stored at 4ºC for two months. After cold storage, somatic embryos were cultured on germination medium consisting of Gresshoff and Doy medium (1972) supplemented with 0.1 mg/L BA and 20µM silver thiosulphate. Conversion of embryos into plantlets was observed under these conditions.

Keywords: *Quercus ilex*, Leaf explants, NAA, *Phytophthora cinnamomi*, Shoot apex explants, Somatic embryo.

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Proliferation and rooting of chestnut under photoautotrophic conditions

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Photoautotrophic micropropagation consists of the elimination of exogenous sugar from nutritive media in order to promote photosynthetic activity in the cultures. Shoots propagated under photoautotrophic conditions are considered to be in a healthier physiological state and better adapted to acclimation than those cultured in the presence of sugar (Xiao et al. 2011).

In this study we used chestnut clones selected for their resistance to ink disease and propagated them under photoautotrophic conditions during both proliferation and adventitious rooting stages.

Explants were cultured in liquid medium in a continuous immersion system using bioreactors adapted in our laboratories from polypropylene food storage containers (Lock&Lock©). The bioreactors were equipped with 0.2 µm filters to receive forced ventilation with CO_2-enriched air and were placed under white light-emitting diodes (LEDs), which provided a photosynthetic photon flux density of 150 µmol m^{-2} s^{-1}.

For multiplication, explants were cultured in 10 L bioreactors with 1 L of Murashige and Skoog medium with half-strength nitrates supplemented with 0.05 mg/L N^6-benzyladenine (BA). Rockwool cubes of 1 cm^2 (Grodan©) were used as inert support material. Rooting was established in 16 L bioreactors containing 2.5 L of media devoid of plant growth regulators, and shoots were dipped in 1 g/L indole-3-butyric-acid (IBA) for 2 min before being inserted in rock wool blocks of different shapes and sizes.

Four chestnut genotypes were successfully micropropagated under photoautotrophic conditions. Basal and apical explants (3 cm of height and at least two expanded leaves) were aerated (1 min/hour) with air enriched with 2000 ppm of CO_2. In the first experiments, explants became necrotic when cultured in media devoid of sugar. This was subsequently prevented by pre-treatment with decreasing concentrations of sucrose for at least three subcultures.

For rooting and acclimation experiments, 15 clones were used. Vigorous shoots of 3-4 cm were treated with IBA and inserted in Kiemplugs or Blocks AO de Grodan®. In several experiments using more than 6000 shoots, mean values of rooting of 71 and 56 % were obtained respectively for Blocks and Kiemplugs. The CO_2 levels of 1200-1500 ppm were suitable for root development and shoot growth, and high survival rates were observed after transplanting. More than 7000 shoots rooted under photoautotrophic conditions were successfully acclimated.

Keywords: Bioreactors, Chestnut, Continuous immersion, LEDs, Liquid culture

Reference: Xiao, Y., Niu, G., Kozai, T. 2011. Development and application of photoautotrophic micropropagation plant system. Plant Cell Tiss.Organ Cult. 105: 149–158.

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Preliminary study of *in vitro* propagation of *Austrocedrus chilensis*

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Austrocedrus chilensis (Patagonian cypress) is an endemic tree belonging to the Cupressaceae family found in Southern Argentina and Chile, across 140 000 ha in a wide variety of ecological niches and different soil types. In Argentina, it grows in a 60 to 80 km wide strip along the Andean foothills across a broad moisture gradient. In the west, *A. chilensis* can be found either in mixed stands with *Nothofagus spp*. or in pure stands on dryer sites. In the north, it can be found mixed with *Araucaria araucana.* It also grows in open, xeric forests or in isolated clumps at the limit of the Andean forest and the Patagonian steppe, acting as a barrier against desert advance. *A. chilensis* is valued not only because of its ecological function, also is one of the few native tree species with high potential to be planted for timber production. It grows relatively fast and the wood has been widely used since it is quite stable and appealing. *Phytophthora austrocedrei* is a recently discovered pathogen that causes severe mortality of *A. chilensis.* Mortality was first registered in 1948 and the cause remained unknown until recently, which generated a deleterious effect on the native forests, which led the species to a serious threat of conservation. Individuals with different degrees of susceptibility to the pathogen are generally observed in affected areas. Since factors associated with the spread of the disease are difficult to control, detection and reproduction of tolerant/resistant individuals seems to be the best solution to the problem. At present, little work with almost no success regarding agamic reproduction of the cypress was done. The aim of this study was to contribute to the development of a micropropagation protocol. Seeds were collected from an open pollinated natural stands. Before use they were pre-chilled for 70 days. Seeds were submerged in 70% ethanol for seconds, and then the asepsis was performed with 25% sodium hypochlorite. After asepsis, seeds were stored in 1% w/v H_2O_2 to stimulate germination during 30 days. Embryos were extracted from the germinated seeds and incubated in a growth chamber at 24±2°C under a photoperiod of 16 h of cold light at a light intensity of 60µmol•m^{-2}•s^{-1}. In the establishment stage the induction media employed was QL medium supplemented with the following combination of the growth regulators: Indole 3-butiric acid (IBA) (0.1 mg/l) and 6- Benzylaminopurine (BA) (1.5 mg/l). For callus induction QL was supplemented with 2,4-Dichlorophenoxyacetc acid (2,4-D; 3.0 mg/l). In all cases sucrose (30 g/l) and agar (8 g/l) were added, pH was adjusted to 5.70 ± 0.05, and media were sterilized by autoclaving. After 30 days of culture, the aseptic procedure performed in the laboratory was effective, showing low contamination (6.25%). Morphogenesis on explants was not induced in the media employed, with the balance and combination of growth regulators used. It is known that different proportions of cytokinins and auxins induce distinct responses according to their natural control in each plant. This was a preliminary study, new studies are necessary to determine the best balance of growth regulators, explant type, and culture conditions to induce organogenic or embryogenic tissue on explants of *A. chilensis.*

Keywords: Cypress, Micropropagation, *Phytophtora austrocedrei*

Ex vitro rooting in *Acacia crassicarpa* micropropagation

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The *Acacia crassicarpa* is the most planted species for pulpwood in Indonesia and active breeding programs are ongoing to improve productivity. Elite genetic material is propagated *in vitro* by microcuttings and protocol has been successfully achieved. Nevertheless, adjustment of the technique is important in large scale propagation. *Ex vitro* rooting in the nursery of shoots produced in the laboratory is an alternative step to simplify the procedure. A feasible method for *ex vitro* rooting was tested and results are hereby presented.

The two main factors analyzed were *A. crassicarpa* family and the time between cutting preparation in the laboratory and transplanting in the nursery for *ex vitro* rooting. Randomized complete design with 4 families of *A. crassicarpa* and 5 time intervals between cutting preparation and transplanting in the nursery (0 h, 24 h, 48 h, 72 h, 96 h) were contrasted with the control *in vitro* rooting. Parameters of plant survival in the nursery, stem height, stem diameter and number of main roots were assessed. The results show that the two factors have significant effects in the *ex vitro* rooting output. Family affects plant survival, stem diameter and number of main roots, but not stem height. The factor time interval also shows significant differences between control and time intervals longer than 24 hours. If the time of transition from the controlled environmental conditions in the laboratory to transplanting does not exceed the 24 hours, set *ex vitro* rooting shows that plants have no significant differences in comparison to the control for survival, stem diameter and height. These results indicate that *in vitro* rooting could be replaced by *ex vitro* without disadvantages in the plant propagation success. Furthermore, the simplicity in the procedure for *A. crassicarpa* large scale propagation is associated with additional benefits, such as cost reduction.

Keywords: Microcutting, Vegetative propagation, Rooting of cuttings

Interaction effects of cytokinin type and 2,4-D levels on callus induction from inflorescences of the giant bamboo (*Dendrocalamus asper* (Schult. & Schult. F.) Backer ex K. Heyne

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Woody bamboos are perennial plants of great importance as genetic resources for sustainable forestry use due to fast growing, annual culms harvesting, and also for environmental services and climate change mitigation. *Dendrocalamus asper* is a tropical sympodial bamboo with economic importance for its high quality timber and edible sweet shoots. Furthermore, it presents potential as energetic biomass source, for fiber and pulp industry, civil construction, and value-added engineered products, such as laminated, composites and fiberboards. However, large scale conventional bamboo propagation is difficult due to seasonal dependence, large propagule size, and low rates of rooting and survival of cuttings. Moreover, seed propagation has low practical applicability because of short-term seed viability and the long and unpredictable vegetative cycle that can take decades. Tissue culture techniques can be reliable alternatives as they allow the mass production of healthy clones from elite genotypes. An important bottleneck for the micropropagation of bamboos is the high level of associated microorganisms which result in culture media contamination. Inflorescences are important for tissue culture research due to less endogenous contamination and higher morphogenetic response rates, especially for somatic embryogenesis. The aim of the present work was to test different cytokinins combined with different levels of 2,4-D on callus induction from *D. asper* immature inflorescences. The culture media were composed of MS basal salts with 30 $g.L^{-1}$ sucrose, 250 $mg.L^{-1}$ PVPP, Morel vitamins; the pH adjusted to 5.8. The culture media were supplemented with 9.3 µM of either 2iP or KIN, combined with increasing concentrations of 2,4-D (0, 9, 18, 27 and 36 µM). The experiment was arranged in a completely randomized design, with ten treatments and 40 explants per treatment. A three-step disinfestation procedure with 2% NaOCl and 70 °GL ethanol was carried out before *in vitro* inoculation. The cultures were incubated at 25 °C in a dark BOD chamber. Contamination, oxidation and callus induction (CI) rates were evaluated after ten weeks. Data were analyzed with binomial regression using R statistical environment. Contamination rates were low (7%), and corresponded only to bacterial occurrence. Oxidation rates were dependent on the type of cytokinin used and on the concentration of 2,4-D. Combined with 2iP, increasing 2,4-D concentrations decreased oxidation rates from 90% on 2,4-D absence, to 16% on the highest concentration. On KIN containing treatments oxidation rates were stable across 2,4-D concentrations, varying from 62% to 51%. CI was dependent on the presence of auxin in culture media, and increased proportionally to the increase on 2,4-D levels, and 2iP containing media showed higher rates of CI as compared to KIN. The culture medium with 9.3 µM 2iP and 36 µM 2,4-D was the most suitable for CI on *D. asper* immature inflorescences, showing the highest CI (82%) and the lowest oxidation (16%) rates.

Keywords: Micropropagation, Sympodial bamboo, Immature inflorescences, 2iP, Kinetin, 2,4-D

Influence of season on minicutting rooting of *Prosopis alba*

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During last years, the interest on foresting arid and semi-arid zones using species of the genus *Prosopis* has increased. Several cloning techniques has been assessed in order to enhance the productivity, quality and uniformity of the material used in the industry, through clonal silviculture as well as reducing cutting turn frequency.

White mesquite presents a high potential for the forestry materials production, which are traditionally and intensively used. Since *P. alba* is nowadays propagated by seeds, it exists a high interest on obtaining new propagation technologies for this species. The season in which woody species propagules are collected exerts an influence on minicutting rooting, in some cases. The aim of this work was to evaluate the effect of season on minicutting rooting of *P. alba*. For this purpose, different indol butyric acid (IBA) concentrations were used: 0, 3.000, 4.500, 6.000 and 7.500 mg L^{-1} dissolved in 1M potassium hydroxide. The present work demonstrated that the season in which *P. alba* minicuttings are collected affects their rooting. Minicuttings collected in spring reached between 98 – 100% of rooting, after 40 days in rooting chamber, at the assessed concentrations, meanwhile minicuttings collected in autumn did not root during the evaluation period.

Keywords: Vegetative propagation, Mesquite, IBA, Species of difficult rooting, Cloning of native species

Adventitious bud regeneration and plantlets production of *Balfourodendron riedelianum* (Engl.) Engl.

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Balfourodendron riedelianum (Rutaceae) is distributed between the latitudes of 10° S and 30º S comprising south of Brazil and northern of Argentina. Due to the high quality, its wood is used in luxury furniture, construction and carpentry in general, triggered the overexploitation of the natural resource. Actually, *B. riedelianum* is included in the category of "endangered species" from the Red Book of the International Union for Conservation of Nature.

In order to develop a protocol for adventitious bud formation and large-scale plant production of *Balfourodendron riedelianum*, hypocotyls and cotyledons obtained from the *in vitro* germination of seeds were cultured on Murashige and Skoog (MS) semisolid medium (Phytagel®, 3.5 $g \cdot L^{-1}$), plus sucrose 30 $g \cdot L^{-1}$ and containing different combinations of α-naphtalenacetic acid (NAA, 0.05 µM), 6-benzyladenine (BA, 0.44 µM), and thidiazuron (TDZ, 0.004-0.4 µM). The cultures were incubated at 27 ± 2ºC and photoperiod 12 h (116 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) for 30 days. Therefore, the regenerative explants were transferred to bioreactors containing 100 mL MS plus gibberellic acid (GA_3, 0.5; 1 µM), BA (0.8, 1.5 µM) for elongation for 30 days. Consequently, the elongated shoots were rooted in MS semisolid (agar 0.65%) medium with either indole-3- butyric acid (IBA, 2.5, 5, 7 µM) or NAA (0.5, 1.4, 2.7 µM) under the environmental conditions described above.

The regeneration frequency (40±10%) and the number of adventitious buds per responsive explants (8 ± 6.15) was greater when the cotyledons explants were cultured in MS plus NAA 0.05 and TDZ 0.04 µM. During the elongation phase, 48.79 ± 4.01% of shoots grown in MS plus BA 0.8 µM provided shoot with more than 5 mm in length. All plantlets raised *in vitro* were phenotypically normal and successfully hardened to *ex vitro* conditions.

Keywords: *Balfourodendron*, Cotyledon, Hypocotyl, Temporary Immersion

Somatic embryogenesis of *Beaucarnea inermis* (Asparagaceae), threatened northeastern Mexico

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Beaucarnea inermis (Watson) Rose, (Asparagaceae), monocot, dioecious tree up to 13 m tall, the trunk base widened shaped balloon, which stores water, tapering to the neck, curved leaves, is known locally as "soyate". Register slow growth, reaching maturation represents several decades of life. It is endemic to northeastern Mexico and is distributed in the states of Tamaulipas and San Luis Potosi, is threatened by illegal collection of seeds, juvenile and adult plants, recorded a high ornamental value inside and outside the country, the price per plant potentially adult, with sizes of 60 cm at the base and 2 to 3 m high, ranging at $ 500.00 us in the regional market, it is therefore part of private collections in residential houses and luxury restaurant. The objective of this work was the establishment of micropropagation of *Beaucarnea inermis* through somatic embryogenesis. The initial explants were seeds from ripe fruit of six wild plants *B. inermis*, from the tropical dry forest in the municipality of Ocampo, Tamaulipas, Mexico. The seeds were disinfected in 70% ethyl alcohol for one min, then a solution of 30% commercial bleach for 30 min. Finally, seeds were rinsed three times with sterile distilled water. They were sown in basal culture medium Murashige and Skoog (MS). Explants were used in plants germinated 90 days. To induce micropropagation through somatic embryogenesis, five types of plant explants, apical, middle and basal blade section, stem apex and previously formed callus were used. Somatic embryogenesis was generated in the culture medium B5 modified with 2,4-D and KIN, incubated at 24 h of darkness. Five types of explants, apex, middle and basal leaf segment and apex of stem and callus previously generated in the presence of 2 were grown, 4-D. Response was recorded only in the basal leaf explants in apex of stem and callus. 2.2 and 3.6 respectively somatic embryos, both in the presence of 2 mg L^{-1} 2,4-D + 0.5 mg L^{-1} were generated KIN. The apex of the outbreak, recorded 6.8 somatic embryos with 4 mg L^{-1} 2,4-D + 0.5 mg L^{-1} KIN; an average of 3.6 somatic embryos was induced when the medium was added 2 mg L^{-1} 2,4-D + 0.5 mg L^{-1} KIN + 60 g L^{-1} sucrose. The presence of light and 2,4-D in callus derived to rhizogenesis in all treatments, inhibiting response somatic embryogenesis. Somatic embryos to be subcultured in fresh medium MS basal, germinated and plants developed normal. It was recorded up to 100% survival 90 days after transplantation under greenhouse conditions. In system of thickening of the stem base it behaved normally, appearing from the early stages of development. The protocol set is presented as an alternative propagation of plants to meet the demand for ornamental use.

Keywords: Endemic, Micropropagation, Seeds, Soyate

Effect of folic acid and culture medium in somatic embryogenesis of *Pinus caribaea* var. hondurensis

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Somatic embryogenesis is a tissue culture technique which consists of the embryo formation from somatic cells, in a similar process to that of zygotic embryogenesis. The advantages of the technique are mass propagation of elite clones, cryopreservation of embryogenic tissues, and *ex situ* conservation of germplasm of endangered species. This study aimed to evaluate the effect of folic acid on the embryogenic potential of megagametophytes of *Pinus caribaea* var. *hondurensis* on different culture media. Explants were placed on induction medium containing QL, DCR or WV5 salts and vitamins, sucrose, myo-inositol, glutamine, casein hydrolysate, 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP) and agar, with or without the addition of 100 mg L^{-1} of folic acid (FA) in Petri dishes. The explants were incubated in the dark at 23 ± 2°C, being transferred to new fresh medium every 21 days. After 90 days the explants were evaluated regarding the percent of embryogenic callus by staining with acetic acid and Evans blue. After 120 days, we evaluated the calli for the presence of somatic embryos. The treatments were compared by Tukey test ($p < 0.05$). The coloring test indicated the presence of embryogenic callus in all treatments, and the formation of pro-embryos were observed under the microscope. The percentage of embryogenic callus was higher in all treatments containing FA (up to 48% on QL + FA). However, the percentage in the media DCR and WV5 with AF were not statistically different from the QL without folic acid, with 26% of somatic embryogenesis. After 120 days, Somatic Embryos-like structures were observed on media with FA and on QL medium without FA. The largest number of calli with structures (10%) was observed on QL medium + AF. The largest average of structures per embryogenic callus was observed on both QL media with or without FA (5). Histological analyzes are underway to prove if the structures are somatic embryos. Although they do not present vascularization with the callus, a macroscopic structure points to abnormal embryos.

Keywords: QL, WV5, DCR, Megagametophyte

Propagation of ornamental forms of Norway spruce (*Picea abies* L. Karst) using rooted cuttings and chip-budding

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Among the normal trees in our forests there are, as rare whims of nature, a variety of peculiar tree forms: spruces with globular crown, golden and red needle trees, and narrow-crowned, weeping spruces. The phenomenon is caused by a mutation, i.e. a change in genetic material. The change in genetic code can take place in gametes prior to fertilization, or in vegetative buds.

Some of the genetically changed forms of trees are very decorative and can be utilized as ornamental trees. The utilization of special ornamental tree forms usually requires vegetative propagation. In this way, the traits of the parent tree are passed as such on to the cloned offspring. Methods used include grafting, rooted cuttings and tissue culture.

Grafting is the most commonly used method when the special tree forms, found in the nature, have been propagated to clone archives or arboretums. However, also in grafting there are different kind of methods, and chances for improvement. Scion grafting (either as side or slice grafts) is in most cases applicable method, but work and material consuming. For bud grafting (i.e. grafting of adventitious buds with a chip of wood = chip budding) less material is needed, and in some cases, when original material has very small scions, bud grafting could be suitable method.

Cutting propagation can also be applied in spruce. Rooted cuttings have, however, not been used in practice for propagation of ornamental forms, at least in our Northern conditions. For the present, there is no cutting propagation method efficient enough for affordable production of ornamental forms. The rooting success is influenced by many factors: the taxa, donor age, timing of propagation, rooting substrates (Nikkanen et al. 2013).

In this work both bud grafting and rooted cutting methods are applied for various ornamental forms of Norway spruce. Preliminary results showing differences in propagation success between the methods, and between forms and clones are shown.

Keywords: *Picea abies*, Ornamental forms, Grafting, Rooted cuttings, Landscaping

Reference:

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Shoot proliferation and organogenesis on strawberry tree (*Arbutus unedo* L.): a physiological analysis under water stress

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Strawberry tree (*Arbutus unedo*) is a small perennial tree that grows spontaneously in several countries of the Mediterranean basin, from Spain to Turkey, as well as in North Africa, in Mediterranean islands and Atlantic coast, including Ireland and Portugal. It is a very important species in Mediterranean ecosystems, as its regenerate after forest fires avoiding erosion and helping recover marginal lands. Strawberry tree is also a very attractive ornamental and can be used for honey production. The fruit is a spherical edible berry that is commonly used in the manufacture of traditional products such as jam and jelly. However, its main application is the production of an alcoholic distillate. Once considered a "Neglected or Underutilized Crop" (www.cropsforthefuture.org/), the demanding for strawberry tree by producers and stakeholders is increasing, as an alternative to other forest species, such as pine and eucalyptus, that are suffering from several diseases. In order to make this species more attractive, an intensive propagation and breeding program is being carried out.

Clones were established *in vitro* through epicormic shoots and micropropagated on solid and liquid medium on a modified De Fossard medium, supplemented with 2 mg/L BAP (benzylaminopurine) as well as through organogenesis from a *calli* obtained from shoots or leaves on a medium supplemented with TDZ (thidiazuron) or BAP. Shoots where rooted with IBA (indole-3-butyric acid) and acclimatized (rates higher than 90%). Due to some phenotypic changes observed on plants produced in liquid medium and through organogenesis, as well as lower levels of chlorophyll, it is essential to assure plant quality and drought tolerance of the micropropagated plants, especially on a changing climate context. For this purpose, plants produced *in vitro* through different methods were submitted to drought stress and several physiological parameters evaluated. Six month-old plants obtained by the three methods of *in vitro* cloning were submitted to different water regimes: WW (10ml water/plant/day during 10 days, and 10ml water each 3 days for 10 days) or WS (5 ml water/plant/day for 10 days, followed by 10 days without water). After this period several physiological and morphological parameters were evaluated. No significant differences have been found in terms of plant biomass, plant size, leaf area, rooting system development or chlorophyll a and b levels. Moreover, most of the physiological parameters evaluated showed to be very similar both between WW and WS regimes and in the different cloning methods. No statistical differences were found in terms of transpiration rates (E) and stress does not seems to significantly affect photosystem II (Fv/Fm). No marked differences were found on CO_2 assimilation rates (A), relative water content (RWC) and intercellular CO_2 concentration (ci). However, water potential was lower on plants obtained on liquid medium and trough organogenesis when submitted to water stress. Stomatal conductance (gs) was also affected on WS plants from the three propagation methods. Overall, the behavior of strawberry tree micropropagated plants produced by different propagation methods was very similar and plants were not strongly affected under drought stress.

Keywords: Drought, *In vitro*, Micropropagation, Physiology, Stress

Vegetative propagation of *Cordia trichotoma, Cabralea canjerana* and *Picrasma crenata* species with potential for productive diversification.

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Misiones has around 1.700.000 hectares of native forest, it belongs to restricted areas of national, provincial or private reserve. Due to the large exploitation of forests, it produces a steady decline in raw material supply, and timber products. There are native species of great economic and social value in the Misiones rainforest such as *Cordia trichotoma* (black parrot), *Cabralea canjerana* (cancharana), *Picrasma crenata* (Palo bitter), which they have been part of deforestation and, selective use of the best individuals, resulting in a reduction in the size of populations in natural conditions, and adversely affect the genetic makeup of the two first species of then.

Previous work carried out by INTA (*National Agricultural Technology Institute*) identified individuals with desirable characteristics from the forestry point of view, fruiting period, pre- germination seed treatment and viverización of these species.

It is necessary to seek alternatives for propagation of these species to increase the availability of plants. An effective and potential tool to carry out multiplication by asexual way is the macropropagation. Favoring minimize the exploitation of native forests, generating a sustainable balance both in economic terms, ecological and social. This the objective was studied different technics of macropropagation.

For the vegetative propagation, were used selected young trees got by seed germination, which were decapitated, to allow lateral bud outbreak. The new cuttings were used for the experiment rooting. Proceeded to disinfect the cuttings with fungicide (Captan 2 g/L). Treatments consisted of assessing the response to exposure to different concentrations of hormone IBA. The cuttings set in containers (trays and tubes), with mixture of substrate (50% composted pine bark + 50% lateritic soil) and slow- release fertilizer (NPK plus micronutrients). The experiment set under greenhouse conditions with controlled irrigation. These experiments were conducted in the laboratory and field Annex Laharrague EEA Montecarlo (INTA). A randomized design with 3 replications (plots) was used each species. The results were interpreted statistically by Analysis of Variance using Mixed Models.

In cancharana it was observed that the highest percentages of rooting were obtained in apical cuttings 95.8% (IBA 0 ppm) and 91.6% (1000 ppm IBA) on subapicals apical cuttings 33.3% (IBA 0 ppm) and 29% (IBA 500 ppm). In black parrot, the best rooting percentages were observed with doses of IBA from 0 of 1000 ppm. yielding 96% values for the case of apical cuttings, not significant difference from subapicals, since they were about 80% rooting for the same concentrations of IBA. In the case of post bitter, the best results were obtained when cuttings were treated with IBA 2500 ppm powder formulation prepared with inert powder (45%) when treated IBA in formulation with inert gel at a concentration of 3000 ppm (40%). Concluding that it is possible the clonal multiplication of these species, it is important to know behavior of cuttings trees against the use of growth regulators such as IBA and concentrations, as it could increase the number of rooted plants.

Keywords: Macropropagation, Native forest, Conservation

Direct shoot regeneration from hypocotyls and cotyledon segments of *Eucalyptus nitens*: Effect of light irradiance during the *in vitro* germination of donor plants

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Eucalyptus nitens Maiden (shining gum) is a frost-tolerant species that can be used as an alternative species of *Eucalyptus* in some regions where winter temperatures are too low. Unlike most eucalypts, *E. nitens* is a shy seed bearer. This study was aimed at propagating *Eucalyptus nitens* using organogenesis.

To developed an efficient protocol of shoots bud regeneration, *in vitro* germination of seeds was achieved on Murashige and Skoog (1962) semisolid medium (agar Sigma-Aldrich® A-1296, 6.5 $g \cdot L^{-1}$) plus sucrose 30 $g \cdot L^{-1}$ at 27±1/24±1°C (day/night) temperature and subjected to darkness or indirect white light (58 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, 12 h photoperiod) for 6, 10 and 30 days. Subsequently, hypocotyls and cotyledons explants were collected and cultured on a fresh medium of similar composition and supplemented with indoleacetic acid (IBA, 1 µM) and 6-benzyladenine (BA, 2.21 µM). The cultures were incubated under controlled light (118 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) and temperature (27±1/24±1°C) conditions.

A completely randomized experimental design was used for the experiment, with three repetitions and an experimental unit of ten cultured explants. The results were expressed as a percentage of newly formed vegetative buds per explant. The results were statistically analyzed by analysis of variance (ANOVA) and Tukey test (α=0.05) using statistical software Infostat.

After 30 days of incubation, 16.7 to 40% of the explants subjected to a short period of darkness become browning and death. However, the oxidative process was higher from the explants grown under indirect light radiation ranging from 60 to 86.7%. A direct pattern formation of adventitious buds was observed from both tested explants. The best results were obtained using hypocotyls collected from seedlings grown in darkness for 10 days; in which, 40±10% of the explants show multiples shoots without callus proliferation.

Keywords:

In vitro propagation of *Pinus taeda* via direct organogenesis from mature zygotic embryos

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Plant regeneration from *in vitro* culture of certain woody species requires look for complex techniques. Organogenesis and somatic embryogenesis have been considered the *in vitro* system of choice for the potential mass propagation of superior genotypes of forest species. Therefore, the aim of this work was to obtain an optimal combination of cytokinins on regeneration and production of buds from mature zygotic embryos of *Pinus taeda*.

Mature zygotic seed embryos were cultured, after being scarified by agitation in H_2O_2 30 vol. for 12 hours, disinfected by immersion in a 70° ethanol solution for 1 minute, then transferred to a 2.2% sodium hypochlorite aqueous solution added with 0.1% TRITON® and finally rinsed three times with sterile distilled water. The experiment used Murashige and Skoog basal medium (1962), with 30 $g.L^{-1}$ sucrose and 6.5 $g.L^{-1}$ Agar, in addition to 0.45, 5, 10, 13.6 µM of Thidiazuron (TDZ) and 0.45, 4.44, 9 y 13.31 µM of 6-benzylaminopurine (BA) alone or combined. The culture media were sterilized at 1.45 $Kg.cm^{-2}$ for 20 minutes. The explants were cultured in laminar flow and incubated under light (116 µmol m^{-2} s^{-1}, 14 h photoperiod) and temperature (27 ± 2°C). Measurements were performed at 30, 45 and 60 days. A completely randomized experimental design was used, with five repetitions and an experimental unit of ten explants. The results were expressed as regeneration rate, oxidation rate of and number of buds per explant. The results were statistically analyzed by analysis of variance (ANOVA) and Tukey test ($\alpha = 0.05$).

Even though bud regeneration was found associated with the presence of BA in the basal medium, there were no significant differences between concentrations of 4.44 µM and 13.31 µM with 56 ± 8.94% and 44 ± 15.2% respectively, although a concentration of 0.45 µM registered a lower regeneration of buds (10±6%). As to the number of buds per explants, it was higher in concentrations of 4.44 µM (9.39±2.25) and 13.33 µM BA (10.88 ± 7.18). In the same way, the oxidation rate was not affected by the presence of this cytokinin. The highest concentrations of TDZ had a negative effect in regeneration and number of buds per explants; with 0.45 µM the regeneration rate was 40.67±11.63% and the number of buds per explants was 10.5 ± 3, while with increased concentration both parameters decreased. The oxidation rate was affected too with the increase in the concentration of TDZ, from 34.6 ± 12.46% with 0.45 µM to 65 ± 10.49% 9 µM, and not being significant compared to 13.6 µM (63.33 ± 12.11%). As for the incubation time, the number of buds per explant was significantly superior at 45 days (10.11 ± 2.8 buds/explant) compared to 30 days, while there was no significant difference at 60 days (9.1 ± 3.5 buds/explant). Regarding the oxidation rate, it was significantly superior at 45 days (29.5 ± 14.3 %), compared to 60 days of incubation (40.5 ± 17.3%).

In conclusion, 4.44 µM BA presented the highest regeneration rate, while the highest number of buds per explant was registered at 45 days of incubation, concurring with a lower oxidation rate.

Keywords: *Pinus taeda*, Regeneration, Cytokinin

Possibilities of somatic embryogenesis for production of hybrid pine and loblolly pine

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Clonal propagation allows to increase productivity of forest plantations by capturing non-additive genetic effects. In conifers, somatic embryogenesis is an alternative technique for cloning of genetically improved trees.

In this study, immature seeds from controlled crosses from loblolly pine and *Pinus elliottii* x *caribaea* var. *hondurensis* F1 hybrid were used to determine the most appropriate culture medium in the stages of induction, multiplication and maturation of embryogenic masses. Since maturation state of zygotic embryo determines the success of induction process and restricts the harvest window, it was also necessary to follow it before in vitro culture. Then, cones were harvested weekly between November and January and five culture media were tested.

Among other results it was determined that the optimum window for harvest immature embryos occurs in the F1 hybrid two to three weeks earlier than in *P. taeda*. However, the culture medium for induction, multiplication and maturation phases were similar for both species.

Keywords: Somatic embryogenesis, *Pinus taeda*, Hybrid, Megagametophyte

Histological analysis of somatic embryogenesis of *Melia azedarach* and *Prosopis alpataco.*

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Histological methods contribute significantly to the understanding of *in-vitro* tissue culture systems, since they provide information since they provide information to be able to make the right decisions to optimize *in vitro* propagation protocols. *Melia azedarach* (chinaberry) and *Prosopis alpataco* (alpataco) are two multipurpose woody species. They provide wood, food and varied use of active principles. Cotyledons placed in the induction medium were used to induce somatic embryogenesis (Sharry *et al*. 2006; Boeri *et al*. 2015). Both species are managed to differentiate between morphogenic and not morphogenic callus. In chinaberry there were no differences between the embryogenic calli to the organogenic, since both processes took place in the same type of callus. This mixed callus originated both organs and embryos. They were analyzed microscopically showing the coexistence of processes of differentiation as meristemoids, shoots, somatic embryos and vascular tissue, as well as still dedifferentiated cells. Prosopis cotyledons produced only somatic embryos. In both, pro-embryogenic (ce) and non-embryogenic (cne) cells were observed. A re-differentiation process took place in different types of cells with intense cell divisions that were located in regions distributed randomly. The cne were rounded or elongated, of thin walls, cytoplasm little dense. The ce were also rounded, small, with relatively thick-walled, dense cytoplasm and prominent nucleus, which is colored in deep red; typical characters of meristematic and pro embryogenic cells. Embryogenesis was initiated in individual cells located in the periphery of the callus and from superficial cells from ce cells existing. Divisions observed in these surface cells adopt the affirmation of the unicellular origin of embryos obtained in alpataco and chinaberry. The different stages of embryogenic development observed in both species were similar to a process of embryogenesis in vivo, suggesting the genetic potential of the plant being used. The formation of somatic embryos was a continuous process during the period of incubation of the explants. Given that not all cells differentiate into somatic embryos at the same time, it was possible to observe all the stages of development of the embryo in a same callus. Finally, somatic embryos germinated normally. Both processes of somatic embryogenesis were highly similar despite the no systematic relationship of the species studied, but the explants and environmental conditions were similar. *In vitro* development of cells and tissues depends on different factors such as: genotype, type of plant, age and developmental stage of an explant, physiological state of an explant-donor plant, and the external environment which includes composition of media and physical culture conditions. The majority of the mechanisms that regulate plant embryogenesis still remain to be clarified. The availability of model systems of plant somatic embryogenesis in woody perennials has created effective tools for examining the details of plant embryogenesis. However, studies that used no model plants for somatic embryogenesis systems also revealed the molecular mechanisms in charge of controlling the expression of some genes during somatic embryogenesis, and with practical applications. Nowadays SE can be achieved for any plant provided that the appropriate explant and adequate culture treatment.

Keywords:

Identification of the hormones involved in the growth and development of Comino Crespo (*Aniba perutilis*) for its clonal multiplication in nursery

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The Comino crespo (*Aniba perutilis*), a species of tree native to the Colombian Amazon, is listed as endangered. Vegetative propagation has been report as recalcitrant both *in vitro* and *ex vitro*. Therefore, a protocol of asexual multiplication of said species that allowed its propagation in nursery conditions (*in vivo*) was implemented. Growth and development of plant apexes in nursery seedlings were analysed, and in turn, two clearly distinguishable states, a dormant state and one with active growth, were found. These states were taken into account in order to see their influence on the formation of adventitious roots. At the same time, the correlation between the size of the apical leaves and the induction of adventitious roots was evaluated. In this study the adventitious root formation percentages were determined: 66%, 33% y 0% in active-younger, active- middle and grown-up dormant apical leaves respectively. It was further determined that these percentages are associated with the carbohydrate concentration (which is lower in the active state) and hormones present in each state. Given the above, it was decided to analyze the presence of the hormones Zeatin, Abscisic Acid (ABA) and Indole Acetic Acid (IAA) in both states and determine their concentration.

This study lets to know the Comino crespo hormone content and its adventitious root appropriate formation states, thus favoring their propagation in the nursery conditions.

Knowledge of this hormonal behavior might be a breakthrough in the search for optimal protocols for *in vitro* propagation of Comino crespo.

Keywords: Dormancy, Carbohydrates, Hormones, Adventitious, Propagation

Generation of epicormic shoots for *in vitro* vegetative propagation of *Peumus boldus*. Mol

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Forest Institute of Chile (INFOR), through their different investigation lines, has developed vast amount of native species research, promoting their assessment and conservation. *Peumus boldus* (Boldo) is a native and endemic species of Chile, characteristic of sclerophyllous forests, widely distributed in central southern zone of country. Previous antecedent of this species have been developed such as silviculture, forest management and medicinal properties, which main value is in their active components contained in leaves, bark and wood, including flavonoids, essential oils and alkaloids, being the Boldina the compound most valued.

Our main aims is the development of regeneration protocol through micropropagation by direct organogenesis for adult trees of P. boldus, previously selected for their high levels of flavonoids, essential oils and alkaloids. Preliminary evidence suggests the inability of establish material directly from field due to high rates of contamination and oxidation present. As alternate method, we were evaluated the epicormic shoots induction in laboratory, to use this material as source explant. Pieces of branches was collected of 3 individuals, of 3 cm diameter and 30 cm length, these were placed under controlled conditions to 22±2°C, 80% RH y 16 h-photoperiod, shoot emergence was presented between 15-45 days. It was possible to reproduce epicormic shoots in the 3 individuals, these was collected and established in vitro conditions, in Murashige-Skoog medium supplemented with 0,5 mgL^{-1} BAP and 0,01 mgL^{-1} ANA, also was added 800mgL^{-1} PVP. The rate of contamination and oxidation reached a 6% and 3% respectively.

The results from this study are highly satisfactory since was able to establish the material with low rates of contamination and oxidation, successfully responding to disinfection methods. This allowed obtain rejuvenated, healthy and vigorous shoots, which can be used in future programs of micropropagation, genetic breeding, physiological studies and conservation of germplasm of selected material.

Keywords: *P.boldus*, Micropropagacion, Epicormics shoots

Somatic embryogenesis and plant regeneration from 20-year-old mature tree in *Prunus serrulata* var. pubescens (Korean mountain cherry)

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Our study was to establish somatic embryogenesis system from plant materials (leaf, petiole and root segments) which in vitro grown plantlets derived from mature cherry tree. In inducing embryogenic callus from various types of explants, it was only produced on the root segments (35% induction rates) which cultured on Murashige and Skoog (MS) supplemented with 1.0 mg/L 2,4-D and 3% sucrose under darkness for 5 weeks. Embryogenic callus and early stage of somatic embryos showed pale yellow in color, relatively compact in texture. Somatic embryo germination and conversion to plantlets were significantly influenced by the concentration of gibberellic acid, types of carbon source and gelling agent. Finally, the acclimatized plantlets in artificial soil mixtures showed survival rate of 70%. Our results indicated that somatic embryogenesis and plant regeneration can be successfully accomplished with root explants of in vitro grown plantlets derived from a mature Korean mountain cherry.

Keywords: *Prunus serrulata* var pubescens, Root segment, Somatic embryogenesis, Mature tree

ABSTRACTS OF POSTER PRESENTATIONS

SESSION 3: **(Epi)genomics of embryo or other vegetative propagule development .**

Enterolobium contortisiliquum (Vell.) Morong (PACARÁ - TIMBÓ)

Native of the north and Mesopotamia of Argentina, have a spreading crown and a bark with lots of lenticels. Its leaves are compound, bipinnate and bicolored. With white flowers grouped in terminal inflorescences. The fruit is a kidney-shaped or spherical black indehiscent legume. Its wood is used for crafts being careful with the powder that can be toxic for those who breathe it.

Pinus radiata protein profile of somatic embryos: effect of the application of abiotic stress at the initial stage of somatic embryogenesis process

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Proteome profiling has been successfully applied to the systematic analysis of protein expression during the different stages of the development of the somatic embryos. Furthermore, proteomics enables the identification and quantification of proteins associated to stress tolerance and comparative studies using proteomics to find a marker have been carried out for different aspects of the somatic embryogenesis process (Correia et al. 2012; Morel et al. 2014).

The general objective of this study was to identify the differential proteins abundance of somatic embryos from embryogenic cell lines (ECLs) originated under different environmental conditions. To accomplish this goal, embryonal masses from *Pinus radiata* were initiated following Montalbán et al. (2012). Different concentrations of Gelrite® were added to the initiation medium (2, 3 or 4 g/l) and the explants were cultured at three different temperatures: 18, 23 or 28ºC. According to initiation percentages, somatic embryos from ECLs initiated at 18-23ºC and 4 g/l gellan gum were classified as "high initiation" (HI), those from ECLs initiated at 28ºC and 2-3 g/l gellan gum as "low initiation" (LI) and those from ECLs initiated at 23ºC and 2 g/l gellan gum as Control. Total protein extraction, 2-D Electrophoresis, Image Analysis and protein identification was carried out following Correia et al. (2012).

The 2-DE images of LI and HI showed different spot distribution. 139 spots were detected. 75 proteins were common to HI, LI and Control. 25 proteins were unique to LI, while only 2 spots to HI, and no spots were unique to Control. 11 differentially abundant proteins from LI samples were precisely identified.

A prominent group of proteins involved in defense responses, such as the osmotically inducible protein OsmC, chaperon protein and vicilins, was identified. Proteins related to response to ROS and carbohydrate metabolic process, or proteins related to gene expression were also found in lower proportions. Protein patterns found in somatic embryos cultured under unfavorable environmental conditions (LI) suggest the influence of stress and metabolic response proteins in the maturation stage.

Future studies confirm the hypothesis that unfavorable conditions during initiation produce plantlets better adapted to *ex vitro* conditions, the proteins described in this work could be used as biomarkers.

Keywords: 2-D analysis, Embryogenic potential, Radiata pine, Tree proteomics

Use of *in vitro* chestnut clones to characterize candidate genes for resistance to *Phytophthora cinnamomi*

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European chestnut (*Castanea sativa* Mill.) is a broadleaved tree species native to Europe and of great economic importance for the Mediterranean region. However, among other threats, chestnut populations are affected by "ink disease" caused by *Phytophthora cinnamomi*, a fungus-like eukaryotic microorganism belonging to the class oomycota. This disease has contributed to the drastic decline of chestnut distribution in Europe, with the greatest reduction in the warm southwestern and southern regions of central Europe. It can also affect plantlets, thus causing severe economic losses in nurseries. Hybrids between European and Asiatic chestnut species have long been used because of the high degree of natural resistance to the pathogen shown by the Chinese and Japanese species. The disease-related resistance genes play an important role in plant defense mechanisms, including the plant response to recognition of the pathogen and regulation of plant immune responses to infection by the pathogen.

In this study, we analyzed the expression patterns of nine chestnut genes after *in vitro* inoculation with *P. cinnamomi* into microshoots from 4 clones of chestnut showing different levels of resistance to this pathogen: CS12 (a pure *C. sativa* clone highly sensitive to infection) and three *Castanea* hybrids, PO43, PO42 and PO11, with different percentage of alleles of Asiatic origin, in which PO11 is the most resistant clone. The analyzed genes encode transcription factors (*CsERF1*, *CsSCL1*), members of the GH3 family (*CsGH3.1*; *CsGH3.2* and *CsGH3.5*), a glycine-rich protein (*CsCPE*), an actin-depolymerizing factor (*CsADF*), a LRR-RLK receptor kinase (*CsRLK*) and a transcriptionally controlled tumor protein (*CsTCTP*). Rooted plantlets were inoculated with a virulent strain of *P. cinnamomi*, and leaf samples were collected 24, 48 and 72 hours after inoculation for qPCR analysis.

Two hormone-signaling genes responsive to auxin, *CsGH3.1* and *CsGH3.2*, were strongly upregulated in the most resistant clone 24h after infection. The expression levels increased throughout the course of infection, indicating that these genes are involved in defense mechanisms. Expression levels of *CsGH3.5*, *CsTCTP* and genes encoding the transcription factors *CsERF1* and *CsSCL1* also increased after infection in the most resistant clone, although they exhibited different expression profiles. On the other hand, induction of these genes was lowest in the most sensitive clone (except for *CsSCL1)*. Strong upregulation of the *CsCPE* gene observed after 72 h of infection in the most resistant clone indicates a role for this gene in the plant response to infection and may prevent the progress of pathogenesis through modifications in cell wall synthesis. The slightly early upregulation of *CsLRK* gene in the most resistant clones (PO11 and PO42) 24 h after infection, together with the downregulation of the gene in the most sensitive clones (Cs12 and PO43), also suggests involvement of the gene in pathogen recognition.

Our results suggest that inoculation triggers a general shift in the pattern of gene expression in the plantlets. In general, greater transcriptional activation is directly related to a higher degree of resistance in the clones.

Keywords: Chestnut, Ink disease, Leaves, Resistance genes

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In vitro chestnut leaves as a model system for studying auxin regulation and gene expression during the regeneration of adventitious roots

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The clonal propagation of ornamental plants and woody species is highly dependent on their ability to form roots, which is frequently lost during plant development. In chestnut, cuttings and shoot cultures derived from mature tissues are difficult to root using auxin treatments. Characterization of auxin-regulated genes involved in the control of adventitious rooting is required in order to improve the rooting of recalcitrant species. However, as these genes may also be involved in other developmental processes that occur simultaneously in response to auxin, the expression levels may not necessarily be correlated with root organogenesis. To address this issue, we developed an experimental system consisting of detached leaves from microshoots, to minimize the tissue complexity and also to exclude other developmental process that may be triggered by auxin in microshoots.

The aim of the study was to analyze the physiological and molecular responses during the induction of adventitious roots in the leaves excised from juvenile and mature microshoots. We compared the rooting ability of juvenile and mature leaves in response to auxin in order to test the suitability of the system to study the loss of rooting capacity associated with the maturation process. The effect of the auxin transport inhibitor NPA (N-1-naphthyl-phthalamic acid) on the rooting response was also investigated in juvenile leaves. To determine the period required for reprogramming the fate of certain cells that can establish the root differentiation pathway, NPA was applied at different times after the initiation of auxin treatment. We also identified three genes: *CsSHR2* (Short-root 2), which encodes a GRAS transcription factor; *CsEND093*, which encodes an early-noduline; and *CsUGT*, which encodes an UDP-glycosyltransferase that catalyzes the transfer of sugar to specific acceptors. Expression of these genes was analysed by qPCR in leaf samples harvested at different times (6, 12, and 24 h) after treatments.

The results indicate that the leaf system is a suitable method for studying the adventitious rooting process, as the rooting response was similar to that of microshoots, which is also ontogenetic-stage dependent. The strongest inhibition of rooting rates by NPA was directly correlated with the earliest application of this auxin polar transport inhibitor. Reprogramming of rooting competent cells to root initials occurs during the first 48 h of auxin induction and requires polar auxin transport, as root initiation is strongly inhibited by NPA during this period. The early auxin-related induction of *CsSHR2* and *CsENOD93* observed only in rooting-competent leaves, together with the inhibitory effect of NPA on gene expression and root induction, suggests an important role for these genes in the initial steps of adventitious rooting.

Keywords: Adventitious rooting, Auxin, Chestnut, Gene expression, NPA

Acknowledgements: this work was funded by Xunta de Galicia (10MRU400033PR)

Polyamines biosynthesis in embryogenic cultures of *Araucaria angustifolia*

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Somatic embryogenesis (SE) is a biotechnological tool for mass clonal propagation and *in vitro* conservation of threatened plants, like Brazilian pine, a native conifer in the southern part of Brazil. Despite several efforts, plant regeneration through SE in Brazilian pine is still a challenge. Therefore, there is a need for molecular and physiological studies on the correct regulation of embryo development. In Brazilian pine, our studies have shown that polyamines (PAs) and their precursors are involved in the regulatory mechanisms responsible for embryogenic competence and structure of proembryogenic masses, affording morphogenetic evolution of somatic embryos. In order to understand the PAs metabolism and its potential manipulation during SE in Brazilian pine, we carried out a study using the incorporation of labelled precursors of putrescine biosynthesis (L-[U- ^{14}C]Arginine or L-[U-^{14}C]Ornithine) during embryogenic culture (ECs) proliferation. To this end, ECs of Brazilian pine were grown on liquid MSG medium supplemented with 2.5 µCi of either L-[U- ^{14}C]Arg or L-[U-^{14}C]Orn, along with cold arginine or ornithine. After 6, 24, 48, 72 and 168h of ECs proliferation, aliquots were collected and PAs were extracted with 5% perchloric acid (PCA). Three fractions were obtained: PCA extract, aqueous and acetonitrile containing dansyl-PAs. Dansyls-PAs were separated using Thin Layer Chromatography, and the respective polyamine bands (putrescine, spermidine and spermine) were scrapped for radioactivity count. The maximum absorption of both L- [U-^{14}C]Arg and L-[U-^{14}C]Orn into PAs was observed after 48h of ECs proliferation. The highest levels of radioactivity from aqueous fraction (containing amino acids and other charged radioactive products) was recorded at 48h with L-[U-^{14}C]Arg and 72h with L-[U-^{14}C]Orn. However, the acetonitrile fraction (containing dansyl-PAs) showed an increase on the incorporation of L-[U-^{14}C]Arg from 6 until 168h of ECs proliferation, while a little effect was observed on the incorporation of L-[U- ^{14}C]Orn into PAs. The highest levels of radioactivity in liquid medium supplemented with L-[U- ^{14}C]Arg were: 6h for putrescine, 48h for spermidine and 72h for spermine. The results indicate that arginine is the preferential amino acid for PAs biosynthesis. However, it is important to consider the involvement of ornithine during putrescine biosynthesis by its conversion into arginine. Expression of PAs metabolism-related genes, resulted from Araucaria transcriptome database, will be integrated with labelled precursors results in order to understand PAs homeostasis during somatic embryo formation.

Keywords: Somatic embryogenesis, Labelled precursors, Polyamines metabolism, Gymnosperms

Same, same, but different - closely related conifer species and even clones vary in their optimal culture conditions

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Somatic embryogenesis (sE) in conifers is considered a very promising tool that offers a perspective for modern breeding strategies in forestry. Nevertheless, there is a constant need of adjusting the system, not least because the mechanisms behind remain largely unknown. For individual species (*Larix x eurolepis, L. decidua, Pseudotsuga menziesii, and Abies nordmanniana)* our group adapted protocols to generate sE clones under standardized conditions. Afterwards a characterization and evaluation enables the creation of a multi-trait repository of genotypes. At this stage we are selecting clones based on their handling characteristics (maintenance, proliferation rate during; quality and quantity of mature embryos as well as plantlets after conversion). In addition, we are continuously analyzing parameters to improve upon the results of induction, maturation and conversion: e.g. concentration and ratio of plant growth regulators (PGR); nutritional factors; duration of maturation; subculture intervals; variations in temperature regimes, the influence of crossing partner combinations. For *P. menziesii* and *L. x eurolepis* we confirmed, that induction is most efficient using globular and early cotyledonary stages of the zygotic starting material, whereas in *A. nordmanniana* sE is reliably induced from mature zygotic embryos, which ensures a seasonally independent induction period. Taken together, a shortened, calculable induction schedule with yet a reasonable yield has been developed for each species. Following a selection of mature embryos with defined morphological qualities, we currently yield an average of above 60 % plantlets with a vigorous habitus in *L. x eurolepis* and *A. nordmanniana*. The conversion efficiency is considerably lower in *P. menziesii* and still requires further adjusting. However, this is compensated by the production of relatively large numbers of embryos per gram fresh weight of proliferating embryogenic materials during maturation.

Despite advances in the field, the yield of somatic embryos varies strongly across clones, as many regulatory influences remain poorly understood. The formation of proper somatic embryos during the maturation remains to be of great interest as this requires a timely and exact sequence of developmental steps. Drawing on the diverse characteristics of established genotypes, we intend to identify marker genes for proper and numerous embryo development, screening known embryogenesis related factors (e.g. *LdLEC1*, *LdWOX2*, *LdBBM*, *LdSERK*, *ABI3*, putative *LdKN-sSTM*), using *Larix* as a model species for (somatic) embryo development. Thereby, we aim to further a basic understanding of key developmental processes.

Along with applicable approaches, these results and the possibility to actively control the efficacy of the sE process would be fundamental to generate plantlets ready to be acclimatized.

For the analysed conifer species, our data show a comparison of our advances of the involved *in vitro* processes in its entirety.

Keywords: Somatic embryogenesis, Induction, Maturation, Conversion to plantlets, Gene expression

ABSTRACTS OF POSTER PRESENTATIONS

SESSION 4: **Preservation and adaptation of wild and selected genetic resources to environmental and socio-economic changes.**

Nothofagus pumilio (Poepp. & Endl.) Krasser (LENGA)

It's a 30 meters high tree belonging to the family Fagaceae which changes its appearance depending on climate and latitude where grows. Form forests with other species such as Araucaria araucana. Serrated edge leaves take on a reddish color before falling. Its fruit is a winged achene.

Conservation "*in vitro*" of Alcornoque (*Bowchidia virgilioide*), a native leguminous savanna tree

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The world of legumes is almost as varied as the diversity of genders of them, so they are often called multipurpose plants. In Venezuela, the agricultural production of legumes is essential for food, and constitutes an important component in the savanna vegetation of our country, for its ecological and economic significance. The regeneration rate for legume trees is very low, so it has been considered necessary to implement methodologies for rapid "*in vitro*" propagation, and genetic improvement. The process of "in vitro" propagation has been complex for forest species, with a tendency to present a high degree of oxidation, and formation of callus on the explants. However, the most successful for these species in the initiation stage of the in vitro propagation, results indicate that they were obtained from apical buds resulted from seedlings generated from seeds germinated in vitro. The main goal of this work was to develop a successful protocol for in vitro propagation of Alcornoque, and then incorporate it into the germplasm of in vitro legumes. Explants (microcuttings) were placed in a base medium MS (1962) and supplemented with cytokinins in different concentrations (0.5; 1.0; 1.5 and 2.0 mg / l)), and a control medium without hormones. These hormone explants were placed in continuous light, presenting responses at the third week of planting, finding that the medium with 0.5 mg / l BA is the most suitable for the in vitro propagation of this species. However, it is important to say, explants placed on medium controls also showed an excellent response. In the multiplication stage, BA concentrations to 1 mg / l and 1.5 mg / l with best results obtained on a concentration of 1 mg / l with approximately 14 shoots per explant. Subsequently, the shoots stopped growing, so it was necessary to decrease the concentration of BA 0.1 mg / l, which achieved the shoot elongation. Specifically, it was observed that concentrations above 1 and 1.5 mg / l BA are inhibitory during the micro propagation process. The development of "*in vitro*" propagation protocols of savanna native legume tree species will allow the strengthening of an "*in vitro*" germplasm bank that will enable the conservation of plant species used in Venezuelan savannas.

Keywords: Leguminous, Savanna tree, *In vitro*, Conservation

Evaluation of induced tolerance to *Phytophthora cinnamomi* in holm oak somatic embryos

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Holm oak trees are challenged by some biotic stresses like fungal infections. The main disease is caused by the oomycete *Phytophthora* spp., which is responsible of forest decline and dieback in evergreen oak forest areas of the southwestern Iberian Peninsula.

Our study is based on the possibility of apply elicitors or oomycete extracts to holm oak somatic embryos to induce epigenetic memory, priming, that may increase tolerance to the pathogen in future infections. To this end, 50µM of the elicitors methyl jasmonate (MeJA), benzothiadiazole (BTH) and para-aminobenzoic acid (PABA) or 10% and 30% oomycete extracts (OCF) were applied to proliferating holm oak somatic embryos for 3-5 days in liquid, or for 60 days on solid media. After treatments, SE were transferred to proliferation medium. The regenerated plants will be tested for tolerance by inoculating roots with *Phytophthora cinnamomi* mycelium.

In previous experiments we assayed the effect of the above-mentioned elicitors and oomycete extracts on the proliferation of SE lines. Also, to quantify oxidative stress produced by these treatments, MDA (malonyldialdehyde) was determined two months after elicitor assay. The application of the OCF did not affect neither proliferation capability nor altered MDA production. In contrast, MeJA, applied in liquid medium, and BTH, applied on solid medium, inhibited growth and increased MDA.

Three months after elicitation, we were able to regenerate shoots from SE treated mainly with MeJA, and to a less extend with PABA or OCF, whereas those treated with BTH produced necrotic tissue and did not develop into plants. Despite this, these shoots failed to root, so the acclimation and later tolerance test is being delayed.

Currently and in order to test the "priming" capacity of the elicitors, dual cultures in which treated SE are being cultured together with *Phytophthora cinnamomi* mycelium are in course. To this end, SE were placed at 0.5 cm from the edge of a 90 mm sterile Petri dish with culture medium, and a piece of agar with *Phytophthora cinnamomi* mycelium was placed in the middle of the plate. Cultures are being maintained in the dark, and checked daily for mycelium evolution. At the final of the process mycelium growth inhibition and H_2O_2 production will be determined. These preliminary results will be presented and discussed.

Keywords: Biotic stress, Dual cultures, Elicitor, Holm oak, Induced tolerance, *Phytophthora* spp.

Acknowledgements: the author would like to thank Dra. Abad and her research group (Phytopathogenic fungi, Instituto Agroforestal Mediterráneo - Universidad Politécnica de Valencia) for providing the strain used in this study (1630 *Phytophthora cinnamomi*).
This work was supported by the research project cofinanced by MICINN and the EU (AGL2013-47400-C4-04-R) and by a predoctoral contract to M.M.

Influence of environmental and endogenous factors on somatic embryogenesis of *Pinus pinaster* Aiton

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Conifers are long-lived organisms with complex life cycles for which epigenetic memory may play an important role in adaptation to environmental stresses. Moreover, acquisition of this epigenetic memory has been linked to zygotic embryo development and could therefore be mimetised by means of somatic embryogenesis (SE). SE is the most useful biotechnology for conifer clonal propagation and a powerful research tool but its practical application in forest industry remains limited since the yield and quality of somatic embryos strongly depends on the line. There is a need to develop molecular markers (such as endogenous hormone levels or gene expression) that could be used to verify the quality of the embryogenic masses (EMs) prior to their maturation.

Pinus pinaster Aiton is one of the most commercial and economically valuable tree species in the Mediterrean Basin. Due to its capacity to live in a wide range of ecological conditions it has been traditionally used in breeding and reforestation programs and as a model organism for the study of stress responses in conifers.

In this work, EMs were initiated from *Pinus pinaster* megagametophytes isolated from cones of 3 open pollinated mother trees collected at middle July. The effect of agar concentration (3 and 6 g/L Gelrite®) and temperatures (18ºC, 23ºC and 28ºC) on the SE process was tested. Both factors significantly affected induction and establishment of SE. Irrespective of the mother tree, no EMs were obtained from megagametophytes cultured at 18ºC and 6 g/L Gelrite® increased induction of EMs. Preliminary results also suggest an improvement on the maturation capacity of the lines cultured with 6 g/L Gelrite® through the induction and proliferation phases.

Plants regenerated from all treatments will be challenged with high temperatures for short periods of time and expression levels of water-deficit stress-related genes such as dehydrins will be determined.

In an attempt to select early markers for a successful SE maturation, endogenous ABA and IAA content in 6 SE lines with different morphology (3 “spiky” vs. 3 “smooth”) is being measured. To this end, samples were collected from calli on proliferation medium and then every 4 weeks after the transfer to ABA-containing maturation medium. Finally, the expression level of *LEC1* and *WOX2* genes on SE lines during the maturation process has been analyzed. Results from these analyses will be presented and correlated with the maturation performance of the lines.

Keywords: Abiotic stress, Epigenetic breeding, Forest Biotechnology, Maritime pine, SE maturation

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Cryopreservation of embryogenic cell lines of *Solanum betaceum* Cav.

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Germplasm preservation has a very important role in current breeding and conservation programs. Duo to either the genetic alterations that plant breeding may impose and to the extinction rate numerous plants are facing, it is crucial to store genetic resources in order to avoid the loss of biodiversity. *In vitro* conservation methods depend on the storage duration that is intended and for long-term storage cryopreservation is the only current method available. A vitrification procedure that allows the cryopreservation of tamarillo (*Solanum betaceum*) embryogenic lines is here presented for the first time. Tamarillo is a solanaceous tree from the Andean region and economically important due to its fruits high nutrient content. One of the most important biotechnological tools that can be applied to this species is somatic embryogenesis (SE) which protocol was elaborated at the Plant Biotechnology Laboratory of the University of Coimbra. Induction of somatic embryos on this species can be achieved through a two-step process. In this procedure leaf segments are first exposed to MS media with an auxin and high concentrations of sucrose forming embryogenic and non-embryogenic masses (that can be subcultured). Embryogenic masses are then then transferred to auxin-free medium to allow somatic embryos development. However, maintenance of the embryogenic masses requires frequent subcultures making the process labor-intensive and space-consuming. Furthermore, in long-term cultures (more than 2 years), karyotype aberrations and other events causing somaclonal variation occur resulting in atypical embryos.

Three different lines of embryogenic masses (EM) were submitted to a 5-day cold hardening stage (4 ºC in the dark) and a 3-day preculture on a hormone-free MS (Murashige and Skoog) medium with an increasing sucrose concentration of 0.25 M, 0.5 M and 1 M, 24 h each (4 °C in the dark). For osmo-protection, after this treatment, at room temperature (RT), a loading solution was added (30 min). The loading solution was further removed and changed by plant vitrification solution 2 (PVS2) for 60 min at 0 ºC, time after which the PVS2 solution was renewed and the embryogenic masses were immersed in liquid nitrogen. Following rapid thawing in a water bath at 40 °C and before placing the tissues in recovery conditions, the PVS2 solution was replaced with a washing solution (30 min at RT). The EM were then transferred to a regeneration medium (MS supplemented with 5 mg l^{-1} picloram, 90 g l^{-1} sucrose and 6 g l^{-1} phytagel). The quantification was carried out by monitoring the samples weight during four months at regular intervals of one month. The quantification of the tissues was performed during the exponential growth stage, therefore, the calli's growth rate was calculated using an exponential regression of mass/time. The average growth rate of cryopreserved EM reached about 0.5 g/month for all lines (50% less than control EM). As for germinated plants, a 50% decrease was observed for cryopreserved EM when compared to the control. The results so far obtained have shown that cryopreservation by PVS2 vitrification is a viable method to maintain embryogenic masses of tamarillo.

Keywords: Cryopreservation, Embryogenic masses, Germplasm storage, Vitrification, Woody species

Elicitation of holm oak somatic embryos and dual-culture with *Phytophthora cinnamomi*

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The holm oak (*Quercus ilex* L.) is the main tree species of the "dehesa" and "montado", typical Mediterranean agroforestry systems of Spain and Portugal. Acorns produced by this species are an important component of the diet of the Iberian pig, a local race that is the basis of a high quality gastronomic industry. In the past decades there has been a high mortality among trees of this species, which is increasing in the last years. This is the consequence of a decline syndrome called "la seca" one of whose causative agents is a root infection by the oomycete *Phytophthora cinnamomi* Rands.

Genetic improvement techniques such as the use of the natural variability capturing tolerance traits or the induction of new variability by the direct transfer of pathogenesis-related genes could be used to produce tolerant varieties. There is another possibility to produce tolerant plants: the induction of epigenetic changes to elicit defense responses. Transgenerational defense induction is a well-known phenomenon. This induction occurs in the parental generation and is transferred to the offspring through maternal signals at the time of seed formation. The formation of somatic embryos in a controlled environment offers an opportunity to give "somatic seeds" suitable cues to prime regenerated plants for tolerance. Recent published studies suggest that transgenerational induced resistance against *Phytophthora cinnamomi* occurs in holm oak. Therefore the elicitation of induced tolerance could be possible in somatic embryos of this species. The objective of this study was to test whether changes in somatic embryos could be induced by elicitors of defense response, and to assess whether these changes could be detected by a dual-culture bioassay.

Embryo clumps from three holm oak embryogenic lines were treated with several compounds well known as elicitors of induced resistance in plants, BABA, BTH, Salicylic Acid and Methyl-Jasmonate. They were also treated with filtrates of a cinnamomin-inducing culture medium in which the oomycete was cultured. Treatments to somatic embryos consisted in the culture on semisolid SH medium supplemented with 50µM of each of the compounds separately or filtrates at 10 or 30% for 60 days, or immersion in the same liquid medium for 3 days followed by culture on medium without elicitor up to 60 days. Data on the number of somatic embryos produced at the end of each treatment and their germination ability were recorded. Single somatic embryos were challenged with the oomycete in a dual culture. They were placed in the periphery of a 90 mm diameter Petri dish filled with SH medium in whose center a piece of 1 sq cm PDA medium with actively growing mycelium was laid. Data of relative growth of mycelium (growth towards the plant tissues minus growth to the opposite side) and of necrosis of somatic embryos (using a predetermined scale) were daily collected from day 2 to day 10 from the beginning.

Treatments with different elicitors enhanced or decreased the production of single somatic embryos depending on genotype and short or long term exposure, but germination ability was not significantly affected. In dual culture the presence of holm oak tissues clearly enhanced in all cases the growth of mycelium. Some significant differences were recorded among treatments for necrosis but not for growth of mycelium.

Keywords: Epigenetic changes, Forest biotechnology, Disease tolerance, *Quercus ilex*, Somatic embryogenesis, Transgenerational resistance

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Optimizing DNA delivery on somatic embryogenic tissue of stone pine

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Stone pine (*Pinus pinea*) is a native tree to the Mediterranean region, occurring in Southern Europe, Israel, Lebanon and Syria. Stone pines have been used and cultivated for their edible pine nuts since prehistoric times. They are widespread in horticultural cultivation as ornamental trees, planted in gardens and parks around the world.

Genetic transformation is the best tool to allow gene functional analysis and for rapidly increasing yield and wood quality. The objective of this work is to develop a protocol to deliver DNA into somatic embryogenic *Pinus pinea* tissue by *Agrobacterium tumefaciens* coculture. Embryogenic lines were initiated from immature megagametophytes and maintained by 2-week subcultures on Litvay medium (mLV). Embryogenic callus was resuspended in liquid mLV and mixed with the same volume of the bacterial suspension. After infection, cells were recovered on filter paper and transferred to the same medium for coculture. Transient Gus Assay was performed following standard protocols three days after coculture. Several factors were tested in order to optimize DNA delivery; *Agrobacterium* strain (AGL1, GV3101, EHA105 y C58), plasmid constructions (pABC, pBIN35SGUSINT, pBINUbiGUSINT y pTAB16), two vegetal tissue concentrations (6 and 12 g of tissue per 50 ml of Litvay modified liquid medium), different infection periods (two treatment with 5 to 10 infection minutes after one minute vacuum), different bacterial dilutions (0.3 to 0.8 OD_{600nm}) and different acetosyringone concentrations (0, 100 and 200 µM) on transformation efficiency. Furthermore, concentrations of selective agents (0, 2, 4 and 6 mg/l phosphinothricin or 0, 5, 10, 20 and 30 mg/l kanamycin) that inhibit growth in untransformed embryogenic calli were also determined.

The proposed protocol for stone pine transformation is infection of 12 g of embryogenic tissue in 100 ml AGL1 *Agrobacterium tumefaciens* strain harboring pTAB16 plasmid at an OD_{600nm} and 200 ml acetosyringone for 5 min, including 1 minute vacuum. Concentrations of selective agents that have yielded better results in selection of transformed callus have been 1 mg PPT for pABC and pTAB16 plasmids, and 5 mg/l kanamycin for pBIN35SGUSINT and pBINUbiGUSINT.

Keywords: Transient expression, Gus assay, *Pinus pinea*, *Agrobacterium tumefaciens*

Acknowledgements: we thank the IMIDRA group (Dr. Mariano Toribio) for providing embryogenic lines. This work was funded by the Spanish Government (MICINN) and FEDER funds (AGL2010-22292-C03-03) Generalitat Valenciana (Prometeo 2009/075) and by a Research Fellowship (FPI) from the MICINN (to M. B.).

Mass by direct organogenesis of a *Eucalyptus* genotype resistant to eucalyptus weevil *Gonipterus scutellatus* (Coleoptera, Curculionidae) spp.

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The Forest Institute (INFOR), from 60' years has carried out a variety of breeding programs of several species of forest interest, such as *Eucalyptus* genus, in order to increase and diversify the forest production in different parts of the country. In such programs have been selected plus tree based on its interest trait such as: volume, form, pulpwood yield, minimal growth stresses, resistance to abiotic stresses (cold and drought) and tolerance to pests and diseases. The water constraints present in the interior dryland in Chile is one of the main factors of predisposition of pests and diseases in forest plantations *Eucalyptus globulus, E. camaldulensis and E. globulus x nitens.* The main pests and diseases experienced by these plantations are often caused by *Gonipterus platensis* and *Phoracantha semipunctata*. Explorations performed in forest plantations of de *E. globulus* and *E. camaldulensis* massively attacked by these insects in the area of Cauquenes, Chile (Latitude -35.888075, Longitude -72.113724) allowed to the identification of 3 plus tree of *E.urophylla* hybrid, which possess a great tolerance to attack of Gonipterus and remarkable growth, for this reason were incorporated into assessment programs to resistance Gonipterus in company CMPC. One of these was selected for obtain micro cutting and subsequently grafted onto *E. globulus*, these were obtained nodal segments which were introduced *in vitro* conditions, using Murashige-Skoog medium supplemented with 2,2 mgL^{-1} BAP and 0,2mgL^{-1} ANA and then maintained in the Germplasm Bank of INFOR. Later experimental work on this material allowed developing of an *in vitro* regeneration protocol which allows obtaining of complete and healthy plants by direct organogenesis through the use of different concentrations of phytohormone (BAP and ANA), additional compounds (Casein hydrolysate and Pantothenic acid) and environmental conditions (photoperiod and etiolation). This represents an excellent option to incorporate tolerance or resistance to *Gonipterus* in the operational programs of breeding of *Eucalyptus* of INFOR and CMPC.

Keywords: *Eucalyptus urograndis*, Micropropagation, *Eucalyptus* weevil

ABSTRACTS OF POSTER PRESENTATIONS

SESSION 5: **Lessons from in vivo growth of vegetative propagules, especially in various pedoclimatic conditions.**

Handroanthus impetiginosus (Mart. ex DC.) Mattos (LAPACHO ROSADO)

Northwest native of Argentina, with stunning silhouette and broad crown. The leaves are composed of 5 leaflets with serrated edge. The explosive pink bloom is prior to the foliation, being these flowers as large bells gathered in terminal inflorescences. Its wood is prized for its color and particular design.

Vegetative propagation and trial planting of clones of selected Philippine commercial timbers

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Interest in the vegetative propagation of trees has grown considerably throughout the world over the last three decades. This has resulted from scientific findings that quick and large genetic gains can be achieved by using vegetative propagated materials in the development of tree plantations. Vegetative propagation produces planting stock called clones, which are identical in genetic makeup with the trees from which the clones were obtained. Thus, producing clones from trees possessing desirable traits such as good form, fast rate of growth, superior wood quality, and high resistance to pests and diseases would, in theory, result in improved plantations.

This study was conducted to generate technology in the improvement of planting stock production for selected native species of known commercial values in Caraga Region in the Philippines. Three rooting facilities namely; mist, non-mist and open mist, and six levels and kinds of rooting hormones were tried to determine the rooting performance of 12 premium forest tree species shoot cuttings. The results showed that different species responded differently to rooting facilities. Bagalunga (*Melia azedarach*), binuang (*Octomeles sumatrana*), dita (*Alstonia scholaris*), loktob (*Duabanga molluccana*), kalantas (*Toona calantas*) and toog (*Petersianthus quadrialatus*) performed significantly better in open mist facility. Kaatoan bangkal (*Anthocephalus chinensis*), malapapaya (*Polycias nodosa*), narra (*Pterocarpus indicus*) performed in non-mist rooting facility and dao (*Dracontomelon dao*) and nato (*Palaquium luzoniense*) were better at mist rooting facility. Banlag (*Xylopia ferrugenia*), however did not perform better in any facilities.

No significant differences on the rooting performance of the species on the application of different kinds and levels of rooting hormones compared to the control. The results suggest that vegetative propagation of these species can be done without rooting hormones.

Results on trial planting showed that growth of planted rooted shoot cuttings were affected by soil fertility of sites. Species of binuang (*Octomeles sumatrana*), dao (*Dracontomelon dao*), dita (*Alstonia scholaris*), kaatoan bangkal (*Anthocephalus chinensis*) and toog (*Petersianthus quadrialatus* showed encouraging result on fertile soils in Ampayon, Butuan City, Agusan del Norte, Philippines.

Keywords: Rooting facilities (mist, non-mist, open mist, six levels and kinds of rooting hormones)

Responses of *Eucalytpus grandis* and interspecific hybrids clones to severe frosts in the Mesopotamian region of Argentina

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E. grandis faces several environmental limitations in the Mesopotamian region of Argentina. Probably, one of the main concerns is frost damage in young plantations (one to three years-old). Some practices to avoid it in prone-to-frost areas are the allocation of frost tolerant genotypes and the application of appropriate silvicultural management to reach the first winter with the maximal tree eight possible.

The main objective of the *Eucalyptus* breeding program of INTA is to select fast-growing clones for solid wood uses. However, given the relevance of biotic and abiotic stresses in a climate changing context, selection traits related to these factors were included in the last decade. Among them, the identification of frost-tolerant genotypes of *E. grandis* and interspecific hybrids through the evaluation of frost damage on field trials was the first to be included. When sub-zero temperatures are not strong enough to kill trees, frost damage can be observed in leaves and shoots, being the latter more relevant in *Eucalyptus* for solid wood porpoises, since lack of dominance and epicormic sprouting permanent affect stem form and bole quality.

Between 2008 and 2009, *Eucalyptus* clonal trials were established in several test sites along the Mesopotamia. One of them, placed in Ubajay, Entre Ríos (31°46'03''S – 58°24'14''W) suffered severe frost damage (-5.1°C) in 2012. In order to identify frost-tolerant clones, diameter at breast height (DBH), total height (TH) and frost damage (FD) were evaluated in the following growth season. The trial has 68 *E. grandis* (EG) clones, three *E. grandis x E. camaldulensis* (GC) clones, three *E. grandis x E. tereticornis* (GT) clones, one *E. grandis x E. dunnii* (GD) cloneand *E. grandis* seedlings from a Seedling Seed Orchard (SSO) as a control. Significant differences were found for all the assessed variables. Both GC and GT clones showed higher DBH and TH than *E. grandis* clones, being the GC clones which showed the best responses for both variables. Among them, the best clone reached 13.4 cm DBH and 13.4 m TH and the worst 3.7 cm DBH and 5.7 m TH at 3.5 years-old. It was observed that frost damage responses were extremely variable among clones, from dead to undamaged trees, and several intermediate levels of epicormic sprouting. Hybrid clones were also superior for FD; however, a small number of *E. grandis* clones showed also good responses for this and the others variables. Both *E. grandis* SSO and the GD clone were heavily affected by frost, though SSO *E. grandis* showed good resilience, since DBH and TH were similar to GC and GT clones. Additionally, it was found that phenotypic correlation was positive and high for DBH with FD (0.71) and for TH with FD (0.76), therefore, it is possible to infer that most of the fast-growing clones were also more frost-tolerant.

In brief, field trials allows to identify better GC and GT clones than *E. grandis* when frosts jeopardize successful plantations establishment. Moreover, the strong positive correlation among growth traits and frost damage stress the relevance of selecting fast-growing *Eucalyptus* clones.

Keywords: Vegetative propagation, Adaptability, Frost tolerance, Climate change, Abiotic stress

Productivity in various pedo-climatic conditions of cold-hardy *Eucalyptus* clones developed by FCBA for plantation forestry in southern France

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FCBA (formerly AFOCEL) is developing researches and a long-term breeding program of *Eucalyptus* species since the seventies. The program includes both traditional breeding and already operating biotechnological inputs such as clonal micropropagation, cryopreservation and DNA fingerprinting. Significant developments in *Eucalyptus* genomics are also ongoing in the frame of partnerships at the national and international levels towards increased knowledge about genes involved in cold-hardiness, growth, development and wood properties of interest for customers (pulp industries, biomass production).

The main objective is to select and deploy fast-growing varieties in clonal plantation forestry that are well-adapted to the various pedo-climatic conditions found in southern France from oceanic to more Mediterranean and arid conditions. One critical issue for breeders in this program is to improve cold-hardiness of new varieties developed for foresters to cope with erratic severe winter frost. Selected *Eucalyptus* species for this program, mostly *E. gunnii* and *E. dalrympleana*, originated from the mountainous regions of their natural distribution area in Australia, especially Tasmania. *E. gunnii* was identified as one of the most appropriate tested species for cold hardiness whereas *E. dalrympleana* has less tolerance but better growth behavior. Breeding was focused on *E. gunnii* and the *E. gunnii x E. dalrympleana* hybrid species (*E. gundal*) using well-selected provenances.

The best selected hybrids clones for both good growth and significant cold-hardiness (up to -12°C at a 50% cutoff threshold for damages) were successfully introduced *in vitro* for conservation purposes (including cryopreserved collections), reactivation or maintenance of organogenic capacities (especially rooting ability) and rapid initial vegetative propagation through micropropagation. *In vitro* rootstocks are then implemented by commercial forest nurseries (e.g. Forelite) to form large stool beds for the production of cuttings from selected varieties. Following this strategy, around 2000 ha of pilot clonal plantations have been established (100-150 ha/year), mainly for the purpose of short rotation coppices (3 rotations of 10-12 years). In this presentation we specifically reported on the productivity at ages 4 to 13 years of 1 control (FCBA-121) and 2 commercially available FCBA *E. gundal* clones (FCBA-208, FCBA-645). These varieties have been tested at various field plots (47 stands) in southern France divided into 3 large pedo-climatic areas from east to west with corresponding rainfalls in the range of 500-1500 mm/year. A map of putative development zones for eucalypts has been established based on pedo-climatic data, especially the frost constraint. Following the first rotation, the productivity of the 3 clones at age 10-12 years is 15-25 m^3/ha/year. Subsequent rotations are even more productive (by 20%), and up to 35 m^3/ha/year. We concluded that soil water availability and rainfalls are the main factors affecting the productivity of these cold-hardy varieties.

Keywords: Micropropagation, Frost, *E. gundal*, Climatic zones, Growth, Volume

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ABSTRACTS OF POSTER PRESENTATIONS

SESSION 6: **Reducing socio-economic and environmental costs of plantation forestry.**

Prosopis alba Griseb. var. alba (ALGARROBO BLANCO)

Native with large spread in the country, from Jujuy to La Pampa. Medium sized with thorny and gray cracked bark. Bipinnate leaves, yellow flowers arranged in clusters pendulums cylindrical. The fruit is a leathery pod coiled with numerous seeds. Its fruits are used as fodder in winter. It's a very long-lived tree.

From Petri dishes to bioreactors
First experiences on optimization of Norway spruce SE-process for bioreactors

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The project *Vegetative propagation of spruce – towards future plant production*, carried out at Natural Resources Institute Finland (Luke), is focused on paving way for practical applications of Norway spruce´s (*Picea abies* (L.) Karst.) somatic embryogenesis (SE). One important aspect related to this goal is improving cost efficiency of the process.

Currently used SE-process at Luke is based on handwork which is time consuming and expensive. Using bioreactors for multiplication of embryogenic tissues and maturation of embryos could reduce the amount of handwork, and also as a result reduce the cost of a single plant significantly. So, what has to be taken into consideration when transferring SE-process based on petri dish culturing into bioreactors?

Selecting the bioreactor type and then the model would be the first step. Temporary immersion system (TIS) is one of the most commonly used bioreactor types in tissue cultures. There are several commercially available TIS models such as Rita, SETIS and Plant Form. Since bioreactors are sterile systems, it is good to bear in mind the autoclaving treatment needed for specific bioreactor model, as well as easiness of aseptic handling when considering between different bioreactor models.

Following the selection of bioreactor model, there are several factors such as support frame for tissue, growth conditions including gas exchange and growing media that need to be optimized. In TIS bioreactors, frequency and duration of medium application will affect nutrient and growth regulator availability, and thus the development of cultures. It might also be necessary to modify the growing media for bioreactors.

Optimizing plant tissue culture method into bioreactors is challenging and time consuming, with requirement of all culture process steps to be optimized separately. The first experiences obtained with embryogenic cultures of Norway spruce at Luke laboratory will be presented.

Keywords: Somatic embryogenesis, Norway spruce, Bioreactor, Temporary immersion system

Scaling-up of cherry rootstock production in temporary immersion bioreactor

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Chile is one of the main countries which exports Cherries off-station to the north hemisphere with a high potential of business growth mainly to the increase demand of this fruit from North America, Asia and Europe markets. To cover this need, thousands of hectares should be cultivate using rootstocks to improve the agronomic features of the fruit and helping with the yield of the crops for export. Although, the conventional propagation or *in vitro* propagation of diverse varieties of cherries does not present issues in those procedures but the propagation of the rootstocks is recalcitrant in both the conventional and *in vitro* methodologies. Therefore, in this research, we have developed Temporary Immersion Systems (TIS) to scaling up the propagation rate of Cherry rootstocks Maxma 14, Colt and YQM1. These varieties are key to obtain grafted plants with commercial yields for export. The best conditions for the TIS were acquire using an experimental design with factorial treatment of 2_3 with 6 replicates and variables of number of immersions, BAP concentration and air supply and with an answer of the number of earn plantlets. The rooting of the plantlets with the best conditions for each variety was carried in an *in vitro* media supplemented with IBA and finally the acclimatization was done in humid tunnels on soil at 25°C. The results for the rootstocks Maxma 14, Colt and YQW1 with an initial inoculum of 40 plantlets reached on average 90, 80 and 60 plantlets in 21 days (duplication rate 2) in comparison with *in vitro* cultures with a duplication rate 2 of 30 to 45 days to obtain the same number of plants as a rootstock. The methodology of rooting and acclimatization reached 90% of success for rootstocks of Maxma 14, Colt and YQM1. During all the process, we use 56 bioreactors, letting us to evaluate the needs and capabilities of the TIS production yields and creating a platform for massive propagation of Cherry rootstocks.

Keywords: Temporary Immersion Bioreactor, Cherry Rootstock

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