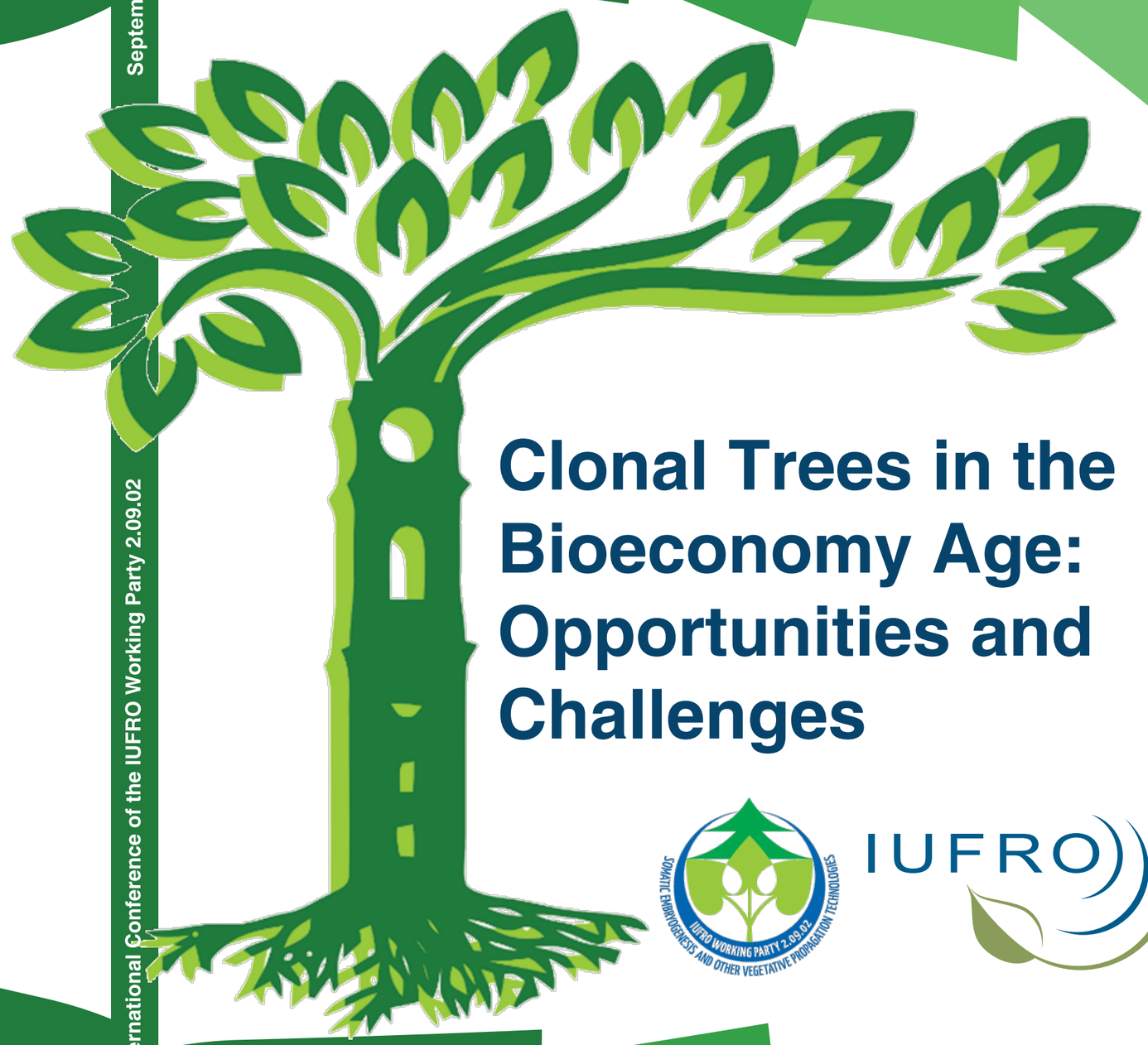
Fifth International Conference of the
IUFRO Working Party 2.09.02

Somatic Embryogenesis and Other Vegetative Propagation Technologies

September 10-15, 2018



Clonal Trees in the Bioeconomy Age: Opportunities and Challenges

Fifth International Conference of the IUFRO Working Party 2.09.02

September 10-15, 2018
Coimbra, Portugal

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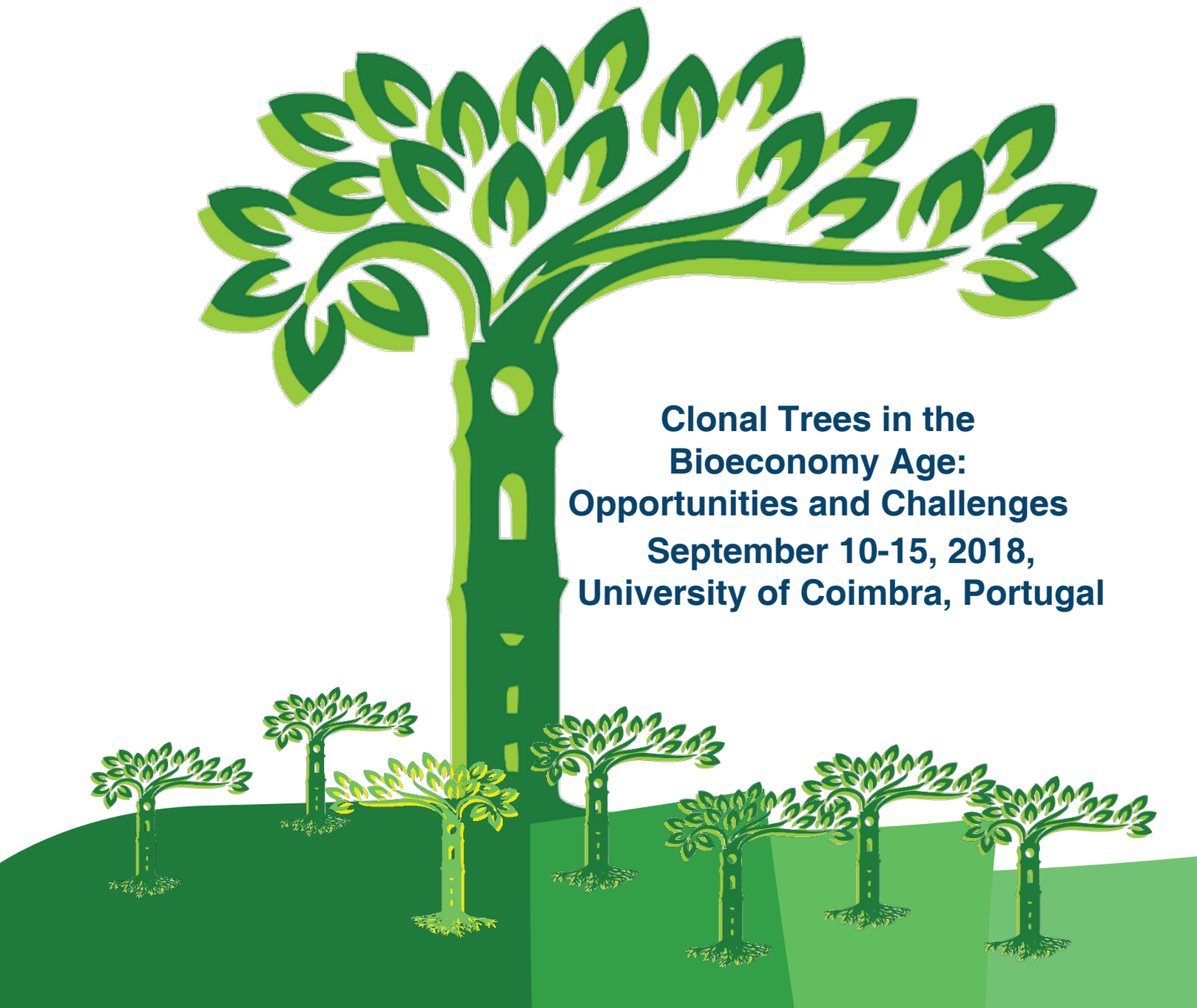
Book of Abstracts & Program

5th International Conference of the IUFRO Working Party 2-09.02 Somatic embryogenesis and Other Vegetative Propagation Technologies



Clonal Trees in the Bioeconomy Age: Opportunities and Challenges

September 10-15, 2018,
University of Coimbra, Portugal

Book of Abstracts - 5th International Conference of the IUFRO Working Party 2.09.02
Somatic Embryogenesis and Other Vegetative Propagation Technologies

Clonal Trees in The Bioeconomy Age: Opportunities and Challenges

Coimbra, Portugal
September 10-15, 2018

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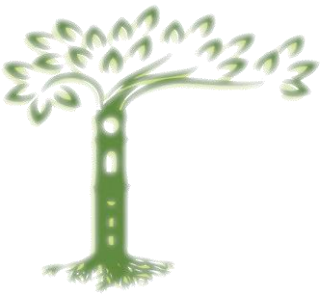

Contents

Welcome Message

Welcome to "Coimbra 2018", the Fifth International Conference of the IUFRO 2.09.02 Working Party: Somatic Embryogenesis (SE) and other Vegetative Propagation (VP) technologies! The main goal of this IUFRO Unit 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.

Since our Unit was launched by Yill-Sung Park about 10 years ago, we already had four successful conferences in Republic of Korea (Suwon 2010, inaugural meeting organized by Heung-Kyu Moon), Czech Republic (Brno 2012, Jana Krajňáková), Spain (Vitoria-Gasteiz 2014, Paloma Moncaleán) and Argentina (La Plata 2016, Sandra Sharry). We are particularly pleased that we have this 5th conference in the beautiful city of Coimbra, the old capital of the Portuguese Kingdom which is full of heritage from the past and classified since 2013 as a UNESCO World Heritage Site!

This year's conference is hosted and funded by the University of Coimbra. It is one of the oldest universities in Europe and in the world (created in the 1290), the oldest in the country (classified as an historical site), and one of the most important research and higher education institutions in Portugal with worldwide reputation. Further funding support was provided by the Fundação Oriente (a not-for-profit organization promoting in particular scientific activities in Portugal), Fundação para a Ciência e a Tecnologia (FCT) and The Navigator Company, one of the leading pulp and paper manufacturer in Portugal and Europe. Various other sponsors also provided helpful support. Many thanks to all these strong supporters of our IUFRO 2.09.02 activities!

Our conference is entitled "Clonal Trees in the Bioeconomy Age: Opportunities and Challenges". Following our last conference in La Plata, the importance of plantation forestry was emphasized as forests are becoming increasingly fragmented and vulnerable in the context of both anthropic- and climate-related pressure. Flexible and popular, low-cost VP methods are considered critical for both sustainable plantation forestry and conservation of genetic resources. So clonal trees have a lot to do with the "bioeconomy" that can be defined as the economy of photosynthesis and more broadly of living organisms. It is based on the sustainable production and mobilization of biomass for optimal valorization. The bioeconomy is part of the larger green economy, i.e. an economy that respects the environment and uses natural resources more efficiently. Forests are a considerable source of biomass and other wood products and our conference therefore logically fall into IUFRO Theme 2 "Forests and Forest Products for a Greener Future". We expect that this conference is the opportunity for all attendees to discuss the latest advances in scientific knowledge and technology 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 to effective implementation in tree breeding and variety deployment. So we wish you a productive conference and happy networking!

On behalf of the Organizing Committee, I would like to warmly thank all the contributors to Coimbra 2018. Our special and warm thanks first go to Pr. Jorge Canhoto and Dr. Sandra Correia (Centre for Functional Ecology, Department of Life Sciences) who worked tirelessly

to achieve a good balance of scientific program and attractive social activities. Jorge, Sandra, you have perfectly taken up this IUFRO 2.09.02 challenge in a difficult economic context and sometimes between two courses at the University and other appointments! We are grateful to all members of the Executive Committee who looks conscientiously after the local arrangements and in particular to Jorge Noro, João Martins, Ricardo Costa and André Caeiro for their great contribution to organization of the conference.

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 two young scientists 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 high priority for the IUFRO 2.09.02 Unit and Coimbra 2018 was the opportunity to organize our Third Biennial Student's Scientific Competition dedicated to graduate students and recent PhD graduates. Nine candidates from 8 countries were in competition this year. This is the largest number of candidates ever reached for this well-contested competition! On behalf of the Evaluation Committee chaired by Dr. Jana Krajňáková, we would like to thank again the winner, Bruno Viana Navarro (Brazil) and all 8 excellent runners-up, Chang-Ho Ahn (Republic of Korea), Ana Alves (Portugal), Rayan Awada (France), Jayeni Hiti Bandaralage (Australia), Biljana Đorđević (Czech Republic), Cátia Pereira (Spain), Wang Quishui (China), and Cheng Wei (China) for actively contributing to the scientific program. All our encouragements and success in your respective project!

Finally, as Coordinator in charge of this Unit since 2015, I would like to take this opportunity to warmly thank all my colleagues and friends of the Conference Organizing Committee, Jorge and Sandra (Univ. Coimbra, Portugal), Jana (Scion, New-Zealand), Paloma (Neiker, Spain), Sandra (Univ. la Plata, Argentina) and Yong-Wook (NIFoS, Republic of Korea). They all provided kind and essential collaborative support based on their strong respective experience to meet this new challenge.

Here we are in Coimbra with over 90 attendees contributing to 13 keynotes, 42 oral presentations and about 70 posters in our scientific program. We hope that everything is in good order to have a successful and productive conference and also to discover the Secrets of Coimbra!

Sejam bem-vindos e desfrutem da quinta conferência IUFRO 2.09.02.

Jean-François Trontin, September 3th, 2018

Coordinator, IUFRO 2.09.02 Unit

Organization

The **5th International Conference of the IUFRO Working Party 2.09.02 – Somatic Embryogenesis and Other Vegetative Propagation Technologies** is an organization of the Centre for Functional Ecology (http://cfe.uc.pt) of the Department of Life Sciences of the University of Coimbra (www.uc.pt/fctuc/dcv), and of the IUFRO (www.iufro.org) . The theme of this conference is: Clonal Trees in the Bioeconomy Age: Opportunities and Challenges. Other institutions involved in the organization are the FCBA (www.fcba.fr/), Neiker-Tecnalia (www.neiker.net/), SCION (www.scionresearch.com), Faculdad de Ciencias Agrarias e Florestales da la Universidad de La Plata (www.agro.unlp.edu.ar) and the National Institute of Forest Science in Korea – NIFoS (http://english.forest.go.kr).

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SOMATIC EMBRYOGENESIS AND OTHER VEGETATIVE PROPAGATION TECHNOLOGIES

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NIFoS
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Committees

Organizing Committee

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CÂMARA MUNICIPAL DE COIMBRA

We also would like to thank to the Mosteiro de Alcobaça (www.mosteiroalcobaca.gov.pt)

Useful Information

Symposium venue(s)

On Sunday (September, 9th) the registration desk will be open at the Department of Life Sciences (Street: Calçada Martim de Freitas, near the Botanic Garden - 15.00h – 18.30h). Coffee and refreshments will be available. Payment at desk must be in cash (Euro Currency). On Monday (September, 10th), registration will be open from 08.00h to 10.00h near the Auditorium where the symposium takes place (Museum of Science). From Tuesday (11th) to Thursday (13th) the session will be at the Department of Mathematics. Last day (14th) the conference will be at the Convento de São Francisco (same place of the Gala dinner)

Public transportation in the city

Most hotels are located in city centre. Touristic places (museums, churches, monutents…) are in a walk away distance. Bus and "trolleys" cover all the interesting places. A touristic bus is also available as well as a small bus that connects downtown to the University through the Botanic Garden.

Oral sessions

Presenters are required to use the computer provided by the organization. Presentations should be provided to the registration staff one day before the presentation. For those presenting their communications on Monday (September 10th) morning the presentations should be send to jorgecan@uc.pt. Oral presentations are scheduled for a maximum of 20 min. (15 min. plus 5 for discussion). Chairs of the sessions will keep strictly to this schedule.

Posters

Posters (Maximum dimensions - A0) should be mounted on Monday 10th, in the Department of Mathematics (Largo D. Dinis), Poster boards will be labeled with the number of the posters as appearing in Abstract Book (*e.g.* P21). Posters should remain on display until Thursday (September, 13th). The organization will provide pins or other materials to fix the posters.

Meals and coffee breaks

Lunches (from Monday to Thursday) will be provided by the organization and are included in the registration fee. Coffee breaks will be served during morning and afternoon. Social activities are listed in the program.

Tour

On Saturday (September, 15th) a tour to the famous village of Óbidos and to the Monastery of Alcobaça will take place. Lunch will be provided during the tour at a local restaurant. The tour will start (08.00h) from Largo D. Dinis, in front of the Department of Mathematics.

Internet

During the symposium the organization will provide wireless internet access.

Banking and currency

The Portuguese currency is the Euro. There are two banks and automated cash machines at the congress venue. Banks are open from 09.00 to 15.00. Banks, hotels and official agencies change foreign currency. Most credit cards are accepted in hotels, restaurants and shops.

Language

All sessions, posters and presentations must be in English.

Certificate of attendance

All participants will receive a certificate of attendance.

Liability and insurance

The registration fee does not include any insurance for the participants (or accompanying persons).

Phone numbers

Emergency number: 112

Taxis: + 351 239 715445, + 351 239 499 090

Hospital: + 351 239 400 400

Departmen of Life Sciences: + 239 240 700

Time-table

Sunday, September 9th

15:00-18:30	Registration - Get together at the Botanic Garden of the University of Coimbra

Monday, September 10th

08:00-10:00	Registration (Science Museum)
10:00-10:20	Opening session
10:20-11:00	**Plenary lecture 1:** The role of rejuvenation through tissue culture in the success of *Eucalyptus globulus* vegetative propagation**. Cristina Marques**
11:00-11:30	Coffee break
11:30-12:30	**Session 1 – Tree breeding and field assays (part 1)**
12:30-14:15	Lunch
14:15-15:00	**Plenary lecture 2:** Cloning systems of traditional and alternative Brazilian forest species**. Ivar Wendling**
15:00-15:40	**Session 1 – Tree breeding and field assays (part 2)**
15:40-16:20	**Plenary lecture 3**: KLON – Plant biotechnology for productivity and sustainability of agro-forestry industries. **Tânia Almeida**
16:20-17:30	Coffee break and posters
18:00-19:00	Visit to the University of Coimbra
21:00-23:00	Coimbra by night

Tuesday, September 11th

09:00-09:40	**Plenary lecture 4**: Industrial implementation of somatic embryogenesis and genomic selection in white spruce in Brunswick. **Yill-Sung Park**
09:40-11.00	**Session 2 – Large scale cloning and industrial production (part 1)**
11:00-11:30	Coffee break, posters
11:30-12:30	**Session 2 – Large scale cloning and industrial production (part 2)**
12:30-14:00	**Lunch**
14:00-14:40	**Plenary lecture 5**: New method of inititation and culture cycling of somatic polyembryogenesis of Loblolly pine. **Pramod Gupta**
14:40-15:40	**Session 3 – Complementary technologies (part 1)**
15:40-16:10	Coffee break, posters
16:10-17:10	**Session 3 – Complementary technologies (part 2)**
16:10-17:10	**Plenary lecture 6**: An overview on somatic embryogenesis and applications in woody plants. **Shri Mohan Jain**
19:30-22:30	Beer party

Wednesday, September 12th

09:00-09:40	**Plenary lecture 7**: Biotechnological tools to produce superb trees in the green era. **Paloma Moncaleán**
09:40-10:40	**Session 4 – Genetics and epigenetics (part 1)**
10:40-11:10	Coffee break, posters
11:10-12:30	**Session 4 – Genetics and epigenetics (part 2)**
12:30-14:00	Lunch
14:00-14:40	**Plenary lecture 8**: Progress in somatic embryogenesis of Japanese conifers. **Tsuyoshi Maruyama**
14:40-15:40	**Session 5 – Somatic embryogenesis (part 1)**
16:00-18:30	Visit to Mata do Bussaco
19:30-22:30	Dinner at Caves Messias

Thursday, September 13th

09:00-09:40	**Plenary lecture 9**: Induction of somatic embryogenesis in leaf and shoot apex explants derived from adult trees: a case study on an oak species. **Vanesa Cano**
09:40-10:40	**Session 5 – Somatic embryogenesis (part 2)**
10:40-11:10	Coffee break, posters
11:10-12:10	**Session 5 – Somatic embryogenesis (part 3)**
12:10-12:50	**Plenary lecture 10**: The ups and downs of developing hybrid sweetgum varieties for the US bioenergy and pulp and paper industries: a 20-year case study. **Scott Merkle**
12:50-14:30	Lunch, posters
14:30-15:10	**Plenary lecture 11**: Somatic embryogenesis and other vegetative propagation of native forest tree species in North China: current status and issues should be striving solved near future. **Hailong Shen**
15.10-15.30	**Session 5 – Somatic embryogenesis (part 3, cont.)**
15.30-16.30	**IUFRO meeting**
18:30-23:00	**Gala dinner at Convento de São Francisco**

Friday, September 14th

09:00-09:40	**Plenary lecture 12**: Embryogenic competence acquisition in tamarillo: from single tissue to single-cell based analysis. **Sandra Correia**
09:40-11:00	**Session 5 – Somatic embryogenesis (part 4)**
11:00 – 11:30	Coffee break
11:30 – 12:10	**Session 5 – Somatic embryogenesis (part 5)**
12:10-12:50	**Plenary lecture 13**: Promotion of rooting rate for commercial planting of *Eucalyptus pellita* clone and enhancement of seedling growth by mychorriza application. **Heung-Kyu Moon**
12:50-13:15	Closing cerimony

Saturday, September 15th

08:00-18:00	Visit to Óbidos Village and Alcobaça Monastery. Short stop in Fátima in the way back to Coimbra. Lunch included.

Program

Sunday, 9th September 2018

15.00 – 18.30 Registration (Department of Life Sciences, Botanic Garden) – Visit to the Botanic Garden, Coffee and refreshments.

Monday, 10th September 2018

08.00 – 10.00 Registration

(Congress venue – Science Museum of the University of Coimbra)

10.00 – 10.20 Opening session

10.20 – 11.00 Plenary lecture 1

PL1. The role of rejuvenation through tissue culture in the success of *Eucalyptus globulus* vegetative propagation **Cristina Marques** - RAIZ, Instituto de Investigação da Floresta e do Papel, Portugal

11.00 – 11.30 *COFFEE BREAK*

Session 1 – Tree breeding and field assays (part 1)

Chairwoman: Cristina Marques

11.30 Superior ideotypes for Christmas tree production – combining quantitative genetic tools, molecular markers and improved somatic embryogenesis methods - Ulrik Bräuner Nielsen.

11.50 Benefits and risks of using clones in forestry. Harry Wu.

12.10 A new generation of chestnut rootstocks with improved resistance to *Phytophthora cinnamomi* Rands. Rita Costa.

12.30 – 14.15 *LUNCH*

14.15 – 15.00 Plenary lecture 2

PL2. Cloning systems of traditional and alternative Brazilian forest species **Ivar Wendling** - Brazilian Agricultural Research Corporation (Embrapa Florestas), Brazil.

Session 1 – Tree breeding and field assays (part 2)

Chairmen: Ivar Wendling & Bruno Navarro

15.00 An assessment of hybrid poplar leaf and wood traits in micropropagated plants and plants propagated from root cuttings: gas exchange, vascular, nanomechanical and cell wall. Jaroslav Ďurkovič.

15.20 Strawberry tree (*Arbutus unedo* L.) breeding: hybridization and polyploidization. João Martins.

15.40 – 16.20 Plenary lecture 3

PL3. KLON – plant biotechnology for productivity and sustainability of agro-forestry industries **Tânia Almeida** - Klón, Innovative Technologies from Cloning, Biocant Park, Parque Tecnológico de Cantanhede, Portugal

16.20 – 17.30 *COFFEE BREAK, POSTER SETUP*

(Congress venue – Mathematics Department, University of Coimbra)

18.00 – 19.00 *Visit to University of Coimbra – Joanina Library and others*

21.00 – 23.00 *Coimbra by night. Walking guided tour to see some of the main Coimbra monuments. Coffee and refreshments at the Department of Life Sciences. Traditional music – student's Tuna*

Tuesday, 11th September 2018

09.00 – 09.40 Plenary lecture 4

PL4. Industrial implementation of somatic embryogenesis and genomic selection in white spruce in New Brunswick **Yill-Sung Park -** Natural Resources Canada, Canadian Forest Service, Fredericton, NB, Canada

Session 2 – Large scale cloning and industrial production (part 1)

Chairwomen: Jana Krajnakova & Ana Alves

09.40 Clones à la carte - how proper organisation of large clone and data collections can support researchers, breeders and customers. Tim Benneckenstein.

10.00 Biofactory movil: New access alternative to the massive propagation of clones, by tissue culture. José A. Cabral.

10.20 Implementing genomic selection for multi-varietal forestry of white spruce (*Picea glauca*). Yill-Sung Park.

10.40 Effects of meta-topolin derivatives and temporary immersion on hyperhydricity and shoot proliferation in *in vitro* of *Pyrus communis*. Mariem Lotfi.

11.00 – 11.30 *COFFEE BREAK, POSTERS*

Session 2 – Large scale cloning and industrial production (part 2)

Chairman: Jorge Canhoto

11.30 Somatic embryogenesis of Norway spruce in Finland – seven years from start to first commercial pilots. Tuija Aronen.

11.50 Field testing clones of North American willow (*Salix*) species for biomass production and ecological restoration on highly disturbed sites. Alex Mosseler.

12.10 Mitigating the loss of genetic material and increasing the yield of emblings in late phases of Norway spruce SE. Mikko Tikkinen.

12.30 – 14.00 *LUNCH*

14.00 – 14.40 Plenary lecture 5

PL5. New method of initiation and multiplication culture cycling of somatic polyembryogenesis of Loblolly pine **Pramod Gupta** – Trees for the future LCC, WA, USA.

Session 3 – Complementary technologies (part 1)

Chairman: Pramod Gupta

14.40 Tree Biotechnologies: What has worked and what hasn't worked? Jenny Aitken.

15.00 Low cost media for improving in vitro propagation of woody plant species. Conchi Sánchez.

15.20 Effect of CPPU-derived inhibitors of cytokinin oxidase on *de novo* shoot formation. Nino Muvarnidze.

15.40 – 16.10 *COFFEE BREAK, POSTERS*

Session 3 – Complementary technologies (part 2)

Chairman: Shri Mohan Jain

16.10 A fluorine containing topolin cytokinin for plant tissue culture. Stefaan Werbrouck.

16.30 Effect of flavonoid on mycorrhyzal synthesis between *Tuber borchii* and *Arbutus unedo* L. *in vitro* plants. Filomena Gomes.

16.50 Progress and prospect of vegetative propagation for *Acer* mono Maxim. Peng Zhang.

17.10 – 17.50 Plenary lecture 6

PL6. An overview on somatic embryogenesis and applications in woody plants **Shri Mohan Jain** – Department of Agricultural Sciences, Helsinki, Finland

19.30 – 22.30 *Beer party*

Wednesday, 12th September 2018

09.00 – 09.40 Plenary lecture 7

PL7.Biotechnological tools to produce superb trees in the green era **Paloma Moncaleán** – Neiker-Tecnalia. Centro de Arkaute, Vitoria-Gasteiz, Spain.

Session 4 – Genetics and epigenetics (part 1)

Chairwomen: Paloma Moncaléan & Cátia Pereira

09.40 Epigenetics in trees: a source of plasticity in the context of climate change. Stéphane Maury.

10.00 The transcriptome of maritime pine accross embryo development. Célia Miguel.

10.20 Transcriptome-wide analysis dissecting transcription factors orchestrating larch tree phase change. Wan-Feng Li.

10.40 – 11.10 *COFFEE BREAK, POSTERS*

Session 4 – Genetics and epigenetics (part 2)

Chairman: Tsuyoshi Maruyama

11.10 Effect of megagametophyte priming on short-term response to high temperatures in somatic embryogenesis-derived plants of maritime pine. Isabel Arrillaga.

11.30 Detecting and expression of somatic embryogenesis regulatory genes in *Tilia amurensis*, *Tilia insularis* and *Tilia mandshurica*. Hae-in Kang.

11.50 Cork oak somatic embryogenesis as a system model to study QsMYB1, a transcription factor highly expressed in phellem. Tiago Capote.

12.10 Transcriptional identification and characterization of differentially expressed miRNAs involved in conifer embryogenesis. Ana Alves.

12.30 – 14.00 *LUNCH*

14.00 – 14.40 Plenary lecture 8

PL8. Progress in somatic embryogenesis of Japanese conifers
Tsuyoshi Maruyama – Department of Forest Molecular Genetics and Biotechnology, Tsukuba, Japan

Session 5 – Somatic embryogenesis (part 1)

Chairman: Scott Merkle

14.40 The role of mitochondrial alternative oxidase (AOX) during somatic embryogenesis of Norway spruce (*Picea abies* L. Karst). Jana Krajnakova.

15.00 Dynamics of DNA methylation and effects of de-methylating agentes on somatic embryogenesis of *Quercus suber*. Pillar Testillano.

15.20 Carbohydrate metabolism modulation in the zygotic and somatic embryogenesis of the subtropical conifer species *Araucaria angustifolia*. Bruno Navarro.

16.00 – 18.30 *Visit to Mata do Bussaco*

19.30 – 22.30 *Dinner at Caves Messias*

Thursday, 13th September 2018

09.00 – 09.40 Plenary lecture 9

PL9. Induction of somatic embryogenesis in leaf and shoot apex explants derived from adult trees: a case study on an oak species **Vanesa Cano** – IIAG-CSIC, Santiago de Compostela, Spain.

Session 5 – Somatic embryogenesis (part 2)

Chairwoman: Vanesa Cano

09.40 Biotechnology and bioprospection of native species from monte desert Patagonia, as strategies for the development of the regional bioeconomy. Sandra Sharry

10.00 Automated active compounds screening systems allows high-throughput optimization of somatic embryogenesis in *Coffea arabica*. Rayan Awada.

10.20 *In vitro* somatic embryogenesis in sweet orange (*Citrus sinensis* L.) Cvs. Maltaise demi-sanguine and Thomson navel for viral sanitation. Kaouther Ben Mahmoud.

10.40 – 11.10 *COFFEE BREAK, POSTERS*

Session 5 – Somatic embryogenesis (part 3)

Chairmen: J-F Trontin & Rayana Awada

11.10 Somatic embryogenesis in *Bambusa oldhamii* and *Dendrocalamus asper* from immature inflorescences. Miguel Pedro Guerra.

11.30 Gamma radiation effect on somatic embryogenesis of Norway spruces (*Picea abies*). YeonKyeong Lee.

11.50 Effect of heavy metal ions on development of Norway spruce somatic embryos. Biljana Dordevic.

12.10 – 12.50 Plenary lecture 10

PL10. The ups and downs of developing hybrid sweetgum varieties for the U.S. bioenergy and pulp and paper industries: a 20-year case study **Scott Merkle** – University of Georgia, Athens, USA.

12.50 – 14.30 *LUNCH*

14.30 – 15.10 Plenary lecture 11

PL11. Somatic embryogenesis and other vegetative propagation of native forest tree species in north China: current status and issues should be striving solved near future **Hailong Shen** – Northeast Forestry University, Harbin, China.

15.10 – 15.30 Improved and synchronized maturation of Norway spruce somatic embryos in temporary immersion bioreactors. Ulrika Egertsdotter.

15.30 – 16.30 IUFRO meeting

18.30 – 23.00 Gala Dinner, Fado de Coimbra show

Friday, 14th September 2018

09.00 – 09.40 Plenary lecture 12

PL12. Embryogenic competence acquisition in tamarillo – from tissue to single-cell based analysis **Sandra Correia** – Centre Functional Ecology, University of Coimbra, Portugal.

Session 5 – Somatic embryogenesis (part 4)

Chairwomen: Sandra Correia & Biljana Dordevic

09.40 Current results in somatic embryogenesis for *Pinus koraiensis*, an ecologically and economically very important pine species in East Asia. Chunxue Peng.

10.00 Stress application to Aleppo pine somatic embryogenesis: Problem or opportunity? Cátia Pereira.

10.20 Repetitive somatic embryogenesis induced cytological and proteomic changes in embryogenic lines of *Pseudotsuga menziesii*. Caroline Teyssier.

10.40 Somatic embryogenesis of long-term proliferating embryogenic cultures and plant regeneration in *Larix sibrica*. Maria Park.

11.00 – 11.30 *COFFEE BREAK*

Session 6 – Somatic embryogenesis (part 5)

Chairman: Heung-Kyu Moon

11.30 Primordial shoots of Norway spruce (*Picea abies*) as explants for somatic embryogenesis. Saila Varis.

11.50 WINNING AGAINST WILDINGS - The role biotechnology is playing in the fight against wilding conifers in New Zealand. Cathie Reeves.

12.10 – 12.50 Plenary lecture 13

PL13. Promotion of rooting rate for commercial planting of *Eucalyptus pellita* clone and enhancement of seedling growth by mycorrhiza application **Heung-Kyu Moon** – R&D Department, PT. Korintiga Hutani, Kalimantan Tengah, Indonesia

12.50 – 13.15 Closing cerimony

Saturday, 15th September 2018

08.00 – 18.00 Visit to Óbidos Village and Alcobaça Monastery. Short stop in Fátima in the way back to Coimbra. Lunch included.

Plenary Lectures

PL1

The role of rejuvenation through tissue culture in the success of *Eucalyptus globulus* vegetative propagation

Marques, C.M. [1] , Ramos, A. [1] , Rodrigues, A. [1], Costa, J. [1], Borralho, N. [2] Pires A. M[3]

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Clonal propagation allows for genetic gains to be captured, based on selected genotypes, with improved productivity, adaptation and wood properties. Adventitious rooting is a complex and multifactorial process, often the limiting factor for tree cloning. In eucalypts, the ability of cuttings to form roots varies within and across species. *Eucalyptus globulus,* the major plantation species in Portugal due to its excellent wood properties for cellulose pulp production, has a particularly irregular adventitious rooting behavior. This constitutes a bottleneck in the production of elite genotypes. In the last 20 years RAIZ has been investigating the role of different factors affecting the productivity of stock mother plants and the subsequent rooting success of its cuttings, including the role of nutrition, substrate, hormones and pruning strategies as well as possible metabolic changes such as maturation effects. Some of these factors have been associated with greater propagation success, but results have often been irregular across time, and strongly genotype dependent. We will briefly review progress and illustrate the role of rejuvenation of stock mother plants on vegetative propagation success. Results are based on experiments followed up for several years in operational and research conditions. Despite the effects of the year, cutting production date, genotype and experimental block, it was possible to detect significant effects of the stock mother plant rejuvenation procedure (through tissue culture after one year) in terms of number of cuttings produced and adventitious rooting success. Increasing the value and productivity of Portuguese eucalypt forest, taking into account current regulatory and climate change issues, requires enhancing eucalypt productivity and adaptability. Improvements in vegetative propagation protocols for *E. globulus* would allow increments in the availability (number and diversity) of improved clones for replanting. Innovative research directions to improve propagation success and stability will be discussed.

Keywords: *Eucalyptus globulus*, future, micropropagation, rejuvenation, vegetative propagation

PL2

Cloning systems of traditional and alternative Brazilian forest species

Wendling, I.

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Cloning has an important place in the production of forest plants in Brazil, and its use is justified mainly when the availability of high productivity and quality genotypes is limited. It is an important tool to aid the plant breeding programs and mass propagation of plants. However, difficulties in obtaining rooting in some species or clones, mainly adults, have made it difficult to use in many cases. Cloning is responsible today for most of the commercial forest area planted in Brazil, mainly for the genus *Eucalyptus*. Numerous methods have been adapted and developed; among the most used and, or, with greater possibilities of application, we can mention grafting, cuttings, micropropagation, micro-cuttings and mini-cuttings techniques. Species where the objective is the production of wood and leaves (*Eucalyptus* spp and *Ilex paraguariensis*) are propagated by cuttings / mini-cuttings techniques for the formation of high production commercial clonal plantations and by grafting of adult trees for the installation of seed orchards. On the other hand, species such as *Hevea brasiliensis* and *Araucaria angustifolia*, where the objective is the production of latex and seeds (pine nuts), are propagated by grafting. Among the techniques of *in vitro* propagation, micropropagation is the most used in Brazil, mainly for the rejuvenation / reinvigoration of adult elite clones, being the high production costs the greater disadvantage. In the micro-cuttings technique, rejuvenated / reinvigorated propagules (micro-cuttings) are produced *in vitro* for later rooting to obtain plants and their continuous multiplication *ex vitro*, reducing the costs. The mini-cuttings technique arose from the limitations of the micro-cuttings technique in obtaining rejuvenated / invigorated material *in vitro*. It is characterized by the use of sprouts of plants propagated by the conventional cuttings method as sources of vegetative propagules for the formation of the mini-gardens, not previously promoting its passage through the laboratory. Over the past few years, several studies have been carried out aiming at the development of cloning systems for different native forest species in Brazil, although most studies have focused on juvenile materials. The results are still very shallow, not providing enough technology for the development of clonal forestry in most of them.

Keywords: vegetative propagation, genetic improvement, clonal forestry, woody species, rooting.

KLON - plant biotechnology for productivity and sustainability of agro-forestry industries

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Smart and *Sustainable* are two keywords that set the top priorities for economic growth. Knowledge, innovation and the efficient use of resources are the cornerstones of modern bioeconomy, being biotechnology one of the key technologies to meet the current demand for biomass in the world and to ensure their availability to the main industrial sectors. The emergent need of biomass in agro-forestry and energy industries has led to the development of plant biotechnology tools that improve the quantity and quality of plant products and increase the productivity, providing an economic growth based on the principle of reducing the environmental impact. Aware of these needs, KLON, Innovative Technologies from Cloning is a company that invests in research, development and innovation in three key areas of plant biotechnology: micropropagation, cryopreservation and molecular biology. In order to offer the most profitable solutions for forestry and energy sectors, KLON has developed in vitro culture techniques using shoots micropropagation and somatic embryogenesis for cloning tropical pine genotypes selected for their rapid growth and productivity. For agricultural sector, KLON has responded to market needs by developing micropropagation methodologies for clone production of fruit species genotypes of Prunus spp., Olea spp., Pistacia spp. and Juglans spp. selected for their characteristics of resistance/tolerance to biotic and/or abiotic factors, thus providing solutions to producers that result in greater adaptability and more profitable productions.Being cryopreservation one of the main research areas in which KLON has invested, the company has developed a robust methodology for cryopreservation of Pinus somatic embryogenic lines with successful recovery and regeneration of clones, thus providing a powerful tool for clonal breeding programs. In order to extend our range of services, KLON is currently conducting cryopreservation assays with in vitro-grown shoot tips of fruit species (e.g., *Olea europaea*) in order to provide to our customers long-term storage of viable, genetically stable and improved phytosanitary plant material. KLON has structural capacity to cryopreserve about 35800 samples of selected germplasm. KLON also holds a molecular biology lab, that seeks to offer differentiated analyzes that represent an added value for producers and contribute to the increase of productivity and sustainability of agro-forestry systems.

Keywords: agro-forestry, micropropagation, somatic embryogenesis, cryopreservation, molecular analysis

PL4

Industrial implementation of somatic embryogenesis and genomic selection in white spruce in New Brunswick

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JD Irving Limited (JDI) is a forest products company with large forest land holdings in Eastern North America. The company has been active in tree improvement since late 1970s. During early 1990s, the company integrated somatic embryogenesis (SE) in its spruce breeding program and developed a multi-varietal forestry (MVF) strategy. The current production of varietal trees by SE for use in MVF is about 600,000 per year; however this number is to be increased to over a million trees per year by 2019 owing to the development of semi-mechanized somatic tree production system. Data obtained from Varietal field tests at age 15 showed large genetic gains. For example, the deployment of MVF using top 10% of varieties (e.g., top 30 varieties out of 315) in the test would result in a realized gain of 13, 21, and 56% for height, diameter, and volume, respectively, when compared to the test average, and the test average is the expected output from the seed orchard breeding. Currently, genomic selection model is being developed for the next generation improvement (Forward GS) that will enable deployment of high-value varieties with mature traits (e.g., wood density, growth at rotation age, etc.) at very young seedling stage. The main challenge for implementing MVF at this time, however, is relatively high cost of SE seedling production. Thus, further refinements of the mechanized embryo handling is necessary.

Keywords: Genetic gain; Genomic selection; Mass clonal propagation; Mechanized SE handling; Multi-varietal Forestry; Tree breeding

PL5

New method of initiation and multiplication culture cycling of somatic polyembryogenesis of Loblolly pine

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Good quality zygotic like somatic embryo development of pine is a major problem. Industries did not able to commercialize this technology because of this problem. In pine system we initiate the cultures from cleaving embryos which are going to degenerate at later stage inside the seed and we do not initiate cultures from dominant embryo which developed full mature embryo. All other conifer species cultures are initiated from dominant embryos. Malin et al 2017 suggested that there is high risk of embryogenesis of Scot pine and probably all Pinus, established cultures from cleavage zygotic polyembryony. Therefore, we must initiate cultures from dominant zygotic embryo. Culture cycling during multiplication is another problem due to same cleavage polyembryony. Cultures do not produce embryos all the time after plating, decline in embryo yield and finally no embryos or poor-quality embryo development. In this presentation I will discuss new method of initiations and possibility of establishing cultures from dominant zygotic embryo instead of cleaving polyembryos. I will also discuss culture cycling to understand multiplication and correct stage of cultures for plating.

Keywords: embryos, initiation, culture cycling, polyembyony

Malin Abrahamsson *et al*. (2017). In Vitro Cell.Dev.Bio.-Plant. 53.86-96

Gupta PK (2016) Method for initiation of somatic embryos of pine. US Patent 9,374,954

PL6

An overview on somatic embryogenesis and applications in woody plants

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The ever-increasing world population is continuously increased demand for tree products and fruits etc., which has put immense pressure on the world's supplies of trees and raw material to the industry. There are already visible sign of the negative impact of climate change, deforestation, environmental pollution on forestry, food and agriculture, and socio-economy. Plant tissue culture has made rapid progress in plant regeneration from different explants in woody plants, e.g. immature zygotic embryos, shoot tips and adventitious buds for somatic embryogenesis, micropropagation via organogenesis, and genetic transformation. The application of these techniques has facilitated readily plant regeneration in major woody plants- pines, date palm, citrus, mango and many others. Somatic embryogenesis has been utilized for in vitro conservation of genetic material, bioreactor for growing embryogenic cells, in vitro mutagenesis, long-term germplasm storage, gene transfer, and germplasm exchange. Recently published book in 2 volumes, Stepwise protocols for somatic embryogenesis of updated important woody plants (1). However, problems still remain such as genotypic influence, maintenance of genetic fidelity, and poor germination rate of somatic embryos that have hindered their commercial uses. Micropropagation via organogenesis is routinely used for clonal propagation and large-scale production of vegetative propagated plants, especially woody and fruit trees. In vitro mutagenesis is carried out by mutagen treatment of explants shoot meristem, embryogenic cultures (2, 3). Mutagen-treated shoot meristems are cultured in vitro to regenerate shoots followed by root formation; mutants are selected under the selection pressure e.g. disease, salt, drought. The regenerated mutant plants, e.g. citrus is grafted on a root stock or in vitro shoot multiplication for 3-4 generations to dissociate chimera in order to develop true mutant lines. The selected mutant plants with desirable traits are evaluated in the greenhouse and finally to the field evaluation; use them for commercial production, germplasm conservation, and crop improvement.

Keywords:. Somatic embryos, micropropagation, organogenesis, in vitro mutagenesis

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2. Jain, S.M., Ochatt, S.J., Kulkarni, V.M. and Predieri, S. 2010. In vitro culture for mutant development. Acta Hort. 865:59-68
3. Suprasanna, P, Ganapathi, T. R., Ghag, S.B. and Jain, S.M. 2017. Genetic modifications of horticultural plants by induced mutations and transgenic approach. Acta Hort. 1187, 219-232

Biotechnological tools to produce superb trees in the green era

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Owing to increasing human population and the increasing global demand for wood, consumption is exceeding the natural rate of regeneration in many areas (Fenning and Gershenzon 2002). For this reason, it is necessary to enrich traditional breeding programmes with biotechnological tools able to increase the quantity and quality of forestry plants produced. FAO's definition of forest biotechnology encompasses different techniques for cloning forest trees. Using in vitro technologies, organogenesis is generally restricted to the young seedling as explant source (Bonga 2017). For this reason, initially, organogenesis techniques in pinus species in order to produce clonal plants from selected seeds were developed (Moncaleán et al. 2005; De Diego et al. 2011; Montalbán et al. 2011). Then, in order to reproduce exactly the genotype of the donor plant, adult trees were used applying various rejuvenating pre-treatments, e.g., pruning, and spraying with cytokinins (Monteuuis et al. 2011), using vegetative buds of different Pinus species (De Diego et al., 2008; 2010; 2010b; Montalbán et al. 2013) or fewer needle primordia of 3- and 7-year-old trees (Prehn et al. 2003). After getting this extraordinary goal, we realised all the problems associated at this technique: low in vitro rooting, small acclimatization percentage, poor growth, etc..For all these reason, in 2007 we comprised all our efforts in the development of somatic embryogenesis systems. Somatic embryogenesis is a fascinating developmental pathway through which plants can be regenerated from bipolar structures derived from a single or a few somatic cells that it was first described more than 50 years ago in carrot by Reinert (1958) and Steward et al. (1958). *Pinus* spp. somatic embryogenesis presents different inconveniences. During the last years, we were focused in overcoming some of the problems: the competence window problem (Montalbán et al. 2014), the low initiation frequencies (Montalbán et al. 2012), the low rates of maturation (Montalbán et al. 2010), poor germination rates (Montalbán and Moncaleán 2018), low regeneration capacity in conserved cell lines (Montalbán and Moncaleán 2017), etc.. Moreover, we developed combined systems to increase the efficiency of SE in embryogenic cell lines with recalcitrance to be cryopreserved (Montalbán et al. 2011) and procedures in different conifers species (Montalbán et al. 2013) including hybrids (Hargreaves et al. 2017). Parallel, one of our main research areas of interest was the study of the physiological mechanism controlling the tolerance to drought conditions in *Pinus* species (De Diego et al. 2012; 2013a, b; 2015). During the last years and taking into account all the knowledge generated as well as the fact that it has been found that different temperatures applied during the process of embryo formation produced clonal somatic plants with different phenology (Kvaalen and Johnsen 2008), our challenge is being to modulate the drought tolerance in *Pinus* spp; Different stressful environmental conditions has been applied along the different stages of somatic embryogenesis [initiation (García-mendiguren et al. 2015; Pereira et al. 2016), proliferation (Pereira et al. 2017) and maturation in order to obtain clonal plants with different degrees of water stress tolerance. Preliminary results have showed that somatic plants coming from EMs initiated at lower temperatures showed higher water use efficiency that control ones (Montalbán et al. 2016). At the same time, aminoacid and sugars analysis and the ultrastructure at cellular level was studied in order to know the structural changes succeed after extreme temperatures (30,40,50 and 60ºC) as well as the metabolites involved in the different SE response.

Keywords: Abiotic stress, conservation, embryogenic cell lines, metabolites, organogenesis, physiological mechanism, somatic embryogenesis.

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PL8

Progress in somatic embryogenesis of Japanese conifers

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Cryptomeria japonica, *Pinus thunbergii, P. densiflora, P. armandii* var. *amamiana, P. luchuensis*, *Chamaecyparis obtusa, C. pisifera, Picea koyamae,* and *P. maximowiczii* 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 (SE) is the most promising technique for mass propagation of clones, and for plant regeneration in genetic transformation protocols used in basic studies and in tree improvement programs. **T**his presentation reports recent progress over the last decade in protocol development for SE in Japanese conifers. After the first paper on plant regeneration *via* SE in *Cryptomeria japonica* (Maruyama et al. 2000), several results on enhancement of somatic embryo production and plant conversion efficiency were published in Japanese conifers. The status in protocol development, including embryogenic cell induction, proliferation of embryogenic cultures, somatic embryo production, germination, and plant regeneration from somatic embryos are described. Embryogenic cultures initiated from megagametophytes containing zygotic embryos were maintained and proliferated in a medium containing 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine, sucrose, and glutamine. Then, somatic embryo maturation was performed on medium containing maltose, activated charcoal, abscisic acid, and polyethylene glycol (PEG). Addition of PEG to the medium dramatically stimulated embryo maturation and resulted in an enhanced yield of mature embryos as the PEG concentration is increased. Although the 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 conifers. Somatic embryos germinated and then converted into plantlets after their transfer to plant growth regulator-free medium. However, for the Japanese pine species, desiccation of embryos after PEG-mediated maturation was found to be essential for achieving both high germination and high conversion rates. In contrast, when somatic embryos of Japanese pines were matured on PEG-free medium but containing a high concentration of gellan gum, embryos readily germinated without any post-maturation treatments. Scale-up production in liquid medium has been also examined in order to improve the propagation rate.

Keywords: clonal propagation, gellan gum, polyethylene glycol, embryo desiccation, somatic plants

Induction of somatic embryogenesis in leaf and shoot apex explants derived from adult trees: a case study in an oak species

Vanesa Cano, Corredoira E, Martínez MT, Cernadas MJ, San José MC

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Somatic embryogenesis (SE) is a powerful tool for forest tree improvement and is considered one of the best ways of regenerating woody plants in vitro (Vieitez et al., 2012). However, the full potential of this micropropagation technique has not yet been developed for woody species, because it is difficulty to induce somatic embryos in explants obtained from adult trees (Ballester et al., 2016).

During the last few years, great advances have been made in inducing SE from selected adult trees of the genus *Quercus*, mainly using leaves and shoot tips as initial explants (Corredoira et al., 2014). Selection of the most reactive initial explant and the time of collection are critical factors for clonal propagation via SE. Leaf explants excised from epicormic shoots and from shoot cultures derived from mature trees have proven suitable sources of tissues for initiating SE in several species of oaks, including the economically important *Q. suber*, *Q. robur*, *Q. alba*, *Q. rubra* and *Q. bicolor*. Similarly, SE is also possible with apex explants isolated from shoot cultures in these species and also *Q. ilex*. The use of in vitro shoot cultures provides a continuous source of explants for SE while avoiding the effects of seasonality, physiological variations, etc. observed in field-grown plants. In addition to the type of explant and its physiological state, the genotype and also the concentration, type and regime of auxin used in induction media are critical factors in the induction of somatic embryos.

Here, we summarize the advances made in inducing SE in leaf and shoot apex explants from adult trees of the most important species of European and American oaks.

Keywords: Forest biotechnology, *Quercus* species, tree breeding, somatic embryos.

Ballester A, Corredoira E, Vieitez AM (2016) Limitations of somatic embryogenesis in hardwood trees. In: Park Y-S, Bonga JM, Moon H-K (eds) Vegetative propagation of forest trees. National Institute of Forest Science (Nifos), Seoul, Korea, pp: 56-74.

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Vieitez AM, Corredoira E, Martínez MT, San José MC, Sánchez C, Valladares S, Vidal N, Ballester A (2012) Application of biotechnological tools to *Quercus* improvement. Eur J Forest Res 131:519-53.

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PL10

The ups and downs of developing hybrid sweetgum varieties for the U.S. bioenergy and pulp and paper industries: a 20-year case study

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Application of hybrid breeding to forest trees has resulted in some very useful and productive genotypes for some forest crops, such as poplars and some pines, but it has not been a very widely used tool in U.S. plantation forestry. Similarly, somatic embryogenesis, which at one time appeared to have great potential for production of clonal southern pines and other conifers in the U.S., has yet to make significant contributions to planting stock. Combining these two technologies, however, is a powerful approach that created a viable product with real economic potential in the southeastern U.S. A project initiated in the late 1990s focused on the production and testing of embryogenic cultures derived from seeds resulting from controlled pollinations between selected American sweetgum (*Liquidambar styraciflua*) and Chinese sweetgum (*Liquidambar formosana*) parents. Once initiated, embryogenic cultures were verified to be hybrids using RAPDs, and suspension cultures were used to produce thousands of somatic embryos. Somatic seedlings produced from the embryos displayed a range of growth rates and habits in field tests established by International Paper Co. and ArborGen Corp. on multiple sites. Some of the clones displayed faster growth rates than elite native sweetgum clones, as well as significantly higher wood specific gravity. Others showed potential for use as landscape trees and ornamentals, with dwarf phenotypes and striking fall color. Copies of the hybrid cultures remained in cryostorage while the field tests were in progress, but the elite clones could not be recovered for scaled-up production. Instead, a method we had previously developed for initiating embryogenic cultures from sweetgum inflorescence tissues was used to start new embryogenic cultures from the top clones growing in a 7-year old field test, which had begun to produce flowers. Plantlets regenerated from these cultures were used by ArborGen to establish hedges for scaled-up production of rooted cuttings. During 2015-2017, approximately 750,000 trees, representing four elite hybrid clones, were planted by private landowners in four states, with the goal of providing a ready source of hardwood fiber for pulp mills in their areas. Although ArborGen recently ceased production of the hybrid clones, at least for the present, if the clones planted so far continue to perform well, they still may provide a new fiber crop for pulp and paper as well as for biomass energy applications.

Keywords: *Liquidambar styraciflua*, *Liquidambar formosana*, hybrid breeding, somatic embryogenesis, cryostorage

Somatic embryogenesis and other vegetative propagation of native forest tree species in north China: current status and issues should be striving solved near future

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The round wood production in 2016 was 3737 million m³ which was increased by 1%, 8% and 19% compared with that of in 2015, 2000 and 1980 respectively (FAO, 2016). Plantation would have to provide 50%-70% of timber in future (Lelu-Walter et al., 2013; Bonga, 2016; Hazubska-Przybyt et al., 2016) and also for most of non-timber forest products such as edible seeds and industrial raw materials. Hence, the development of the improved materials and their related fast and large-scale vegetative propagation system by somatic embryogenesis (SE), rooted cuttings (RC) and grafting are being focus concerned (Lelu-Walter et al., 2013, 2016ab; Hazubska-Przybyt et al., 2016; Park, 2014; Trontin et al., 2016; Miguel et al., 2016). Native tree species like *Pinus koraiensis*, *Picea koraiensis*, *P. crassifolia*, *Abies holophylla*, *Catalpa bungei*, *Fraxinus mandshurica*, *Juglans mandshurica*, *Tilia amurensis*, *Quercus mongolica*, *Q. acutissima*, *Q. variabilis*, *Acer mono*, *Syringa reticulata* var. *mandshurica* are the ecologically and economically very important in north and/or northeast China. They are high-value timber production species and in which *Pinus koraiensis* and *Juglans mandshurica* are very important edible nut species, oaks are starch and potential ethanol production species. These tree species are grown slowly and longevous but with short cultivation and breeding history and almost all of them have year on and year off phenomenon. Thus, the genetic improved materials (seeds, seedlings, cuttings and scions) of them are insufficient, breeding of them should be promoted in fast speed and accurately, and the limited improved germplasm materials should be propagated in fast and large-scale pattern, i.e. developing and applying somatic embryogenesis and other vegetative propagation methods. But there are many issues which are hindered the development and application of these vegetative propagation systems. These issues are resulted from the problems of biological and technological aspects (such as no well-developed and practically applicable SE and RC system for most species, grafting is well developed and applicable for species like *Pinus koraiensis* but it is just suitable for nut production plantation establishment but not suitable for high valued timber production that more than 100 years long time cultivation period is needed, and even so, such grafting system is still lack for most species and some of them are only propagated by seed and seedling system, etc.), as well as social and ecomic aspects (researchers focus on publishing papers but lacks of cognition and sense on practically applicable propagation technique development, high cost for using genetically improved materials and developing and applying SE and other vegetative propagation methods, etc.). These issues should be solved urgently for those native tree species in north China.

Keywords: Native tree species, north China, somatic embryogenesis, rooted cuttings, grafting, timber production, non-timber forest products

PL12

Embryogenic competence acquisition in tamarillo – from tissue to single-cell based analysis

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Plant somatic embryogenesis (SE) is a developmental pathway in which a somatic cell acquires totipotency and evolves into an embryo. Somatic embryogenesis (SE) in tamarillo (*Solanum betaceum* Cav.) is particularly relevant since it allows successful cloning but also developments in cryopreservation and genetic transformation protocols for this species. Also, it has several advantages for molecular analyses and experimental embryology approaches. SE induction in this solanaceous species is achieved by a two-step protocol, by first exposing leaf segments or mature zygotic embryos to MS media with an auxin and high concentrations of sucrose and then transferring the induced embryogenic masses (EM) to auxin-free medium to allow somatic embryos development. The EM formed can be isolated from surrounding non-embryogenic *calli* (NEC) and subcultured, and protocols for the proliferation of tamarillo EM cell suspension cultures were achieved. Based on this system a comparative proteomic profile of EM and NEC of tamarillo was obtained. Moreover, a protein with a putative inhibitory role in the acquisition of embryogenic competence was isolated and characterized. Besides the easy in vitro manipulation of this woody plant, the establishment of a protoplast isolation protocol is also an important tool for functional genomics studies in tamarillo, particularly for cell-type-specific transcript profiling. *De novo* transcriptome sequencing was used to generate sequences from embryogenic and non-embryogenic cells derived from SE induced tissues and obtained through FACS. The de novo assembly generated around 50 000 unigenes, of which 30% were annotated with a significant Blast against the databases. The differential expression of several transcription factors in sorted embryogenic cells revealed a strong epigenetic regulation of cell commitment to embryogenic competence. These results allow the formulation and test of novel fundamental hypotheses regarding the induction of SE.

Keywords: embryogenic competence, FACS, proteome, solanaceae, transcriptome.

Promotion of rooting rate for commercial planting of *Eucalyptus pellita* clone and enhancement of seedling growth by Mycorrhiza application

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Eucalyptus pellita is a native of Iriyan Jaya, Indonesia, and are now commercially planted in Kalimantan and Sumatra, Indonesia. Wood of the tree is known as red mahogany and is used as building, pulp and pellet materials, for charcoal, and others. Increasing wood productivity through commercial planting, requires the development of good clones as well as efficient propagation techniques. Traditionally, *E. pellita* have been mostly propagated through seed. However, in commercial plantations, the use of cuttings of selected clones is preferred to increase productivity. Moreover, vegetative propagation by means of cuttings or tissue culture is dependent on the clone and the rooting capability. Thus, proper cutting technology should be developed according to the target clone for large-scale application. KTH (PT. Korintiga Hutani) has been developing clones of *E. pellita* for the last 20 years and has been planting different clones in large scale since 2003. Clonal productivity at the rotation stage is closely related to the survival rate and individual tree growth. In particular, cutting's propagules, which have poor root development, usually suffer from wind damage in the course of their subsequent growth. Thus, production of good quality cutting propagules with good root system has become an important task. In general, various factors such as mother plant aging (juvenility), mother plant nutrient condition, cutting's environmental (temperature, humidity, lighting etc.) conditions, rooting media (soil), rooting hormone as well as genetic factors are affecting rooting capacity. In this presentation, we have introduced several cutting techniques to improve rooting rate of the clones that are difficult to root, focusing on hedge orchard management, cutting's matrix, IBA treatment, and other acclimatization techniques. We also tested the effect of some mycorrhizal inoculants in order to accelerate the rooted cutting’s growth and improve root system. Four mycorrhizal inoculants from the Philippines and one mycorrhiza species collected from KTH plantations were evaluated in both nursery and field.

Keywords: Plus tree cloning, hedge orchard management, efficient cutting’s techniques, mycorrhizal inoculants

Oral Presentations

O1

Superior ideotypes for Christmas tree production – combining quantitative genetic tools, molecular markers and improved somatic embryogenesis methods

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Christmas trees are a short rotation crop (10y) grown on farmland as well as under forest conditions. A special characteristic compared to timber or fruit production is the fact that the tree itself is the final product purchased by the consumer. Therefore, a number of traits like tree shape, symmetry, branching, color, odor and post-harvest needle retention are of importance. Around 65 million Christmas trees are marketed in Europe every year and a number of species are used - mostly all relying on seed from unimproved material.
In a recent started project we try to overcome shortage in continuous funding and still benefit from breeding initiatives by use of 'Ad hoc breeding' (Xu et al. 2018) where we combine the use of DNA markers and quantitative genetics, and furthermore combine these efforts with vegetative propagation of the best material.
In production stands originating from a clonal seed orchard of *Abies bornmülleriana* (limited number of parents, e.g. 80) parentage was established by use of SSR markers. Breeding values for Christmas tree characteristics were estimated based on measurements in the stands and quantitative full sib analyses. Later, seed where harvested after open pollination on the six best parents in the seed orchard and a total of 1300 individual embryos were isolated for initiation of somatic embryogenesis (SE). Initiation was achieved for around 300. To identify superior clones and limit costs - paternity of all initiating embryos (mothers known) was established using the SSR markers and only the 60 best parent combinations are now under proliferation.
The SE procedures of proliferation, rooting and development of the embryo into small emblings have lately undergone an improvement. Based on the improved protocols clonal experiments are planned for field testing of Christmas tree quality. All genotypes are cryo-preserved for potential commercial large scale use.

Keywords: breeding, somatic embryogenesis,

Reference Jing XU, Ulrik B. NIELSEN, Ole K. HANSEN 2018: Ad hoc breeding of *Abies bornmülleriana* for Christmas tree production using a combination of DNA markers and quantitative genetics—a case study. Tree Genetics and Genomes (accepted).

O2

Benefits and risks of using clones in forestry

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The use of vegetative propagation in forestry has a long history. In this presentation, the genetic gain from clonal forestry relative to family forestry is reviewed. Both theoretical studies and experimental data from progeny and clonal trials indicate that extra genetic gain (5-25%) is possible in conifer from clone testing and deployment relative to deployment of family forestry, effectively doubling that achievable from family forestry within the same generation. There are three perceived risks from using clones in forestry: (1) risk of plantation failure, (2) risk of diversity loss at the forest and landscape levels, and (3) risk associated with success rate of vegetative (or SE) propagation. Three theoretical models are reviewed and described to assess risk and to determine the number of clones required to mitigate these risks. All studies support that a "safe" number of clones is between 5 and 30. Genetic gains and experiences are reported for individual species, particularly in conifers, as well as in Eucalypts. The combination of genomic selection with somatic embryogenesis has the potential to accelerate the development of clonal forestry by shortening clonal testing or omitting long-term clonal testing completely.

Keywords: Clonal forestry, Genetic gain, Vegetative propagation, Risks, Benefits

O3

A new generation of chestnut rootstocks with improved resistance to *Phytophthora cinnamomi* Rands

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The main threats of European chestnut in Portugal and Europe are those resulting from biotic stresses, particularly those caused by oomycetes of genus *Phytophthora* spp. causing root rot, blight disease caused by the fungus *Cryphonectria parasitica* (Murrill) ME Barr and also the pest gall wasp, *Dryocosmus kuriphilus* Yasumatsu, introduced in Europe in 2002. For both blight and gall wasp, biological control takes time but is available, the first through hypovirulent strains of the fungus, the second through parasitoids, as *Torymus sinensis*. For ink disease there is no biological control available and chemical methods applied have not shown lasting efficacy with negative impact on the environment, so the control of the disease requires the use of improved plant materials with resistance to *Phytophthora cinnamomi*. A breeding program for resistance of chestnut to *P. cinnamomi* was initiated in 2006 in Portugal by INIAV, based on controlled crosses, using the Asian resistant species, namely *C.crenata* and *C.mollissima*, as donors of resistance. New genotypes were selected so far, from the breeding program, with improved resistance to *P. cinnamomi*, (1) and large-scale cloning by micropropagation is being performed. Candidate genes and QTLs were also identified (2,3). The comprehension of the host-pathogen system in *Castanea* spp using histopathology is being performed and the functional validation through genetic transformation is being implemented as well. The ultimate goal of the research program is to select molecular markers linked with resistance genes, for an expedite selection of improved genotypes, from the breeding program and to disclose new rootstocks to the market, to supply the great deficit of improved plant materials. An overview of the research program in progress, with the main results obtained so far, together with the pilot unit that is being created in Alto Alentejo region, for the large scale cloning of the new rootstocks, will be presented.

Keywords: *Castanea*, breeding, biotic stress, root rot

1 – Santos C, Machado H, Correia I, Gomes F, Gomes-Laranjo J e Costa R (2014) - Phenotyping Castanea hybrids for *Phytophthora cinnamomi* resistance. Plant Pathology doi: 10.1111/ppa.12313.

2 - Santos C, Nelson CD, Zhebentyayeva T, Machado H, Gomes-Laranjo J, Costa RL (2017) First interspecific genetic linkage map for *Castanea sativa* x *Castanea crenata* revealed QTLs for resistance to *Phytophthora cinnamomi*. PLoS ONE 12(9): e0184381. https://doi.org/10.1371/journal.pone.018438

3 - Santos C, Duarte S, Tedesco S, Fevereiro P e Costa Lourenço R (2017) Expression profiling of *Castanea* genes during resistant and susceptible interactions with the oomycete pathogen Phytophthora cinnamomi reveal possible mechanisms of immunity. Frontiers in Plant Science, doi: 10.3389/fpls.2017.00515

O4

An assessment of hybrid poplar leaf and wood traits in micropropagated plants and plants propagated from root cuttings: gas exchange, vascular, nanomechanical and cell wall compositional characteristics

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Understanding the physiological, vascular and biomechanical processes that allow micropropagated plants to modify their phenotype in response to environmental conditions can help to improve both field performance and plant survival. To identify differences between the hybrid poplar *Populus tremula* × (*Populus* × *canescens*) plants propagated from in vitro multiple shoot cultures and those from root cuttings, we assessed any changes in both leaf traits and woody cell wall composition in the sixth growing season following field planting. Measurements and comparisons were made of leaf growth, photosynthetic and vascular traits, nanomechanical properties, content of cell wall components, lignin monomer composition, and the macromolecular traits of both lignin and cellulose. The micropropagated plants showed significantly higher values for leaf area, leaf length, leaf width and leaf dry mass. The greater leaf area and leaf size dimensions resulted from the higher transpiration rate. Also, the micropropagated plants reached higher values for the content of cellulose, glucose and mannose, as well as for the nanomechanical dissipation energy of tracheary element cell walls which may indicate a higher damping capacity within the primary xylem tissue under abiotic stress conditions. The performance of the plants propagated from root cuttings was superior for instantaneous water-use efficiency which signifies a higher acclimation capacity to stressful conditions during a severe drought, and also for the content of hemicelluloses, xylose and arabinose. Similarities were found among the majority of the examined leaf and wood traits for both stock types, including leaf mass per area, stomatal conductance, net photosynthetic rate, hydraulic axial conductivity, indicators of xylem vascular architecture, modulus of elasticity, adhesion, deformation, and the macromolecular traits of both lignin and cellulose. This research revealed that there was no decrease in the leaf physiological performance which could be attributed to the micropropagated plants. In addition, from a viewpoint of cell wall composition, a lower xylose content in wood of the micropropagated plants was over-balanced with a higher glucose content which may signify a greater industrial benefit in bioethanol production from this lignocellulosic feedstock.

Keywords: cell wall nanomechanics, glucose, *Populus* × *canescens*, transpiration, xylose

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O5

Strawberry tree (*Arbutus unedo* L.) breeding: hybridization and polyploidization

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Strawberry tree (*Arbutus unedo*, Ericaceae) is a small tree growing around the Mediterranean basin and Atlantic coast, including Portugal. It is well adapted to stress conditions and shows a great regenerative capacity following forest fires making it an interesting species for reforestation programs. Due to its ability to prosper in poor marginal soils and its fairly tolerance to common pathogens, strawberry tree has been seen as a good alternative for farmers due to its potential for fruit production as well as ornamental. During the last years a considerable effort has been carried out in order to convert strawberry tree into a species more interesting for fruit production through the selection and propagation of selected trees by *in vitro* culture methods, through hybridization assays and by polyploidization experiments. The objective of this work was the induction of polyploid plants, as well as the understanding of the pollination mechanisms on strawberry and the obtention of hybrid plants.

Selected trees were propagated through different *in vitro* culture techniques such as axillary shot proliferation and organogenesis. 300 *in vitro* shoots (2n) from 3 different genotypes were then submitted to a treatment with c-mitotic agents (colchicine and oryzalin), for different periods of time and at different concentrations. The survival rate was close to 20% and plant ploidy was evaluated by flow cytometry, using *Solanum lycopersicum* as a control. 15 of the induced shoots were found to be mixoploid but only 3 tetraploid plants were produced, a conversion rate of 1%. Moreover, all the mixoploid plants reverted to its initial ploidy level.

A morphological study of the pollen was carried out, as well as germination tests. The pollen tetrads of strawberry tree have a medium size and can easily be germinate on a medium with 15% sucrose. Controlled *in vitro* and field pollinations were used to understand the pollination process and to obtain hybrids between selected trees. No auto-incompatibility barriers were found on the *in vitro* assays and some plantlets from the controlled crosses have been obtained following seed germination and will be transferred to the field for phenotype evaluation. Genotypes selected for productivity and stress tolerance arising from these crosses will be propagated by the *in vitro* culture techniques.

Keywords: breeding, hybridization, micropropagation, pollen, polyploids

O6

Clones à la carte – how proper organisation of large clone and data collections can support researchers, breeders and customers

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The project, DendroMax“ aims at an operative integration of somatic embryogenesis of coniferous timber species into established classical breeding programs in Germany. One prerequisite of large scale implementation of clonally propagated forest material was the establishment of a large repository of clones (ca. 400) of *Larix x eurolepis* derived from controlled crossings. In order to manage information on these clones, a database was assembled which incorporates extensive but concise data on key characteristics of each genotype. This database serves as a nexus between basic scientific data collection and communication for practical utilization by the project partner Staatsbetrieb Sachsenforst, the public enterprise for forestry in the state of Saxony. For that purpose, the data not only comprise information about the *in vitro* process chain, but also the plantlets' performances during acclimatization and in the field. Regarding individual genotypes, characteristics of each step *in vitro* and *ex vitro* were collected in at least two repetitions, including but not limited to: propagation behavior of the embryo-suspensor-mass, maturation yields and quality, germination yields and quality, acclimatization yields, growth behavior in the greenhouse and the field. Especially data about growth habits (i.e. rate of increase of dendromass, drought tolerance etc.) from currently ongoing field trials under diverse ecological conditions are going to be of great interest for potential future customers.

The compilation of such a database greatly simplifies the handling of large genotype repositories for the practical application in industrial scales. It allows for quick metadata analysis across the clones and clonal families to probe for non-obvious influences in the process chain and might help to alleviate bottlenecks during production. Furthermore, it can easily be adjusted to serve as a marketing tool for commercial distribution in such a way, that private and public customers are able to choose clones and/or clonal blends in a quasi *à la carte* manner.

Keywords: Somatic embryogenesis, conifer breeding, data processing, large-scale implementation

Biofactory movil: New access alternative to the massive propagation of clones, by tissue culture

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In the last 30 years the use of Biofactories in Latin America has served for the massive propagation of elite plants, sanitation and rapid increase of agamic seeds in plant species of agricultural interest. The significant initial investment in building and equipments, the high financial cost of working capital in Biofábricas with high productive capacity and the need of highly qualified people, make this type of investment limited to public or big private enterprises. Even so, not always the success of the project and the return on investment in the short or medium term is ensured. A prefabricated and portable BioFactory has been designed as an installation with lower initial investment and low maintenance cost, taking into account the possibility of being located in areas close to the client or agroindustrial producer. This mobile design requires a less intensive use of qualified human resources, maintaining rigorous in asepsis assurance of the in vitro production process and the functionality in the orderly flow of production, guaranteeing efficiency, productivity and therefore, a competitive cost of the final product. Since its installation, the Biofábrica Misiones (Argentina) has gained experience and knowledge in the adjustment and development of application several micropropagation protocols through organogenesis of plant species, and in its aplication and validation in the field. This accumulated experience has allowed to conceive and to design a portable, flexible and functional model of biofactory. This mobile design has been patented and the first prototype has been developed. Its flexibility and functionality makes it possible to adopt and to adapt efficient technologies in terms of productivity indicators by species or varieties, also maintaining or increasing the coefficients of multiplication, with optimal asepsis, a maximum and rational use of the interior space, and a good plants per operator ratio. The portability of the model makes it possible also to provide the final product at the destination site. Successful transfer and explotation of this mobile Biofactory will be supported by the experience of Biofabrica Misiones in the adjustment and scaling of micropropagation protocols and also by knowledge and training of the operators who will work there. In order to ensure the trust of the client and stakeholders associated with the project, the processes and products will be validated and certified under international standards through a quality management system. Technical assistance provided in the field will assure the planting or sowing and efficient development of the products. Finally, the investment return on the short term is projected through the commercialization by sale or lease oriented to small and medium scopes companies and agroindustrial sectors with a high demand of elite plants or healthy and certified agamic seed, and by a network of technical assistance of biotechnologists.

Keywords: Biofactory, laboratories culture, tissue plant, tissue culture

O8

Implementing genomic selection for multi-varietal forestry of white spruce (*Picea glauca*) in New Brunswick, Canada

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Genomic selection (GS) yields a large genetic gain and shortens the duration of tree breeding by avoiding or eliminating the need for lengthy testing periods. For example, in multi-varietal forestry, with the aid of vegetative propagation techniques such as somatic embryogenesis and rooted cuttings, the time savings of GS can be about 15 years. Mature commercial traits such as volume growth and wood density are difficult to assess at an early age, and the early selection for seed orchard based breeding in conifers has been ineffective. We evaluated the feasibility of implementing GS in a white spruce (Picea glauca [Moench.] Voss) breeding program in eastern Canada. For GS modeling, we assessed the growth traits of a clonally replicated genetic test at age 24 that contains 15,000 vegetatively propagated trees. We sequenced 351 clones of these white spruces with ~4,100 single-nucleotide polymorphism markers on the "Picea Infinium chip" that were employed in the "FastTRAC project." We found that GS combined with vegetative propagation, for example, can result in about 75% greater volume growth when compared to the traditional pedigree-based method used with seed orchard production as well as eliminating genetic testing.

Keywords: Breeding value; BLUP analysis; Genetic gain; Genotyping; Somatic embryogenesis; Tree breeding

O9

Effects of meta-topolin derivatives and temporary immersion on hyperhydricity and shoot proliferation in *in vitro* of *Pyrus communis*

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Although several studies have been reported on the micropropagation of pear and its rootstocks, to date none of them had been efficient for mass production of these plants in bioreactor systems. Thus, micropropagation of pear shoots was investigated in a temporary immersion bioreactor system SETIS™ and on a conventional semi-solid medium. The occurrence of hyperhydricity was affected by the immersion interval and the cytokinin type. Although hyperhydricity was inevitable on semi-solid medium and in SETIS™ when 5 µM benzyladenine was applied, it could be reduced in SETIS™ by lowering the immersion frequency to 3 times per day. Applying 5 µM *meta*-Methoxy topolin riboside (MemTR) or *meta*-topolin riboside (mTR) completely avoided hyperhydric shoot formation. The bacteria contaminated 'Mahdia 6' didn't grow at all on semi-solid medium, although 0,1 % Plant Preservative Mixture (PPM™) was added. Among the tested immersion conditions, immersion for 60 s every 8 h reduced vitrification and improved proliferation. For both 'Arbi' and 'Mahdia 6', 5 µM MemTR induced the highest number of shoots per explant whereas the highest leaf area was obtained with 5 µM mTR. Also in liquid medium, the highest shoot length was recorded with 5 µM mTR for 'Arbi' and 5 µM MemTR for 'Mahdia 6' in SETIS™. Furthermore, *in vitro* rooting of pear plantlets (100%) was achieved on rooting media: half-strength MS (Murashige and Skoog, 1962) medium, enriched with 5 µM *t*-CA *(trans*-cinnamic acid).

Keywords: topolins, hyperhydricity, temporary immersion bioreactor

O10

Somatic embryogenesis of Norway spruce in Finland – seven years from start to first commercial pilots

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A targeted effort to adapt somatic embryogenesis (SE) as a complementary propagation technology of Norway spruce (*Picea abies* L. Karst) was launched in Finland in 2011. Motivation for this attempt was raised by a shortage of high-quality, bred seed for forest regeneration, caused by irregular flowering of the species and problems in seed-orchard production. Simultaneously, need for hardy ornamental conifers for growing landscaping sector was recognized. Later on, Christmas tree producers have presented a great interest towards spruce varieties with desired crown form. Building up SE-pipeline in Finland has mostly relied on published protocols. It was observed, however, that cryopreservation of SE materials and the later phases of SE require further development to be successful enough with Finnish material. Thus research has been focused on developing reliable cryo-protocol (see Varis et al. 2017 Cryobiology 76:8–17), and on laboratory-nursery interface (see presentation by Tikkinen). Currently, over 3000 SE-lines originating from the elite trees of the tree breeding program have been initiated and cryopreserved by Luke. Of these over 850 are in testing process covering embryo production in laboratory, embling conversion and early growth in nursery, and also the first field tests with emblings have been established. In addition, over 300 SE-lines from special forms have been cryostored and the selected ones of them are tested for their suitability as ornamental or Christmas tree varieties. Regulatory issues involved in SE propagation have been managed in close collaboration with national authority Evira. The first lot of SE material was registered as forest regeneration material in 2017 (basic material: *Parents of families, Qualified*), and authorized for commercial production. First two master certificates for Norway spruce emblings in Finland were issued during 2018. In practice, this is bulk propagation of SE-lines originating from12 full-sib families. SE-lines with ornamental value are also available for commercial propagation.

To enhance SE propagation, price of emblings needs to be reduced. Cost analysis has shown that manual labor, especially in the later phases of SE, creates majority of production costs. The research for automation is going on.

Keywords: ornamental varieties, *Picea abies*, registration of forest regeneration material, somatic embryogenesis

O11

Field testing clones of North American willow (*Salix*) species for biomass production and ecological restoration on highly disturbed sites

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Willows (genus *Salix*) are widespread across the northern hemisphere with an estimated 350 species. They have special ecological importance on highly disturbed, low-fertility sites where they can be useful in protecting soil and water quality by mitigating the harmful effects of wind and water erosion. Generally, willows are easily propagated vegetatively, they grow fast, and are adaptable to a wide range of site types, making them useful for both bioenergy production and habitat restoration for either wildlife or domestic grazing animals within silvo-pastoral systems. Our common-garden field-testing research on eight widely distributed native North American willows is aimed largely at land reclamation, forest restoration, and protection of water quality on highly disturbed sites. In this presentation, we highlight species and clonal variation in growth traits among several promising willows using a population genetics approach through common-garden field-testing. Willows are underutilized species with great potential for biomass and forage production, as well as for phytoremediation and land reclamation, with tremendous clonal variation in traits related to their many uses.

Keywords: Common-gardens, Land reclamation, Population genetics, *Salix*, Species and clonal variation

O12

Mitigating the loss of genetic material and increasing the yield of emblings in late phases of Norway spruce SE

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Somatic embryogenesis (SE) could provide a solution to intermittent shortages of improved forest regeneration material of Norway spruce (*Picea abies* L. Karst.) and rapidly facilitate breeding results. However, there are still large bottlenecks in SE, e.g. in the germination and acclimatization phases, which greatly affect the final outcome of somatic embryo plants (emblings). To improve yield and quality of emblings the late phases of SE were refined together with the laboratory-nursery interface (Tikkinen et al. 2018 a, b). Refinements included; decreasing ABA concentration in maturation medium, cold storing embryos on filter papers in maturation dishes, short one-week *in vitro* germination media without inorganic nitrogen in 18:6 photoperiod in similar temperatures compared to seedlings, transplanting germinated emblings straight to growing containers in nursery greenhouse. Decreasing ABA concentration resulted in over 100% increase in embryo yield per gram of fresh embryogenic mass. Cold storage on filter paper, lower nitrogen content in the germination medium and one-week in vitro germination – resulted in an 88% higher survival and 28% higher growth compared to the poorest, reference treatment in the same test year. From 12 registered full-sib families (*Parents of families, Qualified*), 853 genotypes have been tested for their laboratory and nursery performance. Genotypes with high embryo production capacity (over 200E/gFW) were found from all families. With the best protocol, survival rate of 80% from 121 genotypes (11 full-sib families) was recorded among 4006 transplanted emblings from 127 genotypes.

Key words: *Picea abies*, somatic embryogenesis, maturation, germination, nursery

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Tikkinen M, Varis S, Peltola H & Aronen T (2018) Improved germination conditions for Norway spruce somatic cotyledonary embryos increased survival and height growth of emblings. *Submitted to Trees*

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O13

Tree Biotechnologies: What has worked and what hasn't worked?

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It is timely to review tree biotechnologies that have worked well for industry and have not worked so well over the past 25 years, and to speculate on the future. Topics covered include the following:

- Tissue culture by organogenesis, somatic embryogenesis and combinations;
- Manual transfers vs automation or semi-automation of Stage II and Stage III tissue culture (as defined by Murashige);
- Hyperhydricity (vitrification), liquid culture and "bioreactors";
- Cold storage and cryopreservation;
- Importing and exporting tissue cultures;
- Embryo rescue;
- Genetic modification (transformation);
- Somaclonal variation;
- Field trials and integration with other vegetative propagation technologies.

It is also important to be aware of other technologies that are complementary to tissue culture now and in the future, where "global warming" and other factors such as "pests and pathogens" could be an issue, including:

- Other species and new cultivars;
- Endophytes for adding new benefits to a cultivar. E.g. disease resistance and new biochemical or biological products;
- DNA technologies to reduce time to market or enhance identification of "solutions";
- Plant factories in controlled environments.

Whether all of these biotechnologies will be useful for industry in the future remains to be determined, however, industry will need trained people to conduct research in these areas and even more trained people to successfully apply them and measure the economic and environmental impacts.

Keywords: tissue culture, somatic embryogenesis, endophytes, DNA

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O14

Low cost media for improving *in vitro* propagation of woody plant species

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Plant tissue culture is used for large scale plant multiplication, disease elimination, plant breeding and production of secondary metabolites. The high cost of the reagents needed for *in vitro* propagation is one of the limiting factors of these techniques. In a previous report, Phytoplant Research SL -a company specialized in developing the industrial chain of medicinal plants- reported *in vitro* propagation of six *Cannabis sativa* L. varieties using two new culture media (Formula *β*-A and Formula *β*-H). *Cannabis* micropropagation was performed in one step (growth of the aerial part and rooting simultaneously) without sucrose, agar and/or vitamins, and obtained better success rates than by using conventional medium plus vitamins (Codesido et al. 2017). In this study, we investigated the possibility of using these new formulations for the *in vitro* propagation of several woody plant species, including forest and fruit trees. The conventional MS (Murashige and Skoog 1962) and GD (Gresshoff and Doy 1972) formulations were used as controls. Both sucrose (2-3% (w/v)) and plant growth regulators regularly used in these plant species were provided to all tested media. Conventional media were supplied with vitamins described in their formulation while no vitamins were added to the low cost media. To avoid interferences due to previous culturing in conventional medium, the explants were subcultured twice in the new low cost medium before recording the multiplication parameters. Then, survival rate, number of shoots, multiplication rate, shoot length, and rooting capacity were recorded during three subcultures. The media prepared by using Formula *β*-H produced negative results in two species (*Eucalyptus globulus* and *Genista tridentata*), while in two others (*Betula pendula* and *Prunus avium)* a certain growth was achieved, but without reaching the levels obtained with conventional medium. In contrast, with the media prepared by using Formula *β*-A good results were obtained in five of the six species studied (*Betula pendula*, *Eucalyptus globulus*, *Pyrus cordata*, *Prunus domestica* and *Salix viminalis*). In four of them, the multiplication ability and plant quality were significantly improved with respect to their conventional media. These results demonstrate that Formula *β* based media, even without addition of vitamins, may represent a good choice for the micropropagation of woody plant species.

Keywords: Formula *β*-A, Formula *β*-H, *in vitro* propagation media, plant tissue culture, vitamins.

Codesido V, Casano S, Meyer S (2017) Cultivo *in vitro* low cost: maximizando la productividad. SECIVTV 2017 Plants *in vitro* for the future. Madrid, September 13-15, 2017.

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O15

Effect of CPPU-derived inhibitors of cytokinin oxidase on *de novo* shoot formation

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The level of cytokinin in the plant is controlled by its biosynthesis and degradation. The irreversible degradation of cytokinin is catalyzed by cytokinin oxidase/dehydrogenase (CKX). Phenylurea derivative synthetic cytokinins among which are Thidiazuron (TDZ) and Forchlorfenuron (CPPU) show strong cytokinin-like activity, by activating cytokinin receptors but also as an inhibitor of cytokinin oxidase/dehydrogenase (CKX), thus increases the lifetime of cytokinins and their effects in vitro application.

Three new experimental diphenylurea compounds were developed with theoretical cytokinin-oxidase inhibitory activity. They were tested for their ability to mimic or interact with a cytokinin by *de novo* shoot organogenesis. As for most plants, de novo organ initiation can be achieved in a two-step protocol including a Callus Induction medium (CIM) followed by a Shoot Induction Medium (SIM). Poplar was chosen as a model because of the threshold of its leaves to react on cytokinins by shoot meristem induction. The idea was to boost a suboptimal 2iP concentration by inhibiting its oxidation. Therefore, the new compounds were added in the SIM, alone or in combination with 2iP.

Without 2iP, explants showed a continued callus growth and subsequently root outgrowth. However, no shoots emerged from these calli after 4 weeks of incubation. Calli and root development on the three experimental compounds alone were similar as for the hormone-free media. When 1 µM 2iP was applied, root formation was inhibited and only callus developed. On the other side, shoot regeneration occurred when 1 µM 2-ip was combined with 1 µM of each of the compounds separately. The three variants could be ranked according to their regeneration rate. These results will be discussed in the broader perspective of other available cytokinin oxidase inhibitors, such as TDZ (derivatives), CPPU, INCYDE and phenyl adenine and their possible implication in plant tissue culture.

Keywords: poplar, cytokinin, de novo shoot formation

O16

A fluorine containing topolin cytokinin for plant tissue culture

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The introduction of fluorine in order to improve the pharmacological properties of a drug is a modern trend in medicinal chemistry. Currently, there are about 200 fluorinated drugs on the market (~20% of all pharmaceuticals), with even higher figures for agrochemicals (up to 30%) (Oslovsky et al., 2017). In nature, organofluorine compounds are extremely rare. However, synthetic molecules containing fluorine can be very active. Generally, adding a fluorine atom significantly changes the chemical properties of a molecule because of the great stability of the carbon-fluorine bond. As a result, the half-life of such compounds is extended and their activity increases by enhanced binding interactions, changed physical properties and selective relativities (Hagmann, 2008) Previously, several fluorinated derivatives of naturally occurring isoprenoid cytokinins have been prepared (Haidoune et al., 1998; Clemenceau et al., 1996) and their superior biological activity (when compared with their naturally occurring counterparts isopentenyl adenine and trans-zeatin) have been shown (Clemenceau et al., 1996). However, aromatic cytokinins (Strnad, 1997) are more widely used cytokinins in the micropropagation industry due to their effectiveness and affordability. 6-benzylaminopurine (BAP), however, has disadvantages in some crops. Therefore, we evaluated the application of previously prepared fluorine containing topolin derivatives (Doležal et al., 2006; Doležal et al., 2007). Topolin is a class of cytokinines, closely related to 6-benzyladenine (BA). The effects of 6-(3-fluorobenzylamino)purine 9-riboside (F*m*TR) were compared to other cytokinins. The results regarding adventitious shoot regeneration and axillary shoot proliferation in a number of herbaceous and woody species will be presented. Also the influence of F*m*TR on hyperhydricity and senescence will be treated as well as its metabolism in selected plant species.

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O17

Effect of flavonoid on mycorrhizal synthesis between *Tuber borchii* and *Arbutus unedo* L. *in vitro* plants

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Arbutus unedo L. (strawberry tree) is a Mediterranean species, which became important for forest programs, due to its stress tolerance, regeneration capacity following fires and fruit production. Selected adult plants for fruit production and quality were micropropagated, clonal trials established for clonal evaluation and next new orchards have been established. *Tuber borchii* allows the establishment of more prolific orchards promoting particular advantages conferred by the production of edible mushrooms. Among flavonoid, quercetin is known by stimulate the mycorrhization, promoting the spore germination and hyphal branching. The objective of this study was to evaluate the effect of quercetin addition on mycorrhization with spores of *T. borchii*. Different micropropagated clones and quercetin levels (0.5 – 10 µM) were tested. Perlite was used as substrate for inoculation. The inoculation was performed during *ex vitro* rooting simultaneously with acclimatization. Inoculation and acclimatization was performed in the culture chamber, using closed transparent containers to keep high humidity level. During plant hardening, the levels of humidity were gradually decreased. Six months after inoculation (in the culture chamber), plants were transferred to field containers and roots were analyzed. Clones showed different behavior to quercetin addition. When quercetin was added from to 2.0 – 4.0 µM, an higher mycorrhizae establishment was observed, with presence of mycorrhizal cruciform branches. The most productive clones were selected to establish a field trial to confirm long term persistence of mycorrhizae and to evaluate the fungal colonization level required to guarantee mushroom production. The results will be presented.

Keywords: Arbutoid mycorrhizae, *ex vitro* rooting inoculation, quercetin, strawberry tree

O18

Progress and prospect of vegetative propagation for *Acer mono* Maxim.

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Mono maple (*Acer mono* Maxim.) is an important accompanied tree species in the temperate mixed-broad leaved Korean pine forests in Northeast China. Mono maple is also a really good ornamental species as its trunk is straight and high, and the leaves change its colors in autumn. Generally, seed propagation is the main method for the propagation of *A. mono*, however, since its segregation of characters, the vegetative propagation seems to be better methods to keep the excellent characters for the ornamental plants. The current advances in vegetative propagation of mono maple were summarized from the aspect of techniques including cutting and tissue culture. Soft wood cutting is an effective vegetative propagation method of mono maple, and 90% rooting rate can be reached by using this technique. Chemicals, such as NAA, IBA, and ABT rooting powder and so on are necessary to promote rooting of the cuttings and the using of mixed chemicals has a positive effect on increasing both rate and amount of rooting. The cutting medium, the treatments of the cuttings are also factors that influence the rooting rate and the quality of the rooted cuttings. However, more and more work about soft wood cutting and hard wood cutting of mono maple are needed to make these propagation systems more effective. So far, tissue culture of mono maple was conducted only by shoot proliferation system using sprouting of dormant axillary buds collected on adult trees. Sodium hypochlorite (NaClO) and $HgCl_2$ are always choose as sterilization chemicals. MS can be used as basic medium for the growth of the sprouting and shoot proliferation and growth. 1/2 MS can be used as basic medium for rooting, and the regenerated plants can be obtained from this tissue culture system. However, more and more work about tissue culture of mono maple are needed to make the micro propagation system more effective and to find other more effective methods, for example somatic embryogenesis.

Keywords: *Acer mono* Maxim.; ornamental plants; vegetative propagation; micro propagation; shoot cutting

Epigenetics in trees: a source of plasticity in the context of climate change

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Ongoing global climate changes will impact forest productivity notably through reduced water availability and heat periods. One possibility to adapt is phenotypic plasticity for which epigenetic mechanisms are proposed to be a main source of flexibility. Our objective is to evaluate the potential of DNA methylation to significantly participate to phenotypic plasticity in trees, sessile and perennials organisms with major ecological roles. Over the 10 last years, using an integrative approach with ecophysiological, biochemical, transcriptomics, epigenomics (MeDIP, WGBS, Mobilome) and reverse genetics (RNAi lines) tools, we were able to dissect in the shoot apical meristem (center of the shoot morphogenesis) or somatic embryo the response of trees to environmental variations. This work was assessed in two species, poplar and maritime pine, for two environmental cues, temperature and water availability, in distinct experimental set-ups from *in vitro* cultures, greenhouse to field plantations as well as during the stress or months post-stress. Our recent data (Conde et al., 2017; Lafon-Placette et al., 2018; Le Gac et al, revision; Sow et al., submitted; and unpublished data) showed that Differentially Methylated Regions (DMRs) are associated to active TE and differentially expressed genes with biological functions related to stress response and phytohormone signaling. Altogether, our data proposed that DNA methylation is a source of flexibility associated to phenotypic plasticity in trees opening perspectives for tree breeding and management. The role of epigenetic mechanisms in tree adaptation and microevolution will be also presented in the frame of the starting national project EPITREE 2018-2021 (ANR-17-CE32-0009-01; https://www6.inra.fr/epitree-project/).

Keywords: Shoot apical meristem, somatic embryo, epigenetics, trees, stress

O20

The transcriptome of maritime pine across embryo development

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There are clear differences in embryo development between angiosperm and gymnosperm species. Most of the current knowledge on gene expression and regulation during plant embryo development has derived from studies on angiosperms species, in particular from the model plant *Arabidopsis thaliana*. The few published studies on transcript profiling of conifer embryogenesis show the existence of many putative embryo-specific transcripts without an assigned function. In order to extend the knowledge on the transcriptomic expression during conifer embryogenesis, we sequenced the transcriptome of zygotic embryos for several developmental stages that cover most of *Pinus pinaster* (maritime pine) embryogenesis. Total RNA samples collected from five zygotic embryo developmental stages were sequenced with Illumina technology. A *de novo* transcriptome was assembled as no genome sequence is yet publicly available for *Pinus pinaster*. The transcriptome of reference for the period of zygotic embryogenesis in maritime pine contains 68,031 transcripts, which likely encode 58,527 proteins. The annotation shows a significant percentage, 30.8%, of predicted proteins exclusively present in pine embryogenesis. Functional categories and enrichment analysis of the differentially expressed transcripts evidenced carbohydrate transport and metabolism over-representation in early embryo stages, as highlighted by **t**he identification of many putative glycoside hydrolases, possibly associated with cell wall modification, and carbohydrate transport transcripts. Moreover, the predominance of chromatin remodeling events was detected in early to middle embryogenesis, associated with an active synthesis of histones and their post-translational modifiers related to increased transcription, as well as silencing of transposons. Our results extend the understanding of gene expression and regulation during zygotic embryogenesis in conifer species and are a valuable resource to support further improvements in somatic embryogenesis for vegetative propagation of conifer species. Specific transcripts associated with carbohydrate metabolism, monosaccharide transport and epigenetic regulation seem to play an important role in pine early embryogenesis and may be a source of reliable molecular markers for early embryogenesis.

Keywords: Zygotic embryo, *Pinus pinaster*, embryogenesis, RNA-seq, developmental stages

O21

Transcriptome-wide analysis dissecting transcription factors orchestrating larch tree phase change

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In a previous study, aging effects on wood formation was investigated by measuring the transcriptomic changes of the uppermost main stems of 1-, 2-, 5-, 10-, 25-, and 50-year-old *Larix kaempferi*. Based on the published transcriptome data, here we investigated the transcriptomic changes between juvenile vegetative (1- and 2-year-old) and adult reproductive (25- and 50-year-old) phases, to study the molecular mechanism of phase change. In total, 12,789 transcripts were identified as differentially-expressed genes, including 573 transcription factors. Further analysis showed that 27 transcription factors, belong to 9 families, are common in all four comparisons. The analysis of their expression patterns in six age categories showed that members from AP2, Dof and ERF family were highly expressed in 1- and 2-year-old trees, while members from C3H, G2-like, GRAS, M-type MADS, MYB related, MIKC MADS family were almost undetectable in 1- and 2-year-old trees. Notably, one member from MIKC MADS family was only detected in 25- and 50-year-old trees. These results suggested that (1) phase change might occur in the early stage of *L. kaempferi* tree lifetime, and (2) be controlled by a complex regulatory network composed of different transcription factors, some of which have been certified to play roles in phase change in model plant. These findings not only provide molecular markers to distinguish different stage of tree growth and development, but also improve our understanding of phase transition with tree age increase.

Keywords: Larch, Phase change, Transcriptome, Transcription factor, MADS

O22

Effect of priming during SE maturation on the phenotype of maritime pine plants after a short-term heat treatment

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Maritime pine (*Pinus pinaster* Aiton) is the most abundant conifer in the Mediterranean basin with a surprisingly ample ecological niche. Biotechnological tools such as somatic embryogenesis (SE) and gene transfer have been developed in this species for breeding, with the aim of generating elite genotypes with improved growth characteristics under stress conditions. The main goal of our research is to obtain maritime pine clones with a better adaptation to high temperatures, by "priming" somatic embryos (SE) during the maturation process.

Maritime pine embryogenic lines, induced and proliferated at 28ºC, were maturated at 18º, 23º or 28ºC. Mature SEs were germinated and transferred to the greenhouse. After one year, plants were exposed during 10 days to high temperatures, which began with a ramp ranging from 22ºC to 50ºC for 4 hours, and then maintained over 3 hours. Data from photosyntetic parameters, malondialdehyde, proline and relative water content, as well as some morphological and anatomical characteristics were determined in sampled leaves at the beginning (T0) and at the end (T10) of the 10 days-heat treatment and after another 10 days to measure plant recovery (TR).

Our preliminary results suggest that a drastic temperature decrease (10ºC) during SE maturation produced plants with a higher adaptation to heat stress in terms of higher water content, which also correlated with higher proline levels in plant leaves. In addition, reduction in net photosynthesis during heat stress was lower in these plants derived from SE maturated at 18ºC than in those derived from SE maturated at 23ºC or 28ºC (at T10) and increased after 10 days recovery (TR). Finally, we also found that after heat stress, the leaf chlorophyllic parenchyma in these plants was wider than in those from SE maturated at warmer temperatures. A possible correlation between priming with low temperatures during maturation and heat stress resilience is under study.

Keywords: heat stress, resilience, photosynthesis, maritime pine.

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O23

Effect of megagametophyte priming on short-term response to high temperatures in somatic embryogenesis-derived plants of maritime pine

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In the context of climate change, efforts on forest research should be addressed to provide improved genotypes with increased resilience to high temperature events. One of the goals of our group is to obtain improved maritime pine (*Pinus pinaster*) clones that include such a characteristic by "priming" our somatic embryos (SE) at different periods of the SE production. To this end, we have applied high temperature pulses (37ºC and 50ºC) to isolated megagametophytes just at the beginning of the SE induction phase. Afterwards, embryogenic masses from these primed megagametophytes went through proliferation and maturation stages. Finally in vitro germinated emblings from one of these clones were used to study whether this initial "priming" would improve resilience after further heat events. In order to do this, in vitro growing plantlets were subjected to different heat treatments, at 23º C, 37ºC or 50ºC for 3 hours, and leaf samples were collected at 0, 24 and 72 hours after the heat treatment. The relative expression of *HSP70*, *WRKY* and *PEROX1* genes, all related to defense responses, was determined through quantitative-PCR (qPCR).

Expression of the *WRKY* gene was higher for in vitro plants derived from primed megagametophytes than in those from control explants, and its expression remained unaffected after heat treatments, indicating some epigenetic changes on this gene. In contrast, expression of the *HSP70* gene raised after the heat treatment on plants derived from megagametophytes primed at 37º, indicating an earlier response to heat stress. Neither of priming nor heat further treatments affected *PEROX1* gene expression.

Our results suggest that priming initial explants might accelerate heat response of plants under in vitro conditions and thereby increase resilience. Further assays will be performed after transferring these plants to ex-vitro conditions.

Keywords: *Pinus pinaster*, heat stress, *HSP70, WRKY, PEROX1*, somatic embryogenesis, resilience.

This work was supported by the MINECO and EU (AGL2016-76143-C4-1-R) and the Generalitat Valenciana (PROMETEOII/2014/052).

O24

Detecting and Expression of Somatic Embryogenesis Regulatory Genes in *Tilia amurensis, Tilia insularis* and *Tilia mandshurica*

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Genus *Tilia* is in high demand as honey trees or landscape plants. Somatic embryogenesis could be used for mass propagation of *Tilia* spp. as an alternative propagation system. While previous researches regarding somatic embryogenesis have been conducted for developmet of inducible condition, there has not been research on genetics about *Tilia* somatic embryogenesis. Hence, it is necessary to define unknown mechanism of somatic embryogenesis of *Tilia* spp. In this research, we studied whether the induction condition of *T. insularis* and *T. mandshurica* was identical to that of *T. amurensis.* Also, seven genes which were known to be associated with SE were tested for their existence in *T. amurensis* genome. Then expressions of detected genes throughout SE development were measured. For the study, immature embryos of *T. amurensis* Rupr., *T. insularis* Nakai and *T. mandshurica* Rupr. *et* Maxim. dissected from seed were cultured on MS medium with 1.0 mg/L 2,4-D. Total RNA was isolated from SE on different developmental stages and cDNA was synthesized. Seven primers from references were used in polymerase chain reaction (PCR) and sequencing of amplified DNA fragment was performed. Then real time qPCR were performed. Changes in gene expression among SE developmental stages were analyzed by analysis of variance (ANOVA). As a result, SE were developed to cotyledonary stage, the final stage of somatic embryogenesis in *T. amurensis* and *T. Insularis*. SE of globular stage was induced in *T. mandshurica*. Partial sequences of putative homologs of *SERK*, *PICKLE* and *VAL1* of *T. amurensis* were identified. The genes were referred to as *pTaSERK*, *pTaPICKLE* and *pTaVAL1*. When translated sequences were compared to those of arabidopsis, poplar, soybean and cork oak, *SERK*, *PICKLE* and *VAL1* showed 97.1%, 87.6,% and 68.8% similarity, respectively. The expression of *pTaSERK* was the highest at the heart stage in *T. amurensi*s and *T. insularis*, while, *pTaPICKLE* was expressed high at heart stage and low at torpedo stage in *T. amurensis*. The gene expressions at all stages in *T. insularis* were similar to the control. *pTaVAL1* expressed in *T. amuerensis* showed high expression at hearth stage and cotyledonary stage, while the expressions of all stages in *T. insularis* were equal to or lower than the control. According to results of sequencing and gene expression analysis, *pTaSERK*, *pTaPICKLE* and *pTaVAL1* genes had homology to other species genes, hence it could be assumed that these genes have similar roles in somatic embryogenesis.

Keywords: *Tilia amurensis, Tilia insularis, Tilia mandshurica*, somatic embryogenesis, gene detecting, gene expression

O25

Cork oak somatic embryogenesis as a system model to study QsMYB1, a transcription factor highly expressed in phellem

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Cork oak (*Quercus suber*) is a woody species with high socio-economic value due to the production and industrial exploitation of cork tissue. Cork derives from phellogen, a secondary meristem, which differentiates in phellem, characterized by suberized cells.

The study of the molecular regulatory mechanisms of cork formation and differentiation are still scarce. QsMYB1 is a R2R3 transcription factor highly expressed in suberized tissues (Almeida et al. 2013). In previously work, we identified the putative DNA-binding of QsMYB1 in a genome wide-scale of cork oak by ChIP-Seq, unravelling the putative genes regulated by this transcription factor (Capote T. 2018). In this context, a cork oak somatic embryogenesis system was established and somatic embryos overexpressing *QsMYB1* were generated. Therefore, it is of interest to understand how overexpression of QsMYB1 affects the expression of genes implicated in cork development. The transcriptomic analysis of somatic embryos overexpressing *QsMYB1* was performed using a NGS platform, in order to study how QsMYB1 is modulating the expression of the target genes.

Results show that a group of genes identified in the ChIP-Seq data are also overexpressed in these genetic modified embryos. In the present work we are analysing the expression level and biological function of these genes as an attempt to explain how QsMYB1 regulate the transcription of its target genes. With the transcriptomic approach and recurring to somatic embryogenesis as system to overexpress QsMYB1 we expect to identify the genes down and up-regulated by this transcription factor, allowing us to screen the regulatory network and mode of action of QsMYB1.

Keywords: somatic embryos, QsMYB1, ChIP-Seq, RNA-Seq, cork

O26

Transcriptional identification and characterization of differentially expressed miRNAs involved in conifer embryogenesis

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The molecular regulation of plant embryogenesis has been mostly studied using angiosperm model species such as *Arabidopsis*. However, gymnosperm and angiosperm lineages are estimated to have driven apart over 300 million years ago and their differences, in particular at the embryogenic phase, are well described. As major regulators of gene expression during developmental processes in plants, it is expected that sRNAs play a relevant role in the regulation of embryogenesis and, most probably, in the emergence of differential characteristics of the conifer embryo. During this transition, conifer embryos develop multiple cotyledons while maintaining a radial symmetry which contrasts with the *Arabidopsis* embryo where a bilateral symmetry is acquired. Based on previous studies in our lab that generated an overview of the *Pinus pinaster* sRNA transcriptome in several tissues including embryos at several developmental stages, a set of candidate miRNAs and corresponding target transcripts have emerged as potential embryogenesis regulators. Centered on the differential expression analysis of identified miRNAs throughout embryo development, we hypothesize that specific miRNA-target regulatory nodes potentially involved in processes such as auxin responses and miRNA biogenesis, are crucial developmental regulators. Among these, *miR160* and *miR162*, putatively targeting an *ARF10* and *DCL1*, respectively, are interesting candidates to further characterize based on their expression patterns, either specific of zygotic embryo tissues/developmental stages or markedly differentially expressed during embryo development. By using a conifer *in vitro* embryogenesis model system, and a combination of cell, molecular biology and genetic tools, we are working on the functional characterization of a short list of selected candidate miRNA-target regulatory pairs. As a first step of this characterization, the validation of *in vivo* interaction of selected miRNAs and their putative target genes predicted mostly by in silico analysis, is being performed using a co-transient expression with the luciferase reporter system in *Arabidopsis* protoplasts. The results already obtained up to now evidencing the existence of *in vivo* interaction between miR160 and its target ARF transcript will be presented and discussed.

Keywords: auxin responses, embryo development, gymnosperms, sRNA transcriptome

O27

The role of mitochondrial alternative oxidase (AOX) during somatic embryogenesis of Norway spruce (*Picea abies* L. Karst)

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Alternative oxidase (AOX), an enzyme of the alternative respiration pathway, was experimentally studied during the proliferation and maturation phases of somatic embryogenesis in Norway spruce (*Picea abies* L. Karst.). The enzyme was inhibited by different concentrations of salicylhydroxamic acid (SHAM) supplemented in the culture media. The changes in cellular ATP levels and the expression of *AOX1* and other genes of the respiratory pathway were measured. The effect of the inhibitor concentration on the proliferation rate of the embryogenic cell masses (ECMs) was also studied, as well as the ability of the somatic embryos to mature after inhibitor treatment in the proliferation and induction phases.

It was hypothesized that (1) when AOX is inhibited, and thus the pathway blocked, the cells would try to compensate for the loss of the enzyme by increased mRNA expression of the *AOX1* gene; (2) when the active alternative pathway is blocked, the electrons will be forced to the main pathway and this would increase the ATP cellular levels; (3) the inhibition of AOX would negatively affect the stress tolerance and growth of the ECMs and either delay, hamper or obstruct the somatic embryogenesis and the maturation of embryos and (4) the concentration of the inhibitor would affect the severity of the hindrance to growth and development.

The highest of the tested SHAM concentrations increased the expression of *AOX1* and *cytochrome C* genes on the third day of the proliferation cycle. The presence of SHAM in the culture media decreased the growth rate of ECMs as the concentration of SHAM increased and prohibited the maturation development genotype specifically.

This is the first case reported of an AOX study during somatic embryogenesis of Norway spruce. In the future, a similar experimental design could be used on other conifer species. A more challenging approach would be the genetic engineering of genotypes under- or overexpressing *AOX* genes.

O28

Dynamics of DNA methylation and effects of de-methylating agents on somatic embryogenesis of *Quercus suber*

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Epigenetic marks are involved in the regulation of global gene expression programs, among these marks, DNA methylation, accomplished by DNA methyltransferases, is a key epigenetic modification of the chromatin, associated with gene silencing that can change during cell proliferation and differentiation processes. Somatic embryogenesis is a widely used biotechnological tool for in vitro plant regeneration, still with limited efficiency in woody species. Cell reprogramming, totipotency acquisition and somatic embryogenesis initiation involve changes in the developmental program of the cell, which affect global genome organization. Recent studies in rapeseed and barley have reported that microspore embryogenesis initiation is associated with DNA hypomethylation, and its efficiency can be improved by the DNA demethylating agent 5'-azacytidine (AzaC) (Solís et al. 2015). However, in trees, little is known about DNA methylation dynamics during somatic embryogenesis (Rodríguez-Sanz et al. 2014; Corredoira et al. 2017). In this work we analyzed the changes in global DNA methylation levels and nuclear distribution during somatic embryogenesis progression in cork oak, by biochemical and immunocytochemical approaches. Also, effects of AzaC treatments on somatic embryogenesis were analyzed. Results showed low DNA methylation levels at early stages of somatic embryogenesis, in proembryogenic masses, and a progressive increase during embryo differentiation. AzaC treatments reduced global DNA methylation and favored the initial stages of somatic embryogenesis, promoting the proliferation of somatic embryogenesis cultures. However, at advanced stages AzaC prevented embryo differentiation, an effect that could revert by eliminating the drug from culture medium. These findings provide new insights into mechanisms underlying somatic embryogenesis in cork oak, a forest species of high economic and ecologic value, opening up new intervention pathways, by using epigenetic modulators, to improve somatic embryogenesis yields for forestry breeding and propagation programs.

Keywords: Epigenetics, DNA methylation, 5-azacytidine, somatic embryogenesis, cork oak

Corredoira et al. (2017) J Plant Physiol. 213: 42-54
Rodríguez-Sanz et al. (2014) BMC Plant Biol. 14:224
Solís et al. (2015) Front. Plant Sci. 6:472

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O29

Carbohydrate metabolism modulation in the zygotic and somatic embryogenesis of the subtropical conifer species *Araucaria angustifolia*

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Systems biology (SB) can be a powerful tool to understand embryogenesis. SB approaches can be used for instance to compare somatic and zygotic embryogenic development by using tools that reveal molecular, biochemical and physiological patterns that appear concurrently during embryogenesis. By examining these pathways, one can study subsystems with high centralities in metabolism that participate in the modulation of cell fate and embryo development. One of the higher centralities in plant metabolism is its modulation by carbohydrates. Thus, we use as a model the endangered conifer tree species *Araucaria angustifolia*, whose seeds are recalcitrant, preventing propagation for conservation purposes. Transcriptome and proteome databases for *A. angustifolia* have been generated. More recently, we adopted an SB approach by performing a series of observations to evaluate the role of carbohydrate metabolism in three zygotic embryogenesis stages (globular, cotyledonal and mature) and during the proliferation and maturation phases of embryogenic cell lines with contrasting embryogenic potential (responsive and blocked). We generated profiles of non-structural carbohydrates, and also identified and characterized genes and proteins involved in carbohydrate-mediated responses, cell-to-cell communication and homeostasis. We found that the TOR/SnRK1 system operates in the modulation of the *A. angustifolia* embryogenic responses. Here we use co-expression networks to integrate a set of metabolomic data to the transcriptome and proteome databases of developing embryos of *A. angustifolia*. The sugar sensing responses during somatic embryogenesis resembled those occurring at early stages of zygotic embryogenesis. Sucrose and starch accumulation during embryo development was an important trait that modulates the responses of embryogenic cell lines. Cell-to-cell communication seems to play a key role to determine the responsiveness and the fate of development during embryogenesis in *A. angustifolia.* The metabolic changes during zygotic and somatic embryogenesis indicate that the regulatory networks involved in growth and development are highly inter-connected at the metabolic, proteomic and transcriptomic levels. Our findings suggest that although carbohydrate metabolism is an important subsystem with high centrality during zygotic and somatic embryogenesis in *A. angustifolia*, the modulation of embryogenesis is systemically controlled by the concerted action of multiple factors.

Keywords: Carbohydrates, Brazilian pine, sugar sensing, embryogenesis, system biology.

O30

Biotechnology and bioprospection of native species from monte desert Patagonia, as strategies for the development of the regional bioeconomy

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The dry-land surface (i.e. semi-arid and arid regions) cover approximately one-third of the world's land area and are inhabited by almost 400 million people. During the last 50 years, there has been growing interest in the cause of desertification due to its impact on the global environment, economy and society. These ecosystems provide a series of ecological services essential for the sustainability of human life and the development of productive activities. In Argentina, the arid and semi-arid environments represent approximately one-third of the continental surface. Particularly, the Patagonia Argentina have 80% of surface containing any deterioration signs. At the Universidad de Rio Negro we carried out an investigation about propagation and bioprospecting of native shrubs species from these ecosystems. The purpose of this work is to contribute to the body of knowledge of native this species from desert and semi-desert regions, particularly on their, in order to provide alternatives for conservation and sustainable use of these resources and to enable them to be included in ecological reforestation and restoration programs in degraded environments. The species studied were: *Prosopis alpataco*, *Prosopis caldenia*, *Geoffroea decorticans*, *Acantholippia seriphioides*, *Condalia microphylla*, *Senecio* sp, *Larrea divaricata* and *Boungavillea spinosa*. The species under study have been systematically used as sources of energy, food or even medicinal use. This has exposed them to extractive type conditions, compromising their future availability, such as *A. seriphioides*, a species that is currently in danger of extinction. This project has allowed to generate knowledge about the propagation of the native flora of the arid and semiarid region, indispensable for the implementation of restoration strategies of degraded ecosystems and the ex situ conservation of these species. This work allowed us to expand the information base of Patagonian biodiversity, recover those threatened species and collaborate with the maintenance of regional biodiversity. Biodiversity is the basis of the bio-economy and the sustainable management of regional resources is a fundamental principle of the new circular economy paradigm. In this context, the development of the bio-resources area has become a strategic axis for the country, with a strong impact on the regional socio-economic and environmental areas.

Keywords: Shrubs plants, vegetative propagation, tissue culture, arid and semiarid region.

O31

Automated active compounds screening system allows high-throughput optimization of somatic embryogenesis in *Coffea arabica*

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Somatic embryogenesis (SE) is a very promising biotechnological tool for the rapid and large-scale vegetative propagation of elite varieties. For *Coffea arabica* F1 hybrids, research has led to successful industrialization and commercial application of SE in Latin America. However, research on *C. arabica* SE remains essentially empirical and at a low-throughput level, resulting in many drawbacks, especially an overall slow technical progress along the last years. While production today can reach 1-2 million SE-derived plants per year, a scale-up is needed to meet market demands (50-100 million per year). A high-throughput approach can be a solution to optimize SE protocols by testing a wider range of active compounds, multiplying experimental conditions, and reducing volumes as well as required spaces and manpower. An automated and miniaturized system is a pre-requisite to establish this approach. We here present an automated high-throughput screening system able to optimize SE in *C. arabica*. Our focus was on the production of embryos from established cell suspensions. This SE stage is classically done in 250 ml flasks so miniaturization in 24-well plates was necessary. Arabica calli were also miniaturized in order to fit the pipetting platform. The automated platform was then programmed in a way to validate homogeneity of calli distribution as well as callus-to-embryo conversion. After a successful establishment of the system, a screening proof of concept was carried out. Four compounds (Oxamflatin, SAHA, Scriptaid and Trichostatin A) belonging to the histone deacetylase inhibitors family were tested at three different concentrations: 0.1, 1 and 10 µM, as potential active compounds on *C. arabica* embryo differentiation. Only calli treated with 1 µM Trichostatin A showed a significant increase in the number of torpedo-shaped embryos (3-fold increase). As a validation, this treatment was re-tested in standard conditions (250 ml flasks) and also showed a significant increase in the number of embryos obtained (3.5-fold increase), their size (30%) and the overall fresh weight (60%). Our results show that our system can be well-suited to screen thousands of compounds in a restrained time period. Further analysis should be carried out to understand Trichostatin A molecular effect on embryo production stimulation and plantlet conversion efficiency.

Keywords: *Coffea arabica*, somatic embryogenesis, miniaturization, high-throughput screening, histone deacetylase inhibitors

O32

In vitro somatic embryogenesis in sweet orange (*Citrus sinenesis* L.) Cvs. Maltaise demi-sanguine and Thomson navel for viral sanitation

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Tunisian citrus crops are faced to several abiotic and biotic constraints among which virus and virus-like diseases are also important. The production of virus-free plants systematically pass through the use of *in vitro* techniques. Indeed, somatic embryogenesis from style and stigma explants is reported to be highly effective in the elimination of these diseases from citrus plants. In this context, somatic embryogenesis and further plant regeneration of two sweet oranges cvs. Maltaise demi-sanguine and Thomson navel were carried out using style/stigma explants excised from unopened flowers. These explants were cultured on MS medium containing BAP (0, 6.65 or 13 µM) and 500 mg/l malt extract. Cultures were held in growth chamber at 25°C ± 2°C under 16h/8h photoperiod. Subculturing was achieved every 4 weeks on the same fresh medium. Light microscopy, scannig electron microscopy and histology were used to illustrate the main morpho-anatomical events associated with embryogenic process. Firstly, callus was induced at the base of the style after about 10 days of culture, before invading the whole explant. Expression of somatic embryos started after approximatly 4 months of culture. They consisted in small and green globular structures corresponding to the globular embryo stage. Anatomically, these structures showed central procombial cells and are surrounded by a protoderm isolating them from the callus. These globular embryos progressed asynchronously giving rise to somatic embryos at different developemnt stages ranging from globular to cotyledonary. At cotyledonary stage, they had bipolar structure illustrated by the presence of shoot and root apices.

In this experimental conditions, many cases of teratological forms affecting embryos ontogeny were scored during their maturation and then hampered their conversion into plantlets. These abnormalities varied from mono to polycotyledonated embryos and fused ones or trumpet-shaped embryos. Nevertheless, some normal embryos developed into normal plantlets and were successfully grafted on the *Citrange troyer* rootstock and acclimatized in greenhouse. RT-PCR carried out on growing grafted plants revealed that they are free of some viroids such as *Citrus bark cracking viroid* and *Citrus dwarfing viroid* identified in the source material.

Keywords: somatic embryogenesis, style/stigma, histology, viral sanitation, *Citrus*

O33

Somatic embryogenesis in *Bambusa oldhamii* and *Dendrocalamus asper* from immature inflorescences

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Bamboos are multipurpose grasses (*Poaceae),* subfamily *Bambusoideae*, that present environmental, economical and social importance. Brazil holds high bamboo diversity and the climate is ideal for its cultivation, and despite of it doesn't yet have a well-established productive chain. The main bottleneck for this is the lack of planting stocks since conventional propagation methods are not efficient and seeds are rarely available. Therefore, tissue culture techniques can be used for mass production of high quality bamboo plantlets. Bambusa *oldhamii* and *Dendrocalamus asper* are priority species with economic importance in Brazil, mainly because the production versatility of edible sweet shoots and culms for multipurpose applications. Protocols for *in vitro* regeneration through somatic embryogenesis derived from young inflorescences of *B. oldhamii* and *D. asper* are being developed at Laboratory of Plant Developmental Physiology and Genetics of the Federal University of Santa Catarina, South Brazil. Embryogenic cultures were induced from immature inflorescences in Murashige and Skoog basal medium (MS), 3% (w/v) of sucrose and supplemented by 13.6 µM of 2,4-D and 9.3 µM of KIN for *B. oldhamii* and 28.0 µM of 2,4-D and 9.3 µM of 2iP for *D. asper*. Selected embryogenic cultures were selected resulting in repetitive somatic embryogenesis in MS medium supplemented with 2.0 µM 2,4-D and 5.0 µM 2iP, and 14.0 µM 2,4-D and 9.3 µM 2iP, respectively. Compact callus and also spontaneous regeneration of somatic embryos were achieved during multiplication phase in both cultures. For *D. asper,* embryos clusters were transferred to MS medium free of growth regulator, in the presence of light. Conversion of somatic embryos to plantlets was achieved after two weeks. Improvements in these protocols are in progress aiming at the large-scale micropropagation.

Keywords: Bamboos, tissue culture, micropropagation, somatic embryogenesis

O34

Gamma radiation effect on somatic embryogenesis of Norway spruces (*Picea abies*)

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Gamma radiation from natural sources or accidental releases may have a strong impact on plant growth and development. Our earlier studies indicated that gamma radiation resulted in DNA damage at dose rates ≥10 mGy h^{-1} and abnormal shoot apical meristem development and phenotype of young Norway spruce (*Picea abies*) seedlings at dose rates ≥40 mGy h^{-1}. In this study, we aimed to assess sensitivity to gamma radiation also during embryogenesis in this species, using somatic embryogenesis as a model system. Proliferating somatic embryonic cells were exposed to gamma radiation from a ^{60}Co source with dose rates at 0, 10, 20 and 40 mGy h^{-1} for 2 days. To evaluate effects of the gamma radiation, morphological and anatomical studies were performed during different stages of embryogenesis. No remarkable morphological difference between the different dose rate treatments were observed for the embryogenic cultures up to the maturation stage II. However, ability of the embryo formation at the highest dose 40 mGy h^{-1} reduced in the maturation stage III. Analysis by microscopy showed development of disorganized cells at the highest dose rate of 20 and 40 mGy h^{-1} already during the proliferation stage and at further embryogenesis development stages. To identify which cell organelles are affected by the gamma radiation, transmission electron microscopy was used and alterations in cell wall polysaccharides during somatic embryogenesis have been determined by immunolocalization using monoclonal antibodies. To further assess sensitivity towards gamma radiation, experiments with even higher dose rates ranging from 1-100 mGy h^{-1} for 6 days where morphological and anatomical as well as DNA damage has been assessed by the COMET assay, have also been performed and will be discussed.

Keywords: Norway spruce, gamma radiation, somatic embryogenesis, morphological and anatomical analysis, DNA damage discussed.

O35

Effect of heavy metal ions on development of Norway spruce somatic embryos

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Norway spruce belongs to the most common and productive conifer species in Europe with large ecological and economic importance. Over the last decades, changes in climatic conditions and pollution have caused intensive decline in spruce vitality and fertility. Somatic embryogenesis as a biotechnological technique is used for production of large number of superior plantlets, but enormous potential exists for this method to be used in various environmental studies, including the effects of heavy metal ions. In this study, two independent experiments were performed, where two embryogenic cell lines of Norway spruce were grown on media enriched with cadmium and lead in the concentrations 50-500 μM and four embryogenic cell lines were grown on medium where copper and arsenic in the concentrations 50-500 μM and 10-50 μM were tested, respectively. Effects of heavy metals were observed during subsequent stages of somatic embryogenesis and evaluated characteristics were proliferation potential, average number of somatic embryos obtained per g/fresh weight and morphology of developed somatic embryos. The highest tested cadmium concentration stopped the growth of embryogenic cell masses (ECMs) in both tested cell lines. However, the proliferation ratio remained fairly high for cell lines treated with lead at all concentrations tested. In the case of copper and arsenic, tested metals in higher concentrations significantly reduced the growth of ECMs. In the case of arsenic, the highest tested concentration (50 μM) stopped the growth of ECMs while lower concentrations caused abnormal development of somatic embryos. Furthermore, from the examination of the effect of heavy metal ions after maturation process, it was shown that all heavy metals in higher concentrations influenced formation, morphology and further development of somatic embryos. Besides studying embryo development in treatment with heavy metal ions, somatic embryogenesis could be a novel method for faster, easier, and/or cheaper selection of genotypes of coniferous species which can be tested as potential phytoremediators. The analysis showed that a simple *in vitro* laboratory test might be an indicative tool to evaluate phytoremediation potential of a considerable number of cell lines in a short period. Moreover, experimentation on tolerance to a metal is the first step towards identification of potential species for phytoremediation.

Keywords: Norway spruce, heavy metals, somatic embryogenesis, conifers

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O36

Improved and synchronized maturation of Norway spruce somatic embryos in temporary immersion bioreactors

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A key rate limiting steps of the somatic embryogenesis process in conifers is conversion from early stage somatic embryos in proembryogenic masses (PEMs) to maturation stage. Immature embryos in the PEMs are present at different developmental stages where some are unable to respond to the maturation treatment thus limiting yields of mature embryos. By dispersing interconnected tissue of PEMs, early stage embryos are singulated and the further development can be more synchronized. A temporary immersion bioreactor designed for Norway spruce together with a specific system for dispersion was used to dissociate connected tissue of PEMs and release smaller aggregates of early stage somatic embryos to a more uniform spatial distribution. Development of mature embryos was significantly stimulated by dispersion compared to controls in both liquid and on solid media. The study shows that in three out of four cell lines of Norway spruce (*Picea abies*), the yield of mature embryos from dispersed PEMs is three to five times higher than from non-dispersed controls.

Keywords: dispersion, bioreactor, synchronization

O37

Current results in somatic embryogenesis for *Pinus koraiensis*, an ecologically and economically very important pine species in East Asia

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Korean pine (*Pinus koraiensis* Sieb. et Zucc.) (KP) is an ecologically and economically very important pine species focused distributed in Northeast China, Russian Fast East and north part of Korean peninsula, and scattered distributed at high mountain areas in Japan. It is the dominant and community-constructive species (edificato) in cool-temperate zonal climax forest of mixed Korean pine and broad leaved trees in its focused distribution areas and produces high quality pine timber, nutritious edible nut and other products like turpentine. Especially, Korean pine nut production has become the supporting industry in Northeast China's forest regions because that timber harvest has had stopped for natural Korean pine forest and no timber can harvest in Korean pine plantations currently in China. There was 3 reports related to somatic embryogenesis (SE) besides our group (Bozhkov et al. 1997; Shen et al. 2005; Wang et al. 2009). Our studies on SE of KP was begun in 2007 and the SE first achieved in 2016 (Gao et al. 2017). At present, a relative high effective KP SE system has been established by using immature zygotic embryo (ZE), but not yet by mature ZE. The main results were as follows: (1) explant collection time for KP SE was at the end of June or at the begging of July, which was strongly depended on the developmental status of ZE that was influenced by stock trees, locating place of cones on stock tree crown and locating place of seeds on cone; (2) good embryogeneric callus could be obtained from suitable explant of immature ZE by DCR medium solidified by 6.5 g/L agar and supplemented with 35.0 g/L sucrose and supplemented with 2 mg/L NAA, 1.5 mg/L 6-BA and 500 mg/L acid hydrolyzed casein; and the embryogeneric callus could be maintained and proliferated well by DCR medium with 30.0 g/L sucrose, 0.5 mg/L 2,4-D, 0.1 mg/L 6-BA, 500 mg/L acid hydrolyzed casein and 500 mg/L glutamine; the subcullture period should be in 15 days; somatic embryos was obtained but not in ideal quantity and good status; (3) improved embryogenic callus, large quantity somatic pre-embryos and good matured somatic embryos were obtained recently on DCR and modified LV media solidified by 12 g/L gellan gum and supplemented with 68.5 g/L sucrose and 21.1 mg/L ABA, but mLV was much better than DCR for somatic embryo development of KP. These results would provide scientific support for the establishment of large-scale high-efficiency micropropagation system of the genetic improved materials of KP via somatic embryogenesis.

Keywords: *Pinus koraiensis*, somatic embryogenesis, explant effects, embryo development, culture conditions, micropropagation

O38

Stress application to Aleppo pine somatic embryogenesis: Problem or opportunity?

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Changes in environmental conditions can provoke epigenetic modifications responsible for evolution of trees plasticity and somatic embryogenesis can be an interesting tool to modulate the drought tolerance in forestry species. In the last years, our research team has worked in order to optimize different stages of the somatic embryogenesis (SE) process in *Pinus* spp. such as initiation, proliferation and maturation. This developed experience gives us the opportunity to manipulate the standard SE process in order to analyse the effect of drought stress. In this sense, temperature and gelling agent concentrations during the initiation (Pereira et al. 2016) and maturation stages were evaluated. The results showed that the concentration of gellan gum affected water availability, but a clear effect in *Pinus halepensis* SE was not observed when the stress was applied along the maturation stage. Culture media used for Aleppo pine maturation at 28ºC showed significantly higher water availability than the medium stored at 18ºC. However, Aleppo pine showed the same maturation rates (up to 90%) at the end of maturation stage, except in embryogenic cell lines cultured at 28ºC with 8 and 10 gL^{-1} gellan gum where 60-70% of ECLs produced somatic embryos. Respect to the number of somatic embryos produced, no differences were found between the values obtained in embryogenic cell lines cultured at 23ºC with the highest gellan gum concentration and temperature (800). Bonga et al. (2010) suggested that reducing or increasing temperatures may improve initiation and proliferation since temperature stress may promote cellular reprogramming. This prolonged effect was not observed for Aleppo pine where the temperatures that gave the lowest initiation percentages did not lead to better result for maturation stages (Pereira et al. 2016). The lack of this selective pressure could be due to the high adaptability of this species to different environments, a characteristic already observed in the field (Botella et al. 2010). Taking into account all the abovementioned results, higher temperatures were applied along the initiation stage in order to obtain a deeper effect in SE process. Furthermore, a micromorphological and ultrastructural study was carried out in order to analyse the differences in organelle size and organization at intra cellular level. At the same time, the endogenous phytohormone concentration in different tissues as well as a proteome study will be part of the future work to carry out in Catia Pereira's PhD work.

Keywords: Acclimatization, embryogenic cell line, embryonal masses, germination, *Pinus halepensis*, water availability.

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O39

Repetitive somatic embryogenesis induced cytological and proteomic changes in embryogenic lines of *Pseudotsuga menziesii*

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In Europe, Douglas-fir is a major species for reforestation with increasing demand for its wood. Therefore, adaptation of new varieties to climate change 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). Somatic embryogenesis, coupled with cryopreservation, is a promising clonal propagation system of selected trees that has been successfully improved in Douglas-fir (Reeves et al. 2018; Lelu-Walter et al. 2018). Repetitive somatic embryogenesis from cotyledonary somatic embryos (SEs) has been obtained for two unrelated Douglas-fir genotypes, producing secondary and tertiary embryogenic lines. Each one exhibited significantly higher embryogenic potential for both genotypes compared to primary or secondary lines, respectively (increase of 63 to 727%). The origin of such differences in embryogenic potential is unknown. Our objective was to study changes induced in embryonal masses (EMs) after repetitive somatic embryogenesis at the histo-cytological and molecular levels (LC-MS-based proteomics). Repetitive somatic embryogenesis improved the EM structure by increasing frequency of small, singulated immature SEs together with reducing the size of polyembryogenic centers. Each cycle of embryogenesis induced a modification of the expression of proteins related with biological processes and already known to be involved in somatic embryogenesis but quite new for EMs i.e.plant development, defense response, metabolism, proteolysis, and stress. The innovative implementation of protein networks in our proteomic analyses had been very conclusive. The networks revealed a global down or up pattern of protein expression after the first or second cycle of somatic embryogenesis, respectively. For both patterns, interactions with different plant growth signaling molecules (flavonoids and associated compounds, jasmonic acid, ABA, auxin, salicylic acid, lignin) were predominant. It is shown that cells have the ability to use different protein regulatory pathways to result in increased embryogenic potential (cotyledonary SE production). In Douglas-fir and in conifers, this is the first report describing cellular and molecular changes in EMs obtained after two successive cycles of repetitive somatic embryogenesis (Gautier et al. 2018). The results contribute to a better understanding of the cytological and proteomic changes associated with to enhanced embryogenic potential of secondary and tertiary embryogenic lines.

Keywords: histology, embryogenic potential, proteomic, Pseudotsuga menziesii plant growth regulators, secondary somatic embryogenesis

O40

Somatic embryogenesis of long-term proliferating embryogenic cultures and plant regeneration in *Larix sibirica*

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The study of development of somatic embryos (SEs) of Siberian larch revealed that transition of vegetative cells to somatic embryogenesis was accompanied by their elongation, polarization and accumulation of IAA at one of the elongated ends of the cell. It was followed by the asynchronous division and formation of the globule of the somatic embryo, on the basal side of which the embryonal tubes were formed. As a result, the embryonal-suspensor mass was formed. In the period from 2008 to 2017 42 proliferating cell lines (CL) from three donor trees were obtained on AI medium (patent RU 2456344, Tretyakova 2012). The most optimal treatment for the somatic embryo maturation was developed: AI medium supplemented with abscisic acid (121 μM), indolebutyric acid (1 μM) and PEG 8000 (10%). Germination of SEs was carried out on a non-hormonal ½AI medium. Seven young and long-term proliferating embryogenic CLs, characterized by different cultivation time, viability, proliferation activity, the number and size of SEs and their ability to maturate, germinate, and form viable plantlets, were selected for cytogenetic analysis. We found two aneuploidy proliferating CLs (2n=25; 2n=28) capable of somatic embryo formation. One of the CLs analyzed in a current study was found to be cytogenetically unstable with a chromosome number ranging from 24 to 35 and containing micronuclei in the interphase cells. Three CLs out of seven (1, 2 and 6 years of cultivation) were cytogenetically most stable with 91-96 % cells having diploid chromosome number 2n=24, typical for this species. One of the genetically stable cell lines is the CL6, which is characterized by low productivity (2040±189 SE 1 g^{-1} ESM fresh weight) and by lowest yield of maturating SEs (0.6%) as opposed to CL4 (33%). Nevertheless, from this cell line we obtained the cloned plantlets of Siberian larch, which successfully grow in the nursery of Experimental Station «Pogorelskyi Bor». According to microsatellite analysis (nine loci), these clones are identical to the CL6, from which they were obtained. Such cell lines form the basis of the collection bank of embryogenic cultures and can be successfully used for clonal plantation forestry. The reported study was funded by RFBR according to the research project № 18-54-00010 Bel_a and by Krasnoyarsk Regional Fund of Science and Technology Support according to the participation in the event: Fifth International Conference of the IUFRO Working Party 2.09.02.

Keywords: somatic embryogenesis, *Larix sibirica*, chromosome number, plant regeneration.

Primordial shoots of Norway spruce (*Picea abies*) as explants for somatic embryogenesis

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The recalcitrance of adult conifer explants has prevented induction of somatic embryogenesis (SE) in trees with known and desired characteristics. SE induction protocol, recently developed for white spruce (Picea glauca), was applied in order to examine the feasibility, frequency and timing of SE induction from primordial shoots of Norway spruce (Picea abies). In 2015 to 2017, 40 genotypes of four to six years old trees of SE origin as explant donors were screened. Two genotypes responded and produced 28 well proliferating embryonal mass sublines from 15 primordial shoots. Sublines from both genotypes had numerous early somatic embryos that were identified in acetocarmine stained samples. However, the number of somatic embryos produced per g fresh mass varied from 8.4 to 78.6 between the two genotypes. Successful SE inductions occurred at the beginning of April, when the temperature sum (d.d.) started to accumulate, and at the end of October or beginning of November when the chilling unit sum was over 500.

Keywords: Norway spruce, somatic embryogenesis, primordial shoots, recalcitrance

O42

WINNING AGAINST WILDINGS – The role biotechnology is playing in the fight against wilding conifers in New Zealand

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Invasive exotic species that have escaped from forestry plantations and established in the wild (wilding confers) are currently threatening more than 1.8 million hectares of New Zealand land. Many individuals and groups across New Zealand are involved in managing the spread of wilding conifers, their work is aided by both regional and national funding. Despite this major effort, estimated to cost more than $15M/yr, the area invaded continues to increase by 4-6%/yr. One such species of concern is Douglas-fir which is also an economically important species, where it accounts for approximately 6% of the sustainably managed exotic planted forests, second only to *Pinus radiata*. To ensure ongoing licence to operate and continuing resource management consents for planting commercial Douglas-fir there is the need for tools that minimise the risk of future wilding conifer spread.

A 5-year Ministry of Business, Innovation and Employment (MBIE)-funded research programme aimed at halting the spread of wildings was initiated in 2017, led by Landcare Research. This programme provides an integrated approach to wilding control in New Zealand. It comprises three main research themes, Detection, Control and Prevention. The detection component involves the development of comprehensive surveillance and monitoring tools to quantify wilding distributions and abundance. Control involves the development of improved, targeted and cost effective methods to control existing infestations. The prevention theme focusses on the development of novel mechanisms to prevent the generation of new wildings, particularly from wilding prone commercial species. This presentation will focus on work undertaken in the prevention area. This includes ongoing work to develop somatic embryogenesis protocols for Douglas fir in order to provide an enabling platform for the propagation of both sterile and low-coning genotypes. A biolistic transformation system for Douglas fir has been developed and protoplast isolation and regeneration protocols, to assist in the development of a gene-editing approach to gene modification, are being investigated. Douglas-fir genomic and transcriptomic data is being interrogated to identify candidate genes for use in developing strategies for engineered sterility based on transgenic and gene editing approaches. The application of research results will be in the future release of low coning or sterile genotypes (non-modified and modified) for planting in areas where wildings are considered a high risk. Strict environmental regulations will likely favour the former of these two options, at least in the short term.

Keywords: wildings, Douglas fir, somatic embryogenesis, sterility

Poster Presentations

P1

Physiological parameters of *ex vitro* acclimation of plants originated from somatic embryos of *Pinus radiata*

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In order to characterize acclimation stage of plants derived from the cultivation of somatic embryos of *Pinus radiata*, parameters associated with photosynthesis and water regulation were evaluated during first two months of installation in ex vitro conditions.The material was placed in containers with substrate peat under conditions of high humidity (75-90% RH), minimum light intensity of 250μmols-1m-2 and a temperature 15-25°C. Weekly maximum photosynthetic capacity, chlorophyll fluorescence, stomatal conductivity and respiration were evaluated. In addition, total soluble sugars, starch, chlorophyll and epicuticular waxes were quantified and the survival and weekly height increment of plants and aerial and root biomass were evaluated.The results show that the embryogenic plants do not present significant differences with respect to the photosynthetic capacity during the first weeks of installation in the greenhouse, whereas the stomatal conductivity and the transpiration are significantly increased during the first two weeks of cultivation. Fluorescence of chlorophyll shows that throughout the period the plants are under conditions of non-lethal stress. Regarding the behavior of soluble sugars, epicuticular and chlorophyll waxes show an increase after 5 weeks. Finally, the growth parameters show a take-off of the material after 8 weeks of cultivation. The previous results show that the embryogenic plants are in higher stress conditions during the first two weeks of culture and that the physiological parameters begin to show recovery at 5 weeks which is reflected in activation of aerial and root growth at 8 weeks of installation in greenhouse.

Keywords: callus, cell proliferation, tissue culture, tobacco

P2

In vitro propagation and *ex situ* conservation of *Phytolacca tetramera*, an endemic component of the "Talares" from Buenos Aires, Argentina

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It is called "talar" to the native forest formation of the northeast of the Province of Buenos Aires that develops on hills composed of shell deposits of the alluvial valley of the Río de La Plata. Anthropic pressure is the greatest danger faced by these forests in the most populated area of the country, and therefore their situation is clearly in decline. *Phytolacca tetramera* Hauman, or "Ombusillo", is a shrub endemic to the Talares from Buenos Aires that belongs to the Phytolaccaceae Family. By anthropic action, it was included in the category CR (critically endangered) of threatened species. (Delucchi, 2006). It has fungicide active ingredients. The methanolic extract of its berries possesses antifungal activity against opportunistic pathogenic fungi. With the aim of conserving this species, in CEAMSE´s *in vitro* Culture Laboratory, we are dedicated to the development of propagation protocols via Organogenesis and Somatic Embryogenesis, plant nursery culture and ground planting. As a source of explants, mother plants with good health status and optimal growth were selected. For the tests that allowed micropropagation, 30 explants were used per treatment, obtained from nodal and internodal sections of 1 to 1.5 cm in length and 1 to 2 mm in diameter, washed with running water for 5 min and placed in 0, 3% Benomyl for 90 minutes. Its disinfection consisted in the immersion of the explants in commercial sodium hypochlorite (55 g of Active Chlorine L -1), at a concentration of 30% and drops of Tween 20 (surfactant) for 30 min. Subsequently, they were washed with sterile water and submerged in 0.6% of Erythromycin prior to sowing in the culture medium. The nodal and internodal disinfected sections were placed, maintaining their polarity, in the basal culture medium of Murashige-Skoog (1962) (MS), in order to induce the process of adventitious organogenesis. The culture medium supplemented with 3% Sucrose, 0.5 ppm of Indol Butyric Acid (AIB) and 0.5 ppm of 6-Benzyl Amino Purine (BAP), showed the best results. After the acclimation period and the subsequent growth in the plant nursery for two years, 20 individuals were selected to carry out survival and development tests in the soil cover of the Villa Dominico Sanitary Landfill, Province of Buenos Aires, Argentina. They were divided into 4 groups. Each group consisting in 5 clones obtained from different mother plants. They were then planted in the landfill soil cover at elevations from 6 to 19 meters. For each specimen, its sex, filiation, height and stem width were recorded. At the end of its latency period, between the months of September and October, the status of the individuals will be evaluated, taking into account their health and air development. The following actions prepared for the conservation of *Phytolacca tetramera*, will include collaboration with public welfare organizations dedicated to its protection and awareness of the state in which it is located and its possible causes: Botanical Gardens, Urban Nature Reserves, educational entities, etc.

Keywords: *Phytolacca tetramera*, endemism, conservation, Talares, native forests.

P3

Performance of culture lines established *in vitro* from a monumental birch tree

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Biotechnological approaches by using in vitro culture techniques can be applied for clonal propagation and genetic transformation. Furthermore, they offer an alternative for ex situ conservation of elite genotypes, including monumental trees which are characterized by their high biological, ecological, historical and cultural values. It is well known that clonal propagation and in vitro morphogenesis of adult trees is negatively affected by the maturation-related loss of rooting and regeneration abilities. However, little is known about the effect of the position of the initial explants (based in their location or topophysis on the branch) on their in vitro performance. The aim of this work was to micropropagate a mature white birch tree (*Betula pubescens* ssp. celtiberica), and to study the in vitro response of shoot culture lines established from different crown branches. The tree is included in the catalogue of monumental trees (https://www.monumentaltrees.com) with nº 17602. For in vitro establishment, newly grown shoots were collected in June from top branches as well as from "epicormic shoots" originated on thick lower crown branches near to the trunk junction. Nodal segments, bearing one axillary bud, were labelled "1 to n" from the first uppermost (1) node position to the bottom (n) of the shoot, and used to establish different culture lines that were maintained separately in subsequent proliferation cycles. Woody Plant medium supplemented with 0.5 mg L^{-1} N^6-benzyladenine and 0.001 mg L^{-1} naphthalene acetic acid (NAA) was used for establishment of cultures, and Murashige and Skoog (MS) medium supplemented con 0.4 mg L^{-1} meta-topoline and 0.01 mg L^{-1} NAA was used for proliferation. After 4 and 6 weeks of culture, the nº of shoots, length of longest shoot and rooting percentage were recorded. We successfully established seven culture lines, three of them from top branches and four from thick branches. Although all lines exhibited high morphogenetic capacity and there were no great differences among them in shoot multiplication, the highest proliferation rate was achieved in one line derived from epicormic shoots. Results showed that the use of MS medium and meta-topoline are suitable for axillary bud propagation of white birch. Roots were formed in the proliferation medium and rooting rates ranged from 87 to 100%, depending on the culture line. Rooted plants were successfully acclimatized in greenhouse.

Keywords: *Betula pubescens*, mature, meta-topoline, monumental, topophysis

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P4

In vitro assays for cloning and breeding *Jatropha curcas* L. (physic nut)

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In the last 30 years, a large number of works reporting *in vitro* culture protocols of *Jatropha curcas* have been published. This crop has several traditional and some more recent uses, but the most exploited ones are biodiesel production and the production of secondary metabolites. Therefore, there is an increasing interest in promoting breeding programs that contribute to enhance the yield of this crop, and in clonal propagation protocols that allow the potentiation and maintenance of desirable characteristics. In our lab we have been studying the application of different tissue culture protocols to the micropropagation of this species as well as the use of anther culture trying to obtain haploid plants. Concerning micropropagation, the results showed that axillary shoot proliferation can be achieved but rooting is still a limiting step to large clonal propagation. Other techniques of cloning such as organogenesis and somatic embryogenesis gave poor results and could not be envisaged as useful tools for physic nut cloning. For breeding programs haploid plants are a high valuable material. Attempts to induce haploidy in this species were carried out through anther, pollen or ovule culture. The results showed that callus could be obtained from the anthers and from isolated pollen whereas ovular tissues were unable to proliferate. In the case of the anthers callus the exact origin of them could not be determined whereas the callus from isolated pollen culture did not show any regenerative potential. Cell cultures were obtained from root cultures of physic nut. They morphologically resemble the well-known BY-2 tobacco line used as control. Both cultures were kept for long periods and tested with the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Results showing similarities and differences of the two cell lines will be presented. This *J. curcas* cell lines are quite stable and may be considered as a possible new and complementary plant cell model for the production of plant secondary metabolites *in vitro*.

Keywords: cell line, *in vitro* culture, *Jatropha curcas*, micropropagation, microsporogenesis, pollen.

P5

Micropropagation of *Tilia cordata* Mill. including verification of genetic diversity of donor trees

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Tilia cordata Mill. is historically considered to be a Czech national tree, which is appreciated for its wide use. Linden is interspersed broadleaved tree species in Czech forests, ecologically important for biodiversity conservation and as a stabilizing element in the forest ecosystem. In the past, this species has been reduced due to the extensive change of forest to agriculture land and as a consequence of artificial reforestation with preferences for conifers (ÚRADNÍČEK et al. 2009). Regarding to its a great adaptability to climatic factors, linden is becoming more important in the forest ecosystem. Nowadays, micropropagation represents the most effective biotechnology enabling the clonal reproduction of parent trees with high quality of phenotype and also the preservation gene resources in the National bank of explant for further utilization. The organogenesis, as a particularly suitable method for clonal propagation of broadleaved trees, was verified for the establishment of *in vitro* cultures from selected elite parent trees growing in South Bohemia. The linden microshoots were developed on Murashige and Skoog (MS) medium supplemented with growth regulators BAP 0.4 mg/L, IBA 0.1 mg/L and glutamine 100 mg/L, glycine 2 mg/L, 30 g/L of sucrose. In order to obtain knowledge about the level of genetic diversity of donor trees, DNA analyses by nuclear microsatellite markers have been optimized. Nuclear microsatellites are highly polymorphic, codominant markers and can be also used to verify the clonal identity and somaclonal variation in the micropropagated cultures. Clear, reproducible PCR products were produced for eight microsatellite loci (Tc4, Tc5, Tc6, Tc7, Tc915, Tc920, Tc937, Tc963), whose oligonucleotide sequences primer pairs had been published by PHUEKVILAI and WOLFF 2013. They were separated by capillary electrophoresis using the Applied Biosystems 3500 genetic analyser. The genotyping of selected trees was evaluated by the statistical program GenAlEx 6.501 (PEAKALL, SMOUSE 2012). All tested linden trees showed different genotypes. Shannon's information index calculated for genetic diversity ranged from 0.84 at locus Tc937 to 2.48 at locus Tc963. Analyzed trees have shown high levels of genetic diversity, so they can be involved to *in situ* conservation strategies.

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Keywords: *Tilia cordata* Mill., clonal micropropagation, organogenesis, genetic diversity, microsatellite markers

P6

"Hybrid pine seed: what do you prefer, mother or nurse?"

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Development of hybrid pines of *Pinus radiata* D. Don for commercial forestry presents an opportunity to diversify the current resource. Challenges, which include the effects of climate change and different land use, make species alternatives necessary to guarantee wood and non-wood products in the future. *Pinus radiata* var. cedrosensis X *Pinus attenuata* hybrid possess different attributes, such as tolerance to drought conditions (De Diego et al. 2012; 2013a,b; 2015), better growth and resistance to snow damage at higher altitudes, and importantly, different wood quality characteristics (Dungey et al., 2013). Embryogenic cell lines were successfully initiated from first generation reciprocal hybrids using *P. radiata* protocols (Hargreaves et al. 2017). This was the first report where *P. radiata* somatic embryogenic tissue was used as a nurse to facilitate somatic embryogenesis initiation from hybrid pine excised zygotic embryos. It was clear that the excised embryo was better for somatic embryogenesis cell line initiation than when the whole megagametophyte was cultured. But, the question is: Does the presence of the megagametophyte during initiation inhibit or enhance the maturation success months later? In the present work we analysed the maturation rate, number of somatic embryos, germination success, *ex vitro* survival and growth in cell lines derived from embryonal masses initiated from excised zygotic embryos or intact megagametophytes. No differences were observed in *in vitro* parameters such as maturation and germination or in *ex vitro* parameters such as growth in relation to the treatments. However, significant differences were observed due to the species, with somatic embryos being smaller from those cell lines initiated from *P. attenuata* mothers compared to those from cell lines initiated from *P. radiata* mothers.

Keywords: hybrid pine, *Pinus attenuata*, *Pinus radiata*, somatic embryogenesis, embryonal masses, embryogenic cell lines.

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P7

Development of an *in vitro* screen for *Phytophthora cinnamomi* resistance in hybrid and transgenic chestnut trees

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The lack of resistance to *Phytophthora cinnamomi*, an oomycete pathogen that causes Phytophthora root rot disease in American chestnut (*Castanea dentata*) and other woody species, may pose a major barrier to introducing the products of The American Chestnut Foundation's (TACF) breeding program to the southern portion of the American chestnut's original range in the U.S. The integration of genes for *P. cinnamomi* resistance from Chinese chestnut (*Castanea mollissima*) into TACF's hybrid backcross chestnuts is now proceeding, but will take additional years of breeding and selection. Combining somatic embryogenesis-based propagation of chestnuts with a reliable *in vitro* assay for resistance to *P. cinnamomi* would help to more rapidly evaluate hybrid backcross chestnut clones thought to carry resistance genes—which in turn will accelerate the production of elite chestnut varieties with resistance to both chestnut blight (*Cryphonectria parasitica*) and *P. cinnamomi* for planting throughout the eastern U.S. Our goal was to define a quantitative *in vitro* screening approach that could be applied to identify *P. cinnamomi*-resistant hybrid backcross chestnuts (B3 and BC3F3 generations), and to test transgenic American chestnut carrying the candidate *P. cinnamomi* resistance genes *RPH* and *NPR3/4*, using pure American chestnuts and Chinese chestnuts as susceptible and resistant controls, respectively. Clones were screened using micropropagated shoots "planted" into agar gel in test tubes and inoculated with a 3 mm plug of a locally-collected isolate of *P. cinnamomi*. In three experiments, the growth rate of a dark lesion from the base of the shoot to the tip was used to compare resistance among the different chestnut genotypes. The results indicated that within 30 days of inoculation, Chinese chestnuts showed significantly shorter stem lesions compared to pure American chestnuts and hybrid backcross chestnuts. In addition, it appeared that some of the hybrid backcross chestnuts had intermediate resistance between the susceptible American chestnut and Chinese chestnut genotypes tested, as might be expected, although others were no more resistant than pure American chestnuts. None of the tested transgenic chestnut shoots showed evidence of enhanced resistance compared to pure American chestnut.

Keywords: *Castanea dentata, Castanea mollissima, Phytophthora root rot, shoot cultures*

P8

Flowering intensity and phenology in clonal seed orchards of maritime pine

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The third generation orchards currently producing in France the maritime pine VF3 (Vigour-Form) variety were established between 2002 and 2006 using source materials propagated through grafting (clonal seed orchards) or by seeds (seed family orchards). These orchards have been producing improved seeds since 2011 in an open pollination setting, a method that drastically reduces production costs compared to controlled crossings. The full achievement of the expected genetic gain however assumes that pollen contribution from outside the orchard (pollen contamination) is low, while that of the orchard's progenitors is well-balanced. A recent study (Bouffier et al. 2017) used SNP markers (Single Nucleotide Polymorphism) to estimate the pollen contamination and mating structure of seedlots harvested in 3 VF3 clonal orchards (Beychac, Saint-Sardos and Saint-Laurent2) composed of about fifty parental genotypes and grown under contrasting pedoclimatic conditions (Beychac and Saint-Sardos are flowering earlier than Picard). The pollen contamination rate is high and variable according to the orchard (site), the year and the maternal genotype. How the flowering intensity and phenology of orchards progenitors can affect pollen contamination rate and parental contribution in seedlots is still poorly understood. Early female flowering (development stage) as well as intensity of female (number of flowers) and male (score) flowering of all parental genotypes were estimated in the 3 clonal seed orchards during springs 2015 and 2016 (overall more than 7500 data collected). Although annual differences are observed among sites, strikingly, average ranking of each clone for both female phenology and flowering intensity is similar in the 3 orchards for the 2 investigated years suggesting a strong genetic control of flowering traits. There is no apparent relationship between early female flowering and pollen contamination rate. In contrast male flowering intensity could partly explain the heterogeneity of paternal contributions to seedlots as revealed by the SNP analysis. A positive relationship was found between early female flowering and flowering intensity and it is discussed in reference to putative observation bias or selection effects.

Keywords: *Pinus pinaster*, clonal orchard, flowering, reproductive successs, grafting

Reference: Bouffier L. et al.(2017). Pollen contamination and mating structure in maritime pine clonal seed orchards. IUFRO 2.09.01 Seed Orchard Conference, 04-06 sept. 2017, Bålsta (Suède). Poster, pp. 65.

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P9

What technical improvements are needed to achieve industrial application of conifer somatic embryogenesis?

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Even though somatic embryogenesis (SE) of conifers has long been considered to be a potentially useful technique to mass produce high quality planting stock, it has been difficult to develop the technique to a level where it can be applied effectively on an industrial scale. In the following we outline a number of problem areas and we indicate a few areas of research focus that need attention in our efforts to improve the situation. The SE initiation rates are low for many conifers, and often the capacity is restricted to only a small number of genotypes, except for in a few spruce species. The basal medium primarily used in initiation may not be optimal for the maturation step and thus may result in embryo abnormalities and vitrification. As generally practiced today, manual transfer of germinating embryos to soil is labor intensive and expensive and, therefore, requires automation. Furthermore, long-tern cryopreservation of clones during lengthy field testing is expensive. We suggest potential solutions to these problems, including media modifications, the use of capable genotypes obtained by breeding to improve SE initiation rate, automated SE production systems, and the implementation of genomic selection to circumvent or reduce the length of the field testing period.

Keywords: Conifer SE, Cost, Automation, Cryopreservation, Genomic selection

P10

Clonal propagation by somatic embryogenesis in *Pinus rigida*×*P. taeda*

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Zygotic embryos at different developmental stages were tested for their potential in the initiation of ESM (embryogenic suspensor mass) lines using immature seeds of *Pinus rigida*×*P. taeda*. The highest frequency (1.1%) of ESM was obtained with seeds collected on July 1st. All excised embryos of the July 1st collection were at the early proembryo stage. Two different culture media were compared. Forty eight ESM lines were initiated on *Pinus taeda* Basal Medium (P6) (0.97%) with 13.5 μ*M* 2,4-dichlorophenoxyacetic acid (2,4-D) and 4.4 μ*M* benzyladenine (BA). Most of the ESM arose from the seeds that were at the stages ranging from late cleavage polyembryony to the early staged proembryo. Out of 52 lines (0.46%) that were produced from 11,388 explants, only two viable lines (0.018%) (PRT11 and PRT28) survived. As for somatic embryo maturation, the highest number (224/g^{-1} FW) of matured cotyledonary somatic embryos (line PRT 28) was obtained on a medium containing 100 μ*M* abscisic acid (ABA), 0.2 M maltose, and 1.2% gellan gum. For germination of the somatic embryos, the cotyledonary somatic embryos were transferred on half-strength Litvay medium (LM) plus 0.4% gellan gum. The germination rates were high (71.4-96.3%) regardless of the concentrations of either ABA or gellan gum in the maturation medium. The somatic plants were regenerated from the germination medium, acclimatized, finally them were successfully transplanted to the experimental field.

Keywords: embryogenic tissue initiation, somatic embryo maturation, somatic plant regeneration

This text was adapted from the original article of Yong W. Kim published in In Vitro Cell. Dev. Biol.-Plant (2007, 43:335–342).

P11

Commercial micropropagation of selections of a walnut hybrid progeny (Mj209xRa): (1) What about after 12 years?

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As current available walnut seed progenies have associated high phenotypic variability, the use of clonal materials is the base for the establishment of valuable timber plantations. Since 80th of last century, several advances have been done in walnut micropropagation; however, its commercial application is still limited. Walnuts are considered recalcitrant species to tissue culture. Despite in vitro establishment, rooting and survival are critical stages for walnut micropropagation, the relative low multiplication rate and the management of acclimated vitroplants hinder the massive plant production. In 2005, Bosques Naturales initiated a program for micropropagation of selected trees for wood production from a provenance of hybrid Mj209xRa. As a principle, only asexual starting materials, both from field growing trees and grafted potted plants, were used for in vitro initiation. Although these kinds of explant sources have associated low success for in vitro establishment, several tenth of genotypes have been in vitro cultured. Thus, an experimental protocol was designed that has allowed the obtaining more than 10000 vitroplants from at least 16 different genotypes. However, though functional, this protocol is affected by the execution complexity, reducing then its commercial application. In the frame of the EU project "Second generation of planted hardwood forests in the EU", the assessing of commercial scale up of the micropropagation protocol have been approached. Achievements, failures and future actions are here presented and analysed.

Keywords: *Juglans*, timber, rooting, acclimation, survival

P12

New tools for high quality in vitro mass production of pear

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In Tunisia, *Pyrus communis* 'Arbi' is one of the best rootstocks, because of its salt and drought stress tolerance and pear decline and fire blight resistance. The aim of this study was undertaken to develop a high quality micropropagation, rooting and acclimatization systems for pear. For this, we applied all modern tools such as new generation cytokinins and auxins, LEDs, temporary immersion systems and mycorrhizal colonization.

Micropropagation - Combinations of monochromatic blue (B), red (R) and far red (F) LED light were compared with fluorescent (FL) light during micropropagation and rooting of a recalcitrant pear. During the micropropagation phase, R gave some particular advantages: maximal shoot length and leaf area were obtained. Although FR was advantageous for shoot number, shoot quality was inferior because of hyperhydricity and a pale green colour as indicated by a low Chl a+b and carotenoid content. Micropropagation of pear shoots was investigated in a temporary immersion bioreactor system SETIS™ and on a conventional semi-solid medium. The occurrence of hyperhydricity was affected by the immersion interval and the cytokinin type. Hyperhydricity was inevitable on semi-solid medium and in SETIS™ when 5 µM benzyladenine was applied, it could be reduced in SETIS™ by lowering the immersion frequency to 3 times per day. Applying 5 µM *meta*-Methoxy topolin riboside (MemTR) or *meta*-topolin riboside (mTR) completely avoided hyperhydric shoot formation. For both 'Arbi' and 'Mahdia 6', 5 µM MemTR induced the highest number of shoots per explant whereas the highest leaf area was obtained with 5 µM mTR. Rooting - Adventitious rooting of *in vitro* cultured pear plants was highly affected by different light spectra and the addition of a new rooting compound. Without this compound, limited rooting was observed under R, B and BR. In combination with this compound, 100 % rooting was achieved under R. Acclimatization and mycorrhization - Pear plantlets were pre-mycorrhized *in vitro* by mean of the Mycelium Donor Plant system. Heavy *in vitro* root colonization of the pear plantlets was observed after three weeks. One and three months after transfer to a substrate, the survival rates were higher than those of control plants grown on substrate without AMF.

Keywords: topolins, temporary immersion bioreactor, LED, mycorrhization.

P13

Clonal test of *Sequoia sempervirens* (D. Don) Endl. in southern Brazil

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Sequoia sempervirens (D. Don.) Endl., popularly known as sequoia or redwood, is a species with valuable wood, has reddish color, easy workability and high natural durability. It has the potential for planting in places outside its natural habitat, as shown by deployments in Chile and New Zealand. The successful implementation of a species such as sequoia may represent a forest alternative for Brazil, especially for the southern half, a place that presents climatic analogies with the place of origin of the species. Cloning of the species suggests a greater ease of genetic gain and the possibility of selecting specific clones for different sites. The objective of the study was to implement sequential clonal tests in two regions of Santa Catarina (Brazil), seeking information on adaptation and growth. The implantation occurred in two locations in the State of Santa Catarina: Campo Belo do Sul (Cfb climate, average temperature 16ºC, and occurrence of strong frosts) and Arabutã (Cfa climate, average temperature 19.2ºC, and rare occurrence of frost). The material of origin (cuttings) were obtained by means of the rescue of trees with approximately 40 years of age (São Joaquim - SC and São Francisco de Paula - RS), propagation by cutting, assembly of clonal mini-garden, and finally production of cuttings by minicutting. Planted cuttings were around 30cm in height and lap diameter of more than 4mm. In Campo Belo do Sul, implantation was carried out in December 2015 with three clones (SJ, A140 and A228), with evaluation of growth and frost damage at 6, 12 and 24 months. Four clones were used in Arabutã (A127, A138, A140 and A228). Planting was carried out in December 2016, with growth assessment at 6 and 12 months. The sequoia cuttings implanted in the colder region (Campo Belo do Sul) presented severe frost damage, with survival of only 2% observed after 24 months of implantation. In contrast, in the warmer area (Arabutã) (there was no record of frost in the period) survival was greater than 80% at 12 months, with an average height of more than 50cm and 7.5mm in the lap diameter. Other strategies should be adopted to seek the establishment of sequoia in colder areas, seeking to minimize the effect of frosts on cuttings. More evaluations are needed; however, the species initially shows potential for areas without occurrence of frost.

Keywords: redwood, clonal silviculture, conifer, field establishment, frost damage.

P14

Micopropagation of willow shoots under photomixotrophic and photoautotrophic conditions: proliferation in liquid medium and acclimation in different soils

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Salix viminalis L., also known as basket willow, is a promising candidate for bioenergy and phytoremediation applications. The species has been sucessfully micropropagated and acclimated (Regueira et al., 2018) and could therefore be used to develop emergent techniques in the field of plant propagation. The aim of the present study was to investigate the propagation of willow shoots in liquid medium and the acclimation of willow plantlets in different types of soils that could be used as alternatives to commercial peat. Propagation of willow was carried out both by temporary immersion (TIS) under photomixotrophic conditions and by continuous immersion (CIS) under photomixotrophic and photoautotrophic conditions. For TIS, we used RITA® and plantform™ bioreactors containing Murashige and Skoog medium with half-strength nitrates, 0.05 mg L^{-1} N^6-benzyladenine and 3% sucrose (w/v) (photomixotrophic conditions). For CIS, the basal sections of willow were placed in rockwool cubes soaked in the above medium (photomixotrophic conditions) or in the same medium devoid of sucrose (photoautotrophic conditions). Vessels (10 L) were made from food storage containers and were equipped with 0.2 µm filters to receive forced ventilation with CO_2-enriched air, as described for chestnut (Vidal et al. 2017). Photosynthetic photon flux density (150 µmol m^{-2} s^{-1}) was provided by white LEDs. For evaluating different soils during the acclimation step, we used rooted shoots obtained by CIS under photomixotrophic and photoautotrophic conditions. Both groups of plants were first transferred to a phytotron and planted in commercial peat. One month later, the plantlets were transferred to a greenhouse, in which both groups of plants were planted in 1) commercial peat, 2) soil from an oak forest, with high organic matter content, and 3) a crop soil with low organic matter content. Six weeks later, the plants were removed from the soil, and parameters related to the growth response of the 6 groups of plants (2 micropropagation systems and 3 soils) were recorded. In addition, the enzymatic activities of the soils were determined as described by García et al. (2003). The results indicate that liquid medium under photomixotrophic and under photoautotrophic conditions can be satisfactory used to culture willow axillary shoots. Although there were some differences in aerial growth, the willow plants were successfully acclimated in all three soils.

Keywords: bioreactors, continuous immersion, enzymes, rhizosphere, temporary immersion.

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P15

Production of cell suspensions of peanut (*Arachis hypogeae*) for the production of resveratrol

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Resveratrol, a phytoalexin abundantly found in grape seeds, wine and peanut, is an antioxidant and anticancer compound. This studies aims to produce this valuable compound by peanut cell suspension cultures in bioreactors. Peanut is known to be a potent producer of stilbenoïds compounds such as resveratrol. Seeds of three peanut varieties were initiated. To induce callus formation, leaf explants were isolated and transferred to MS medium containing 3 % (w/v) sucrose, 0,7 % agar and supplemented with picloram (10 µM). Callus induction rate and morphology were recorded and cell suspension culture was established by transferring the 8 weeks old callus into 250 ml flasks containing 50 ml of the respective culture lacking agar and supplemented with 0,1 g l^{-1} citric acid and 500 µl l^{-1} PPM. Vigorous suspensions were obtained and subcultured in an orbital shaker at 110 rpm at 25 ±1 °C and in constant darkness during 30 days. Fresh weight, growth index and relative growth rate were routinely quantified by periodical subculture every 10 days. To induce stilbenoïds, an elicitor was added after 3 subculturing and cell viability was assessed before and after elicitation by incubating the cells for 1-2 min in fresh medium containing 100µg ml^{-1} fluorescence diacetate.

Keywords: *Arachis hypogeae*, auxins, callus, cell suspension, cell viability

P16

Comparative analysis of primary metabolome and secondary metabolites in the somatic embryogenesis of tamarillo (*Solanum betaceum* Cav.)

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Tamarillo (*Solanum betaceum* Cav.) is a solanaceous tree endogenous to South America and grown globally for its edible fruits. The emerging economic importance of this plant explains the need for efficient tools of mass propagation. This plant can be cloned using several methods, including *in vitro* techniques namely somatic embryogenesis (SE). The SE process, although achievable in a variety of species, remains poorly understood in some of its molecular mechanisms. The induction protocol used in tamarillo originates embryogenic (EC) and non-embryogenic (NEC) callus from the same explant, allowing a direct comparison of the molecular features responsible for embryogenic competence. Therefore, several approaches have used these samples, namely comparative proteomics and hormonal analysis. Metabolomic studies have also been carried out in other plants, but are to our knowledge lacking in tamarillo. Key metabolites have been assigned to embryogenic competence, and the importance of secondary metabolites, such as phenolic acids and flavonoids has been hinted. The objective of this work was the comparative study of the primary metabolome assisted by nuclear magnetic resonance (NMR) spectroscopy and the screening of phenolic and flavonoid compounds in EC and NEC derived from the same type of explants (leaves). The EC and NEC tissues were analysed by unidimensional ^{1}H-NMR spectroscopy and multivariate analysis methods were used to construct models that explain the difference between the cell lines in terms of key metabolites. The secondary metabolites specific for each family were quantified using spectrometric methods. The results showed an increase in sugar content (glucose, fructose, sucrose), and some amino and organic acids (Proline, Alanine, malic acid, 4-aminobutyrate) in NEC, while chlorogenic acid, phenylalanine and choline content was found increased in EC. In terms of secondary metabolites, both compound classes assayed (phenolic and flavonoid compounds) appeared about two-fold higher in EC, suggesting the importance of these compounds in the maintenance of embryogenic competence, although the specific mechanism of action cannot be inferred from the data presented. Taken together the results confirm a clearly different metabolome between the two cell lines, with EC tending to a more diverse anabolic metabolism, as evidenced by the higher phenolic content and the presence of organic acids and phenylalanine, while NEC metabolome seems to be more catabolic.

Keywords: tamarillo, somatic embryogenesis, metabolome, NMR

P17

A clonal propagation and cryopreservation of Korean arborvitae via somatic embryogenesis

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The genus *Thuja*, a member of the Cupressaceae family, consists of five species. Among them, Korean arborvitae (*Thuja koraiensis* Nakai) is found only in northeast China and in the high mountains of Korea. Korean arborvitae is a critically endangered coniferous tree and an important Korean native conifer. One option to help conserve this species is the use of cryopreservation systems via somatic embryogenesis. The aim of the work reported here was to optimize an in vitro propagation and cryopreservation system for *T. koraiensis* via somatic embryogenesis. We initially tested experiments to determine the effects of the seed collection date on induction of SE in *T. koraiensis*. To compare the difference between pre-freeze and post-thaw regrowth of embryogenic tissues, we also investigated the effect of cryopreservation on maturation capacity and genetic stability of the regenerated plantlets using flow cytometry analysis and inter simple sequence repeat analysis (ISSR).Whole megagametophytes with zygotic embryos from immature fruits were used and the initiation frequency was roughly 23.7%, however, we could not observe the stages of zygotic embryo development becasue of the small size of *T. Koraiensis* embryos. We tested the effect of cryopreservation on maturation capacity to compare differences between non-cryopreserved and cryopreserved cell lines. Overall, analysis of variance results indicated that maturation capacity was not negatively influenced by cryostorage ($P < 0.1896$). We also performed flow cytometric analysis to confirm the genetic stability of the regenerated plants from non-cryopreserved and cryopreserved cell lines. The results revealed no differences or variations among the regenerated plants. To determine the number of chromosomes in KAV, somatic plants regenerated from non-cryopreserved and cryopreserved cell lines were randomly selected. There were no alterations in chromosome number (2n = 22). Finally, ISSR analysis was conducted to characterize the effect of cryopreservation on genetic variation of the regenerated plants from non-cryopreserved and cryopreserved cell lines. All primers amplified the same band patterns from either non-cryopreserved or cryopreserved cell lines and no ISSR fragment pattern variations were detected. The SE system described here has the potential to aid in the restoration of Korean arborvitae to its native habitat.

Keywords: *Thuja koraiensis* Nakai, endangered conifer, somatic embryogenesis, cryostorage, genetic stability

P18

Optimization of stable cryopreservation system of Ulleungdo hemlock via somatic embryogenesis

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Ulleungdo hemlock is an endemic coniferous species in Korea which is distributed naturally in the Korean island (Ulleungdo). In Korea, this tree had been treated as *T. sieboldii* before it was considered as new species, *T. ulleungensis*, by Holman et al. (2017). As rising global temperatures, the wild populations of Ulleungdo hemlock have been decreased. To date, however, no study has as yet been reported of restoration for Ulleungdo hemlocks via either traditional or modern breeding program. Conventional breeding methods require a long period of time due to their long maturation times and slow growth rates. Biotechnology tools such as *in vitro* culture, genomics and gene transfer, are potentially powerful tools that may help overcome these problems in forest tree improvement. In particular, somatic embryogenesis (SE) has shown promise for improvement of important coniferous species. In conifers, cryopreservation in liquid nitrogen has been applied to species with ETs and somatic embryos which are highly amenable to cryopreservation. However, *in vitro* propagation and cryopreservation system for Ulleungdo hemlocks via SE have not been reported yet.

The aim of this study was to optimize a method suitable for the cryopreservation of embryogenic cultures of Ulleungdo hemlock and the subsequent regeneration of the tissue into plantlets.

Whole megagametophytes with zygotic embryos from immature Ulleungdo hemlock cones were cultured on Litvay's medium (Litvay et al. 1985) supplemented with 9 μM 2,4-dichlorophenozyacetic acid and 4.5 μM 6-benzylaminopurine. Cone collection date had significant effects on induction of embryogenic tissues ($p < 0.001$), which ranged as high as 24%. We also conducted experiments to determine the effect of cryopreservation on maturation capacity of somatic embryo. There were no statistical differences on the production of somatic embryos between non-cryopreserved and cryopreserved cells ($P = 0.129$), but the highest mean number of somatic embryo production was from non-cryopreserved cell line (21.0 ± 5.5). Our results such as the propagation and cryopreservation system, described here show the high potential to contribute the conservation of Ulleungdo hemlock to its native habitat.

Keywords: *Tsuga ulleungensis*, somatic embryogenesis, cryopreservation, endangered conifer

P19

Improvement of micropropagation by micrografting in vitro cultured mature scions of *Castanea sativa* Mill. on physiologically juvenile rootstocks of the same genotype

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It is known that micrografting of mature plant material on a juvenile rootstock can improve micropropagation parameters, as multiplication rate and rooting ability (Monteuuis, 2012). On the other hand, it is also known that, for many tree species, due to topophysis, explants taken from the basal region (e.g. basal sprouts) of the mature tree respond better to micropropagation than those taken from the crown (Bonga, 1982). The differences of behaviour in vitro of materials from both origins can remain for many years (Sánchez et al., 1997). In most works, micrografting for rejuvenation/ reinvigoration purposes is carried out on rootstocks of juvenile origin, and their genotype is different to that of the mature scion. In this work, micrografting was performed using as rootstocks explants of a mature clone taken from basal sprouts (BS "topoclone"), expressing physiologically juvenile behaviour in vitro (high multiplication and rooting rates), and scions were explants of the same clone, taken from the crown of the tree (CR "topoclone"), and expressing significantly lower in vitro multiplication and rooting rates. The results show that micrografting significantly increased multiplication and rooting rates of explants of CR topoclone. This increase was not observed inmediately after reisolation of the scions but after a certain number of subcultures. The multiplication rate remained significantly higher than that of ungrafted material for as long as 25 subcultures (2 years), while difference in rooting rate tends to decrease from subculture 19 to subculture 25. These results show that physiologically-juvenile mature material can have a similar effect to juvenile material when used as rootstock for rejuvenation/reinvigoration through micrografting, and that the rejuvenation effect can consist not of an inmediate effect on increasing micropropagation performance, but in making the explant "responsive" to a possible reinvigoration / rejuvenation effect of continuous subculturing.

Keywords: micrografting, rejuvenation, topoclones, *Castanea sativa*

P20

Cryopreservation of *Podocarpus lambertii* Klotzsch ex Endl. (Podocarpaceae) zygotic embryos by droplet-vitrification technique

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Podocarpus lambertii is indigenous to one of the 25 biodiversity hotspots of the world, the subtropical moist forest ecoregion of the Atlantic Forest biome – Brazil. The characterization of the genetic diversity and structure of natural *P. lambertii* populations in Southern Brazil showed low genetic diversity and high mean fixation index. Furthermore, *P. lambertii* conservation status is classified as near threatened in the IUCN red list, requiring the development of strategies for *in situ* and *ex situ* conservation. In the present study, a protocol of cryopreservation of *P. lambertii* zygotic embryos using the droplet vitrification method was successfully established. Different incubation times in Plant Vitrification Solution 2 - PVS2 (without freezing) did not show significant difference on embryo germination and subsequent plantlet formation. Explants treated with PVS2 (20 and 40 min) and subsequently immersed in liquid nitrogen indicated high green embryos formation, reaching the same rates observed in treatments not subjected to liquid nitrogen, showing 53.33% and 46.66% of green embryos, respectively. Longer exposure time to PVS2 (60 min) showed deleterious effect and decreased rate (20%). A rupture of root apical zone was observed in all treatments subjected to liquid nitrogen, possibly due to the presence of more vacuolated cells in that region, increasing the possibility of intracellular ice crystals formation. However, the rootless plantlets could be successfully rooted after indole-3-butyric acid (IBA) treatment. The cryopreservation of *P. lambertii* embryos might enable the long-term conservation of genotypes from endangered populations as a strategy to overcome the seeds relatively high moisture content. This protocol represents the first *ex situ* conservation approach reported for this species, an endangered Brazilian conifer. In addition, this is the first cryopreservation protocol described for the family Podocarpaceae. Thus, this cryopreservation protocol may be tested end useful for future studies with other Podocarpaceae species.

Keywords: conifer; conservation; seeds cryopreservation; Podocarpaceae

P21

Phenyl adenine as a tool to induce adventitious shoots in *Nicotiana tabacum* and *Luffa cylindrica*

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To increase adventitious shoot induction, there are two strategies. The cytokinin can be modified or its oxidation can be blocked. In 2008, Zatloukal discovered interesting phenyl adenine (PA) derivatives. They were called inhibitors of cytokinin degradation (INCYDE). The most powerful types were INCYDE-Cl (2-chloro-6-(3-methoxyphenyl) aminopurine) and INCYDE-F (2-fluoro-6-(3-methoxyphenyl)aminopurine). They combined a high CKX inhibition cytokinin receptor activation. The core molecule, PA popped up later in a chemical screening of a library of 10,000 small molecules using arabidopsis root explant. It was the only compound being a potent inducer of adventitious shoots on roots. PA activated the cytokinin receptors AHK3 and AHK4 in a bacterial receptor assay. Additionally, PA turned out to be a competitive inhibitor of CKX, leading to an accumulation of endogenous cytokinins (Motte et al., 2013). Here we demonstrate how a single application of 10 µM PA can induce shoots on tobacco leaf explants. We show how it enhances the shoot induction capacity of 5 µM 2iP. Combining with 10 or 20 µM 2iP makes no difference, as the leaves reached their maximum at these concentrations. The results of similar experiment in a recalcitrant species such as *Luffa cylindrica* will be discussed.

Keywords: cytokinin, oxidase inhibitor, in vitro, leaf explant

P22

Effect of liquid nitrogen on the viability and germination of teak pollen.

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With the development of this research, we sought to adapt a pollen conservation methodology of *Tectona grandis* (Teak). It was proposed to standardize and adapt collection methodologies; pollen dehydration using silica gel; tests to determine the percentages of viability and germination of pollen before and after freezing; and finally the technique of dehydration and rapid freezing in liquid nitrogen. In addition, the viability and germination of four clones were evaluated during 18 months of storage in liquid nitrogen. The above in order to offer teak breeding programs; a pollen storage option that guarantees its availability at any time and for any use; mainly for conducting controlled crosses. The results indicated that pollen dehydrates up to 39.5% moisture content when stored for 2 years in liquid nitrogen (NL +), behaves like pollen only dehydrated (39.5% CH) (NL-), obtaining percentages of viability and germination of 80% and 40% respectively, in both types of sample.

Keywords: Teak, pollen, cryopreservation, genetic improvement

P23

Introduction of banana (*Musa*) varieties in the Lubumbashi region (DR Congo): The xcase of the hybrids 'FHIA-01' AAAB, 'FHIA-23' AAAA, 'Pelepita' ABB and 'Grand naine'.

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In the Democratic Republic of Congo, bananas are a significant source of vitamins for the tribes who consume them as a staple food. They have very complex sexual reproduction regime and it is vegetative propagation remains until now the only way of multiplication. However, even this path of propagation is subject to two major problems that limit the extension and development of this crop, namely, the low rate of multiplication resulting in the lack of propagation material and viral diseases propagated by vegetative propagation. In vitro propagation of clean stock represents an attractive pathway for control infestations. The present evaluate in vitro culture media and cytokinins that allow to obtain healthy vitroplants and to remedy the lack of planting material in the studied area.

Keywords: banana, vitroplants, topolin cytokinin.

P24

Simplified method to store embryogenic cells: silver nanoparticles and cryoprotectors elimination effect

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Cryopreservation of embryogenic tissues is an essential requirement to maintain the competence of embryogenic cell lines. In our laboratory, a new method to store *Pinus radiata* embryogenic cell lines at -80ºC was developed successfully using an ultra-freezer (Montalbán y Moncaleán 2017). The methodology carried out was simple and similar to the standard cryopreservation protocols used for embryogenic tissues in several plant species:

1.- Preculture in a highly concentrated sucrose solution supplemented with Me2SO,
2.- Immersion of the samples in liquid nitrogen for 5 minutes.
3.- Storage at -80ºC.

The technique was tested on 62 lines, through the assessment of regeneration capacity and plant conversion ability of the regenerated tissues after a year of storage at this temperature. Although the success of the methodology followed was high, as recovery percentage and plant conversion ability were both above 75%, we sought to improve our results and to simplify the methodology.

For these purposes, we analysed the possibility of eliminating the liquid nitrogen step and the removal of Me2SO from preservation solution.

Furthermore, in a second experiment we tested if the use of silver nanoparticles in the culture medium and in the preservation solution had a beneficial effect on the regeneration ability of embryogenic cultures.

Keywords: Conservation, embryogenic cell lines, *Pinus radiata*, somatic embryogenesis.

Acknowledgements: This research was founded by MINECO (Spanish Government) project (AGL2016-76143-C4-3-R), DECO (Basque Government), CYTED (P117RT0522).

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P25

CYTED NET: BIOALI, Biotechnology to improve breeding programs of species with socioeconomic interest

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The Ibero-American Program of Science and Technology for Development (CYTED) has approved the financing of the BIOALI network "Biotechnology to strengthen improve programs of species with socioeconomic interest". The network that groups 18 research groups, 3 companies of the agri-food and 1 of the environmental sector and a total of 85 researchers from research centers and universities including professionals interested in the development and application of biotechnological tools (tissue culture, genetic, physiological and molecular techniques) for the breeding of species with social and economic interest. The general objective of the BIOALI network (www.bioali.es) will be to contribute, with the generation and exchange of knowledge among members and their environment, to the sustainable improvement of forest trees productivity and nutritional value of food species with social and economic interest. This improvement will be carried out through the exploration of their genomes, the fortification of their products, the development of early diagnosis methods of its main pathogens through phenotyping, physiological and metabolomic techniques and obtaining indicators of abiotic and biotic stresses derived from climate change. This big goal is carrying out by establishment of research consortium, training, exchange of information between multidisciplinary groups in different fields, education and social awareness, actions that will be carried out satisfying the needs of present generations, without endangering the future ones.

Keywords: Breeding, food, productivity, social benefit, trees.

P26

Growth and mineral uptake of *Eucalyptus pellita* in response to inoculation with different mycorrhizal inoculants

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This study determined the response of *Eucalyptus pellita* cuttings to different mycorrhizal inoculants from the Philippines as compared with ectomycorrhizal fungi collected under *E. pellita* plantations in Kalimantan, Indonesia. Shoot tips (2-3 inches) of *E. pellita* were collected from the hedge orchard, dipped in rooting hormone, inserted in rooting materials and incubated under mist system. After two weeks, the rooted cuttings were transferred into containers filled with soil. During the rooting propagules transfer to individual container, they were inoculated with mycorrhizal inoculants: KTH (contains spores of *Scleroderma* and *Pisolithus* sp. native in Kalimantan, Indonesia), MYKOVAM, MYKORICH, MYKOCAP, Mycogroe, Mycogroe+MYKORICH and MYKORICH+MYKOCAP from the Philippines following the recommended dosages as stated in the label. Mycogroe contains spores of *Scleroderma* and *Pisolithus* while the others contain spores of arbuscular mycorrhizal fungi. Height of the control plants consistently the shortest throughout the two months growth in the nursery (prior to field planting), while those inoculated with MYKORICH or Mycogroe+MYKORICH inoculated plants outgrew the other mycorrhizal plants. Height of plants inoculated with KTH was increased by 17% whereas the other mycorrhizal inoculants gave higher (24-51%) height increases except MYKOVAM that gave 14% only relative to the uninoculated control (8.86 cm) counterpart. MYKOCAP and Mycogroe+MYKORICH inoculated plants were 51% and 49%, respectively taller than the control (8.86 cm). Moreover, total dry weight was increased by 104 and 111% with these two treatments. The control plants had the lowest (0.87 g per plant). MYKORICH increased root dry weight by 100% while Mycogroe and Mycogroe+MYKORICH increased root dry weight by 85%. In terms of mineral components, highest increases in total plant N uptake by 131%, K by 111%, Mg by 106%, Fe by 92%, and Mn by 86% were observed in plants inoculated with Mycogroe+MYKORICH. Mycorich alone gave the highest percent increases in total plant uptakes of B (86%), Cu (76%) and Zn (104%). Root P uptake was highest (75%) in plants inoculated with MYKORICH but similar to those inoculated with Mycogroe+MYKORICH (72%) relative to the control (0.035 mg g^{-1}). The results clearly showed that growth and mineral composition of *E. pellita* were greatly improved by inoculation with mycorrhizal fungi particularly Mycogroe+MYKORICH. Currently, the mycorrhizae seedlings are transplanted into the field and undergo a growth test.

Keywords: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, mineral elements, field test

P27

Evaluation of three Temporary Immersion Bioreactor Systems for the micropropagation of a commercial eucalyptus hybrid

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Due to the great economic importance of eucalyptus, its rapid growth, wide adaptability and the multipurpose of its wood, a production increase becomes crucial to deliver seedlings of quality and quantity to supply the internal and external market. The micropropagation of plants using the liquid culture medium is considered to be more effective than semi-solid culture medium because plants have greater accessibility to the medium components, allowing greater gains in biomass and time reduction for propagation; moreover it is ease of handling, with possibility to scale and automate and no expenses with gelling agents. The Temporary Immersion System (TIS), one of the systems that use liquid medium, presents advantages such as avoiding continuous immersion of explants and providing adequate oxygen transfer to the culture, hence, reducing physiological disorders such as hyperhydricity. In this sense, the objective of this work was to evaluate the micropropagation of an eucalyptus hybrid in the temporary immersion systems Plantform, SETIS and ElecTIS (prototype first tested by our research team at IVALSA, CNR, Sesto Fiorentino, Florence, Italy) and also in the semi-solid culture medium. Fifty shoots of the commercial hybrid of *Eucalyptus grandis X Eucalyptus urophylla*, with no apical meristem, were introduced into each bioreactor containing the liquid medium JADS with 0.1 mg L^{-1} of NAA (Naphthaleneacetic acid) and 0.3 mg L^{-1} of BA (6-benzyladenine) and the same number of shoots and culture medium were used for the semi-solid medium, but with the addition of 1.8 g L^{-1} of phytagel. The bioreactors were programmed to submerge the shoots for 10 minutes every 8 hours and the experiment was repeated two times. After 3 weeks of proliferation, the number of shoots per explant and the fresh mass per explant were calculated. ElecTIS presented the highest number of shoots per explant (14.61), followed by the semi-solid culture medium (12.36), Plantform (11.46) and SETIS (10.93). In relation to fresh mass, the treatments presented the same behavior, with the ElecTIS presenting the largest increment of fresh mass per explant (0.41 g), semi-solid (0.27 g), Plantform (0.17 g) and SETIS (0.11 g). None of the treatments showed shoots with symptoms of hyperhydricity. ElecTIS presented promising results for the micropropagation of a commercial hybrid of *E. grandis X E. urophylla*, thus demonstrating its ability to micropropagate this hybrid on a large scale.

Keywords: temporary immersion system, micropropagation, eucalyptus.

P28

In vitro callogenesis in anthers of *Hevea* spp.

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The production of double-haploid rubber plants using anther culture represents an important contribution to the genetic improvement program of this perennial crop, producing in a single generation 100% homozygous plants, and improving efficiency in the production of new cultivars. In our preliminary tests it was verified that the anthers should be isolated without fillet residues, as well as the presence of antioxidants, 2,4-D and kinetin in the culture medium. However, these results are still insufficient for the promotion of calluses that manage to reach the morphogenetic route resulting in somatic embryos and the regeneration of haploid plants. Aiming to contribute to the definition of rubber anther culture protocols, this work had the objective of evaluating the influence of different growth regulators on the production of primary calli in anthers of selected genotypes of rubber tree. For this purpose, anthers of immature floral buds were isolated and cultivated in RT culture media with plant growth regulators. The callogenesis obtained was considered low, not exceeding 15%, being null in the presence of activated carbon in the culture medium. The obtained results allowed to conclude that in RT medium, 2,4-D (2,0 mg L-1) associated with KIN (1.0 mg L-1) and AIA (2.0 mg L-1) or 2,4-D (2.0 mg L-1) associated with Picloram (2.1 mg L-1) and AIA (2.0 mg L-1) promoted the formation of small primary calli in rubber anthers.

Keywords: Rubber tree, haploid culture, somatic embryogenesis, plant growth regulators.

P29

Using in vitro culture for conservation of genetic resources: micropropagation of a monumental *Prunus dulcis* tree

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The need to conserve biodiversity has been granted increasing political and social attention in the last years. Monumental or emblematic trees, both those "wild" trees living in forests and the century-old, "domesticated" agricultural trees, should be preserved *in situ* for its intrinsic value, its cultural legacy, the rich diversity of microhabitats they generate, and the quantity of organisms that depend on them to life. Also, they should be preserved *ex situ* for the study and the conservation of their genetic resources, for educational issues and for reintroducing plant material of high quality in their natural areas, most of them currently degraded or threatened. Within *ex situ* conservation methods, *in vitro* culture presents special advantages in the case of emblematic trees, such as the small quantity of plant material needed to begin the micropropagation procedure and the possibility of implementing long-term conservation techniques as cryopreservation.

The aim of this study was to micropropagate mature material from an ancient almond tree, named “Gladiador”, located in Membrilla (Central Spain), together with juvenile material proceeding from its seeds. This monumental tree, probably 300-years-old, dominates a landscape formed by hundreds of olive trees, and has a special emblematic meaning for the population of the area.

For establishment of axillary shoot cultures, plant material was provided by the FIRE foundation. Three types of material were used: 1) shoots flushed at the tree at the beginning of spring, 2) shoots forced to flush in a phytotron from branch segments collected in late winter, and 3) seeds collected in autumn and stored at a cool place for six months before being germinated in vitro. Murashige and Skoog medium supplemented with 0.5 mg L^{-1} N^6-benzyladenine and 0.5 mg L^{-1} indole-3-butyric acid was used for culture establishment and stabilization. Different combinations of plant growth regulators were evaluated for shoot proliferation, elongation and adventitious root formation. Rooted shoots from cultures obtained from the Gladiador mother tree and from lines originated from seeds were successfully acclimatized in the phytotron and the greenhouse. For mid-term conservation, shoots from mature and juvenile origins were submitted to cold storage at 4-6 ºC. So far, we have obtained 78 plantlets that are currently being acclimated, 70 corresponding to clonal material from the mother tree and 10 corresponding to clonal material from seeds.

Keywords: almond, biodiversity, education, divulgation, mature tree

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P30

Ex situ conservation of *Prunus lusitanica* by micropropagation techniques

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Prunus lusitanica is a woody plant of high ecological and botanical interest. It is considered a characteristic species of the relict subtropical flora that persists in Southern Europe, currently in regression due to both human action and climate change. *P. lusitanica* is listed on Appendix I of the Bern Convention and in Annex II of the EU Habitats Directive. In Azores and Madeira this species is being threatened by the alteration of the water regime, the cutting of trees, invasive alien species and lack of pollinators and dispersal agents (Duarte et al. 2011). Both subspecies that occur in Spain (ssp. *lusitanica* and spp. *hixa*) are listed as Vulnerable in the 2008 Spanish Red List.

With the goal of preserving and increasing its genetic diversity, both *in situ* and *ex situ* approaches must be developed and combined. Advances in plant biotechnology may provide new alternatives to establish germplasm collections, and to facilitate the long- and short-term multiplication and storage of the species biodiversity. At present, only a protocol for *in vitro* germination of immature seeds of this species has been reported (Schulze et al., 2017). The aim of this work was to develop specific protocols for *in vitro* establishment and propagation of juvenile and mature material of *P. lusitanica* populations. Actively growing shoots were harvested from three mature plants of different ages (3- and 30 year-old plants) and were used for *in vitro* establishment. For each genotype, different culture lines were established from a single-node or apex that was subcultured and propagated separately. For propagation, different media as MS (Murashige and Skoog, 1962), DKW (Driver and Kuniyuki, 1984) and WPM (Lloyd and McCown, 1980), supplemented with N^6-benzyladenine (0.5 and 1.0 mg L^{-1}) or Metatopoline (0.4 mg L^{-1}) in combination with indole-3-butyric acid (0.05 mg L^{-1}) were used. Proliferation was evaluated in terms of number of shoots, length of longest shoot and rooting percentage. The effect of genotype, culture line and the different media and plant growth regulators combinations was studied. *In vitro* establishment was successfully achieved in the three genotypes evaluated. The *in vitro* response of the initial explants was dependent on the age of the starting material. Proliferation rates indicated a culture line-dependent response to culture medium. Rooting was achieved in proliferation medium and rooted plants were *ex vitro* adapted and successfully to field conditions.

Keywords: biodiversity, genetic resources, Portuguese cherry laurel, vulnerable species

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P31

Public perception in University Education Institutions about clonal forestry and forest biotechnology in Argentina and Venezuela

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The issue of clonal planting and biotechnology has captured unprecedented public interest and concern throughout the world, where South America has not kept out of this debate. The discussion includes scientific aspects, ethical issues and possible environmental, economic and social impacts, as well as health, technological dependence and sovereignty. Biotechnology in South America has been growing rapidly during the last years, highlighting the micro propagation between the different applied technologies. However, forest biotechnology (mainly GM trees) and clonal forestry face constraints that limit their social acceptance, due to aggressive campaigns by environmental NGOs and environmentalists, directed at people who are unaware of the issue, and based on information from unreliable sources. The objective of this work was to conduct a KAP (knowledge, attitudes and practices) survey on Forest engineering students and Environmental Sciences (Argentina) and Biological Sciences., Environmental Sciences and Agro ecology (Venezuela) on the acceptance in relation to the use of forestry and forest biotechnology. There were differences in responses according to country, career and gender. The results show that environmental awareness is the most important concern, focused on the loss of biodiversity. 76% of students know what a plant clone is, but they do not know how they are produced. However, they identify different vegetative propagation techniques, the best known being the propagation through cuttings and grafts, and the somatic embryogenesis as the least known in the in vitro area. All the students answered that they know what biotechnology is, but they could not select the correct definition. Points of view on these issues differ within countries, because South America is a cultural mosaic, where questions about the acceptance of new technologies have a different basis depending on what each nation considers most important. In conclusion, this preliminary study showed that these subjects should be included in the curriculum of the surveyed careers, incorporating in each country, characteristic local elements of importance to the environment.

Keywords: clonal trees; forest biotechnology, KAP survey; public perception.

P32

What the Latin American people think about the clonal forestry and forest biotech?

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Significant progress has been made in LAC regions in the development of appropriate technologies to improve agricultural productivity in sustainable systems. However, these results rarely reach the field, mainly due to misunderstanding of the relationships between the components of the agricultural and forestry systems by those who run the sector, including professionals and those entities in charge of interaction with other sectors. The innovation processes propose that key actors of society be summoned and supported technically, so that they reach a consensus understanding on key issues related to the management of natural resources and the sustainability of agriculture and forestry. Framed in this context, biotechnology is emerging as a useful alternative in these development processes, even within the complex process of perception that exists of it in society. Public acceptance of technologies is based not only on technological strength and scientific, but also in their social, political and economic perception, being this aspect of great influence for investment in technology, and its influence on the quality of life in society. The use of rapid assessment methods allows us to generate fast data already "which is better information faster than none at all". The objective of this work was to conduct a rapid assessment procedure (RAP) to the public, in order to know the perception they have about clonal forestry and forest biotechnology. For this, an exploratory opinion web-survey was made, evaluating different aspects on the subject. The results indicate that, 84% know that it is a cloning of a tree; 60% know forests of clonal plantations, and 62% think that plantations are not forests. However, 82% think that plantations reduce the pressure on native forests. Increasingly, planted forests will have to be recognized within the community in general by the range of values provided, not just by wood. Communication and community participation and dialogue between forestry companies and stakeholders is increasingly important, where we know that public attitudes towards these issues are influenced by different factors, including information, social context, cultural norms, beliefs, values and perceptions.

Keywords: forest biotechnology, clonal forestry, communication, people, opinion poll.

P33

In vitro germination of ***Brachychiton populneus*** (Schott & Endl.) R.Br.

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Brachychiton populneus, commonly known as the Kurrajongo bottle tree, is a tree that belongs to the Sterculiaceae family. It is a native to eastern Australia with much value in cultivation. Plants are tolerant of dry condition and have many interesting feature. It is usually used as an ornamental, appreciated for its high forage value supplementing the diet of animals in winter when the grass is scarce. The seed contains linoleic acid and oleic acid. It has not been found any study of tissue culture of this species. Seeds can be used as explants but only for culture initiation and getting sterilized plantlets for further experiments of tissue culture where the plantlets germinated in vitro will be used as explants. The use of seed as explants is simply germinating under in vitro conditions to reduce the chances of contamination which is otherwise very high if we take other plant parts as explants. The aim of this study was establish the conditions for in vitro germination of Brachychiton seeds to obtain aseptic seedlings as a source of explants. The seeds were collected from mature fruits, disinfected with fungicide (Kasumin and Captan), 70% ethanol (1 minute) and 30% commercial bleach (30 minutes).They were cultivated in Isolation medium (agar 7,5 g/L with 30 g/L of common sugar). After 15 days, the 80% of the seeds germinated. Of the vitroplants were cut explants (cotyledons, hypocotyl and radicle) and were placed in an induction medium of organogenesis (1/2WPM with or without NAA/ BA at different concentrations). As expected, morphogenesis processes were induced in media with a greater concentration of cytokines than of auxins.

Keywords: seeds, in vitro germination, tissue culture, Brachychiton.

P34

In vitro propagation and callus formation in *Morus alba*.

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Mulberry is a multi-purpose tree with protein rich leaves, that traditionally has been used to feed silkworm and cattle, and is valuable as well as for its fruit and wood production. An initiation and micropropagation protocol was established for *M. alba* 'Acorazonada' and callus was obtained for somatic embryogenesis induction. Culture media containing Murashige and Skoog salts (1962) were used for establishment, multiplication and rooting stages. In the multiplication stage, 67% of the established nodal segments reacted on 2 $mg.l^{-1}$ of BAP. In the rooting stage, 1 $mg.l^{-1}$ of IBA was added and this yielded 72.7% of rooted plants. Forty % of the plants survived the acclimatization. Different explants for callus formation were also evaluated, of which the petioles reacted the best on a medium with 1.0 mg.l-1 mg 2,4-D.

Keywords: micropropagation, callus, tissue culture, morus

P35

Callus induction in *Austrocedrus chilensis*, a vulnerable conifer from Patagonia

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*Austrocedrus chilensis (*Patagonian cypress) is an endemic tree in the Cupressaceae, found in southern Argentina and Chile. In Argentina, it grows in a 60 to 80 km wide strip along the Andean foothills across a broad moisture gradient, in a variety of ecological niches. In the west, *cypress* can be found either in mixed stands with *Nothofagus spp*. or in pure stands. 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 foresst getting into the steppe, preventing desert advance. Cypress is valued not only because of its ecological function but because of the quality of its wood. *Phytophthora austrocedri* is a soil pathogen that causes severe mortality of *the species.* Mortality was first registered in 1948 and its cause remained unknown for 60 years, 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 asexual propagation of tolerant/resistant individuals is the best solution to the problem. At present, little work with no success regarding vegetative micropropagation of the cypress was done. The aim of this study was to contribute to the development of a micropropagation protocol. Seeds were collected from open pollinated natural stands. Before sowing, seeds were pre-chilled for 45 days. Germinated seeds were submerged in 25% sodium hypochlorite solution for 10 min and then transferred to 70% ethanol for a min, for seedlings disinfection.

Cotyledons and adult leaves were used as material for explants, and three basal initiation media were tested: modified LP, WP and MS. Media were supplemented with 2,4-Dichlorophenoxyacetc acid (22,6µM), sucrose (30 $g \bullet l^{-1}$) and Bacto agar (8 $g \bullet l^{-1}$), pH was adjusted to 5,7 before autoclaving. Incubations were performed in the darkness in a growth chamber at 24±2°C.

Green to translucid, or white, calluses, were first observed after 20 days in all assayed media.

This preliminary study evidenced that *A. chilensis* might be able to be propagated by indirect organogenesis or somatic embryogenesis, which will allow to address its application in conservation and restoration programs, as well as in capturing genetic gain from elite genotypes for production purposes. However, more studies are needed to get insight and optimize the process.

Keywords: Cypress, micropropagation, *Phytophtora austrocedri*.

P36

Conservation and vegetative propagation of forest genetic resources from Talares and Monte Blanco ecosystems in Argentina.

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Deforestation and forest degradation have led to widespread biodiversity and environmental losses in Argentina. The Talares and Monte Blanco forests are a relict vegetation type throughout Northeast of Buenos Aires province. These forests have suffered extensive deforestation and fragmentation. It is demonstrated that there is a low capacity of regeneration of the indigenous species after clearing, given that the growth of other previous species causes water deficit and lack of light. That is why selection and successful propagation of native tree species are important for improving ecological restoration of these forests. At the Facultad de Ciencias Agrarias y Forestales (FCAyF), Universidad Nacional de La Plata (UNLP), we carried out an investigation about the propagation requirements of indigenous tree species from these ecosystems. The purpose of this work is to present new approaches for characterization, propagation, domestication and conservation of native tree species from Buenos Aires Talares and Monte Blanco forests. We adjust system of propagation by seeds, cuttings and/or in vitro morphogenesis in several native trees. Our work in plant vegetative propagation started in 1983 through cutting, grafting, microcutting and micropropagation (organogenesis and somatic embryogenesis). We worked with: *Celtis ehrenenbergiana Gill. ex Planch* (Tala), *Scutia buxifolia* Reissek (Coronillo), *Jodina rhombifolia* (Hook. & Arn.) Reissek (Sombra de toro), *Schinus longifolius* (Lindl.) Speg. (Molle), *Erythrina crista galli* L.(Ceibo), *Sesbania punicea* (Cav.) Benth. (Acacia mansa), *Phytolacca tetramera* Hauman (Ombusillo), *Parkinsonia aculeata* L.(Cina-cina), *Salix humboldtiana* Willd. (Sauce criollo), *Citharexylum montevidensis* (Spr.) Mold. (Espina de bañado), *Terminalia australis* Cambess and *Acacia caven* (Molina) Molina. We propagated all of them with different techniques. This native species were recommended as appropriate for propagation and afforestation. We describe evidence of protocols through the major methods being used, developed and applied to propagate and conserve native species. Results showed that *ex situ* strategies and propagation skills conserve unique species. These actions can lead to a greater and positive impact in the conservation of our native woody species.

Keywords: woody plants, vegetative propagation, tissue culture, native species,

P37

In vitro propagation of pine (*Pinus caribaea* vr. Hondurensis) through indirect organogenesis

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Forest species have become high demand crops nationally and internationally, which has led to the need to manage this type of crops efficiently. Within this type of propagation, tissue culture offers a great potential to support traditional methods, by increasing the production of genetically superior varieties, coming from the selection of populations, from conventional breeding or from a limited number of pollination seeds. controlled Induction of indirect organogenesis was carried out using juvenile buds, which were sown using the 100% Schenk and Hildebrandt (1972) salts as the base medium. This medium was supplemented with Morel vitamins (10 ml / l), cysteine (60 mg / l), coconut water (5%), ANA (2 mg / l) and gelrite (2.5 gr / l) and he called him MA. At the same time, a similar medium was used, to which the concentration of ANA (4 mg / l) was modified, and was called MB. Significant differences were observed between treatments, the MA being the medium where the best results were observed, with an approximate average of 20 shoots from each explant. The alternative with the best results for the induction of the rooting process was to use a decrease in the concentration of salts, obtaining between 30-40%, a percentage that improved with the use of anti-stress substances (50%). Additionally, it is important to note that this is a difficult process to induce in in vitro-grown forest plants, so that the results obtained can be shown as favorable. The results indicate that indirect organogenesis could be an efficient alternative to obtain pine plants on a large scale, reducing the difficulties that this crop presents for the mass production of plants with specific characteristics.

Keywords: propagation, organogenesis, pine, forest,

P38

Propagation and rooting of *Prunus avium* by temporary and continuous immersion systems

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The conservation of wild and local varieties of plants contributes to sustainable agricultural production, healthy and diversified diets, healthy ecosystems and sociocultural stability. However, FAO estimates that over the last century about 75 percent of the genetic diversity of agricultural crops has been lost. An increasingly number of local varieties of fruit trees are in danger of loss through habitat destruction or socio-economic pressures towards the use of commercial and more uniform varieties. Tissue culture represents a method for conservation and propagation of these genetic resources, promoting their reintroduction in the agricultural sector. The aim of this study was to micropropagate three varieties of cherry growing at local farms in Galicia, Northwestern Spain. Plant material of the varieties named "de Viño", "Negra de San Cristobal" and "Negra de Fene" was provided by a local association of fruit growers (Agfa do Eume). Shoots developed in ramets of three-four-year old trees were used for the initiation of in vitro cultures.

For proliferation, cherry germplasm was cultured in semisolid medium gelled with agar and in liquid medium by temporary immersion, using RITA® bioreactors as recommended by their manufacturers.
For rooting induction, indole-3-butyric acid (IBA) was applied at 9.8 µM for one month or at 122 µM for 24 h. For rooting expression, shoots were cultured in glass jars in semisolid medium or in plantform™ bioreactors with rockwool cubes soaked in liquid medium. One min aerations with CO_2 enriched air were provided 16 times per day to plantform™ bioreactors.

The three varieties of cherry were successfully proliferated in semisolid and liquid medium. Multiplication coefficient and shoot length were affected by genotype in both systems, and some extent of hyperhydricity was detected in RITA® bioreactors. Rooting ability was also genotype dependent. Shoots rooted in plantform™ bioreactors showed more roots and performed better during the acclimation process than those rooted in semisolid medium.

Keywords: acclimation, bioreactors, cherry, liquid medium, local varieties

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P39

Effect of sucrose supplementation and culture system on growth and stress status of *Prunus domestica* and *Castanea sativa* x *C. crenata* shoots

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The acclimatization step remains a critical stage for using *in vitro* culture for large-scale propagation of woody plants. It has been claimed that the elimination of sugar from nutritive media promotes photosynthetic activity, produces a healthier physiological state and increases the adaptation of the plantlets to greenhouse conditions. The aim of this study was to explore the possible benefits of decreasing sucrose supplementation during the micropropagation of woody plants. For that, we used shoots from a *Prunus domestica* cv. "Claudia Blanca País" tree and from two clones of hybrid chestnut (*Castanea sativa* x *C. crenata*). These explants were cultured in different conditions of light and sucrose supplementation, in order to evaluate their growth response by morphological parameters and their stress status in terms of the total phenolic content and the antioxidant activity. In the first experiments, the initial explants were obtained from shoots grown in semisolid medium (SS) with 3% sucrose (w/v) for both species. Then, plum shoots were cultured in liquid medium (LM) by temporary immersion, using RITA® bioreactors, whereas chestnut shoots were propagated in LM by temporary or continuous immersion, using planform™ and 6 L vessels adapted from food containers, respectively. Three concentrations of sucrose (0, 1 and 3%) were evaluated. The bioreactors were placed either under conventional lights providing a photosynthetic photon flux density (PPF) of 50 µmol m^{-2} s^{-1} or under white LEDs to increase PPF to 150 µmol m^{-2} s^{-1}. In this latter case, CO_2-enriched air was provided. In subsequent experiments, the explants were obtained from shoots cultured in SS with 3% sucrose or from shoots cultured in LM with different sucrose concentrations. The results suggest a direct relationship between the sucrose concentration and the content of phenolic compounds, the antioxidant activity and the morphological development reached for the explants. The explants that performed better were those cultured with 3% sucrose under high PPF, followed by those cultured with 1% sucrose in the same conditions. However, when these latter explants were used as initial explants for a second or third culture cycle in the same conditions (LM and 1% sucrose), their growth reached similar levels as the explants cultured with 3% sucrose. These results indicate the need of a progressive adaptation to the new conditions for a successful propagation of woody plants with low sucrose levels.

Keywords: antioxidants, bioreactors, chestnut, phenolics, plum

This research was partly funded by the Xunta de Galicia (Spain) through the Contrato Programa 2017-2018 and the project IN607A 2017/6. We thank Alejandro Díaz, Rafael Sánchez, and Patricia Val for technical assistance.

P40

Physiological and molecular juvenility markers for birch *in vitro* shoot culture

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Silver birch (*Betula pendula*) is economically important tree species in Latvia. Micropropagation of birches is used in breeding programs, in order to effectively propagate selected genotypes, which can then be used for future scientific evaluation and establishment of faster growing and more productive forest stands. Plant material derived from mature birch trees exhibits low morphogenic potential *in vitro*. Various *in vitro* cultivation conditions including different medium composition have to be used to achieve successful rejuvenation and axillary shoot proliferation. Mechanisms underlying rejuvenation process and factors affecting it are not fully understood. Appropriate markers indicating rejuvenation of birch *in vitro* shoots could greatly increase the effectiveness of the research on this phenomenon and help to investigate factors affecting rejuvenation. In this study morphological, anatomical, and molecular juvenility markers were developed for birch *in vitro* shoots. Juvenile and mature birch *in vitro* shoots were examined for differences in shoot morphogenic potential, leaf and stem morphology and anatomy. For molecular examination the expression level of the master regulators of phase change - highly conserved micro RNA miR156 and miR172 and their target genes, were analysed. Expression of five predicted miR156 and three miR172 precursor sequences from different silver birch contigs and their target genes - *SPL1, SPL9, RAP2-7, AP2* was determined. Numerous morphological and anatomical traits differed between juvenile and mature birch *in vitro* shoots. The juvenility markers that characterize juvenile birch *in vitro* shoots are high rooting potential, high shoot multiplication rate, small and thin leaves, small shoot radius, decreased lignification of sclerenchyma, undeveloped periderm and small phloem width ratio to stem radius. Expression level of two miR156 precursors and two of their target genes (*SPL1* and *SPL9*) can be used as molecular birch *in vitro* shoot juvenility markers. The largest differences in expression levels between juvenile and mature shoots were observed for one of the miR156 precursors (miR156_789) and its target gene *SPL1*. Juvenile birch *in vitro* shoots were characterized by higher miR156 precursor and lower *SPL1* expression level compared to mature shoots.

Keywords: *Betula pendula*, plant tissue culture, rejuvenation, miRNA, gene expression

P41

Genetic characterization of oak trees with limited natural recovery potential and their effective reproduction by the organogenesis

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Quercus L. (Oak) belongs to the large genus in the family *Fagaceae*. *Quercus petraea* and *Q. robur* are most common oak species in Europe growing in a wide range of habitats. The landscape matrix, climate conditions, biotic and abiotic stresses heavily impact aboveground vegetation and reproduction potential of several oak trees in the Czech Republic. Nowadays, the sensitive and outstanding individuals may be vegetatively propagated, which may be crucial for many oak species, in which seed production or growth opportunities are limited.

The aim of our present work is to determine genetic variability of selected oak trees using nuclear microsatellite markers (SSR) and to find out appropriate *in vitro* propagation method of oak species, in which conventional vegetative propagation by cuttings is problematic. To reveal potential selective effects of local environment on the variability, thirty individuals of *Q. petraea* and *Q. robur* collected from the rocky (dry area with a low-nutrient availability) and forest area (well moisture conditions with enriched soil) in the Czech Republic will be analyzed. For evaluation of genetic diversity among the selected oaks, 12 polymorphic SSR markers will be used to calculate genetic diversity parameters using the statistical program GenAlEx 6.501. The next step will be development of micropropagation method to multiply selected oak clones. For this purpose, collected oak bud explants were sterilized and transferred into media with different cytokinin concentrations (BAP, 6-benzylaminopurine). After obtaining the results, we will be able to establish suitable micropropagation method for effective shoot growth and proliferation.

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Keywords: oak tree, SSR markers, vegetative *in vitro* reproduction

P42

Regeneration of unique grey poplar population by organogenesis and characterization of individuals by SSR analysis

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Present research was aimed to verify the possibility to regenerate Grey poplar (*Populus ×canescens* Aiton Sm.) mature trees by means of micropropagation technique. Natural population of grey poplar located in floodplain forest at Dyjákovice (South Moravia of the Czech Republic) is characterized by unique and valuable phenotype traits. The very low success of grey poplar cuttings was the reason for developing method of vegetative propagation based on *in vitro* organogenesis. Dormant buds were sampled from the selected old trees in spring and autumn. The extirpated full-grown tops were sterilized and put on the nutrient medium. We observed that the same composition of MS media used for induction of organogenesis and multiplication characterized by a lower concentration of BAP (0.2 mg/l) and glutamine (10 mg/l) was very efficient for grey poplar optimal growth under *in vitro* conditions. The high number of adventitious shoots (20 – 30) was formed in one multi-topped culture. The losses were minimal, around 2 %, during rooting and acclimatization occurred. Up today, we obtained several healthy plantlets that are growing on the outside bed of the experimental nursery and on the plots at Dyjákovice.

To acquire more detailed knowledge about the genetic diversity and for clonal identification of selected gene units of grey poplar we used the Simple Sequence Repeats (SSR) method of DNA analyses. We analyzed 157 grey poplar trees from unique population, 25 individuals were male and 132 were female. Grey poplars samples were screened by nine selected polymorphic microsatellite markers from SSR resources provided by the IPGC. PCRs were optimized for tested primers that have been scanned in publications (van Loo et al. 2007; van der Schoot et al., 2000; Smulders et al., 2001 and Tuskan et al., 2004). Fragment sizes were determined on capillary electrophoresis (Applied Biosystem 3500). The obtained data were analyzed using the statistical programs CERVUS and GenAlEx 6.501. Based on data evaluation, we detected only 31 different genotypes in all samples tested. Among 132 female individuals were found only 6 different genotypes while 25 male genotypes were completely different. The identified genetic loci have been verified as polymorphic and could be further used for clonal identification of grey poplar trees.

Keywords: grey poplar, organogenesis, clonal micropropagation, SSR markers

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P43

ABI3 and *VAL2* genes action during somatic embryogenesis of *Coffea arabica*: competence acquisition and developmental marker genes

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The employment of biotechnology-based approaches such as somatic embryogenesis (SE) has been applied to numerous plants including *Coffea* sp. Despite the economic importance of this genus, to date, little information exists on the role of key regulatory genes in somatic embryogenesis in coffee. Our work provides information about performance of *ABI3* and *VAL2* genes by RT-qPCR during indirect somatic embryogenesis of *Coffea Arabica.* To achieve this, a bioinformatics analysis was performed to identify the genes in the coffee genome. The cell suspensions presented the same histological pattern and increased regeneration rate in culture time function, yielding up to 6.6 embryos per 1 mg of embryogenic aggregates at 7 months. We have identified possible orthologs for *VAL2* (Cc06g00410) and *ABI3* (Cc01g17380) as well as the other members belonging to superfamily B3. The *CaABI3* expression was dramatically higher in embryogenic calli and embryogenic cell suspension at all culture times (2 to 7 months) as compared to non-embryonic calli, corresponding to 122 up to 522 times more transcripts. Whereas the Ca*VAL2* had highest expression in the embryogenic stages of cotyledonary and plantlets, showing its clear performance in the embryogenesis late stages. The present study suggests that the *CaABI3* can potentially be used as a biomarker for acquisition of embryogenic competence during somatic embryogenesis process. In the case of *CaVAL2*, the gene can play an essential role in regulating the transition from embryo maturation to plantlets growth, serving as developmental marker. The results presented here may help to elucidate the process of induction and development of embryos, facilitating the establishment of efficient plant regeneration procedures.

Keywords: Somatic embryogenesis (SE), Coffee, RT-qPCR, Abscisic acid-insensitive 3, Viviparous1/abi3-like2.

P44

Genetic transformation of *Quercus suber* and *Quercus ilex* somatic embryos with a gene encoding a Gnk2 protein

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Cork oak and holm oak are economically and ecologically the most important tree species in the Mediterranean ecosystem. Cork oak provides cork, a renewable product that is widely used in the wine industry, while holm oak produces acorns, used to feed cattle and Iberian pigs. In the last few decades, *Quercus* populations have been increasingly affected by the pathogen *Phytophthora cinnamomi*, one of the main causes of the syndrome denominated oak decline, among many other fungal diseases.

Ginkbilobin2 (Gnk2) is a seed storage protein that occurs in seeds of the gymnosperm *Ginkgo biloba* and is known to possess antifungal activity. It is also found in *Castanea crenata*, a species of the *Fagaceae* family that shows resistance against attack by *P. cinnamomi* (Santos *et al.* 2017). The objective of the present study was to produce cork oak and holm oak somatic embryos that overexpress the Cast_Gnk2-like gene.

The Cast_Gnk2-like gene was isolated from *C. crenata* roots and cloned into pK7WG2D under the CaMV35S promoter by using the Gateway cloning system (Invitrogen, NY, USA). The resulting plasmid was transferred into *Agrobacterium tumefaciens* strain EHA105.

Small clumps of 2-3 somatic embryos at globular and/or torpedo stages, obtained from three different cork oak embryogenic lines (ALM6, ALM80 and TGR3), and 2-3 proembryogenic masses isolated from three holm oak embryogenic lines (Q8, E2 and Q10) were used as target explants. These explants were co-cultured for 5 days with EHA105pGnk2 and then cultured on selective medium containing kanamycin and carbenicillin. After 14 weeks on the selective medium, the transformation efficiency was determined on the basis of the fluorescence of surviving explants. In both species, the transformation efficiency was genotype dependent, and its values were higher in cork oak [TGR3 (9.2%), ALM80 (6.7%) and ALM6 (2.5%)] than in holm oak [Q8 (2.5%), E2 (2.5%) and Q10 (0.0%)]. The presence of Gnk2 gene on transgenic embryos was verified by PCR.

Keywords: antifungal activity, cork oak, holm oak, somatic embryogenesis, *Phytophthora cinnamomi*.

Santos C, Duarte S, Tedesco S, Fevereiro P, Costa RL (2017) Expression profiling of *Castanea* genes during resistant and susceptible interactions with the oomycete pathogen *Phytophthora cinnamomi* reveal possible mechanisms of immunity. Front. Plant Sci 8:515.

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P45

Characterization of the influence of nitric oxide donors on metabolism of polyamines and amino acids in embryogenic cell cultures of Brazilian pine (*Araucaria angustifolia* (Bertol.) Kuntze)

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High levels of stress and/or the application of exogenous plant growth regulators have been used for the induction of embryogenesis in cell cultures. Stress response accelerates somatic embryo formation and increases the transcription of genes related to stress treatment. Thus, the levels of stress tolerance exhibited by individual cell lines may influence both embryogenic cell viability and metabolism, as well as their adaptation to environmental conditions during *in vitro* culturing. Polyamines (PAs) and nitric oxide (NO) are molecules associated to several plant growth and development processes, including adaptive responses to biotic and abiotic stress. PAs (putrescine, spermidine, and spermine) are biochemically related to NO through arginine, a common precursor in their biosynthetic routes, suggesting that alteration in NO homeostasis can affect PAs bioavailability and vice-versa. Moreover, NO has been shown to be produced from PAs through a still uncharacterized mechanism (Tun et al. 2006; Silveira et al. 2006). The overlapping roles between PAs and NO raise the question of how both molecules may act in coordination during plant development. Somatic embryogenesis (SE) associated to cryopreservation can represent a useful strategy for ex situ conservation of Brazilian pine, a native endangered conifer of South America. In Brazilian pine, PAs and NO seem to be involved in the regulatory mechanisms responsible for proliferation of proembryogenic masses and differentiation in somatic embryos. In order to elaborate an appropriate condition to improve SE in Brazilian pine, we analyzed the influence of NO donors (GSNO and SNP) during somatic embryo formation in three embryogenic cell lines. In addition, endogenous NO production, PAs and amino acids levels were recorded after cultivation in the presence of NO donors for 30 min, 24 and 72h. These findings will be important for evaluation of embryogenic culture responses to changes in media culture formulation and increase of somatic embryo formation in different Brazilian pine genotypes.

Keywords: nitric oxide, polyamines, somatic embryogenesis, Brazilian pine

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P46

Auxin signaling homeostasis in proembryogenic masses of Brazilian Pine early somatic embryos

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Somatic embryogenesis (SE) is an important tool to comprehend the process involved in the initial stages of embryogenesis. However, in non-model species, the molecular and biochemical mechanisms that control the SE process are not complete elucidated. This fact become a challenge for an effective SE protocol, specially for *A. angustifolia*, an endangered conifer species. The embryo development is highly dependent of optimal hormonal homeostasis and signaling pathways. Auxin gradient and auxin-mediated signaling is essential to the establishment of the polar axis and morphological adaptations to the environment. In this context, our aim is to uncover the regulatory mechanisms underlying the morphological processes that allow embryos to form. We performed a curated identification of auxin metabolism-related genes in two different embryogenic cell lines (responsive and blocked to form embryos) using the Araucaria transcriptome database. Additionally, we investigated the disturbance effect in the auxin flow by supplementing the proliferation culture medium with 2,3,5-triiodobenzoic acid (TIBA - auxin transport inhibitor) and phloroglucinol (PG - auxin synergist). Our analyses revealed a set of differentially expressed genes involved in auxin perception (AUXIN RESPONSE FACTOR) and homeostasis (AUXIN RESPONSIVE GH3 GENE FAMILY) in the responsive cell line. This set might be involved in embryonic patterning and embryogenesis capacity. Additionally, the responsive cell line showed an influence in cell polarity of proembryogenic masses in response to supplementation of TIBA, increasing the number of suspensor cells. On the other hand, PG promoted an increment of embryogenic cells reducing significantly the suspensor cells, in both cell lines. Thus, both TIBA and PG have strongly morphology effect in both cell lines. Further analyses are needed to understand molecular and biochemical mechanisms that lead the establishment of cell polarity. These analyses include auxin related genes mRNA profile, auxin gradient and content, and their implication to somatic embryo development.

Keywords: auxin mediator, cell proliferation, tissue culture, Brazilian pine.

P47

Theobroma cacao L.: Effect of myo-inositol on somatic embryogenesis efficiency

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The Nestlé Cocoa Plan (NCP) launched in 2009 has the three following main goals (1) enabling farmers to run profitable farms, (2) improving social conditions in cocoa communities and (3) supporting the production of sustainable, good quality cocoa. Nestlé from 2010 to 2015 distributed trees in two countries: in Ecuador to support the production of fine cocoa, and in Ivory Coast, which is the main producing country with 33% of the world production.

The accelerated propagation method based on somatic embryogenesis (SE) has been developed for cocoa tree by R&D-Tours since 2000. Despite all the achievements obtained from this technology, the conversion rate of cocoa somatic embryos into "normal" plantlets remains low. Only 20% of these embryos – in average –converted into plantlets meeting Nestlé quality requirements and farmers expectations. In order to improve this conversion rate, we decided to test myo-inositol addition in our standard media.

For that, an experimental design was executed to evaluate at both physiological and transcriptomic levels the impact of myo-inositol addition at different times in our standard medium. Here, we report that the addition of myo-inositol at 50 g/L during the expression phase of SE process significantly increased the conversion rate for the selected genotype (40% versus 20%). In addition, the results show that the addition of myo-inositol clearly induces important transcriptomic changes currently under investigation. Their identification will highlight major metabolic pathways involved in SE process potentially pointing new candidate molecules to test.

Keywords: somatic embryogenesis, cocoa, myo-inositol, transcriptomic

P48

The effect of different air humidity during desiccation on the development of Norway spruce somatic embryos

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The protocols of coniferous somatic embryogenesis (SE) are constantly evolving with the aim of its applicability for the wide spectrum of species. Although individual genera, sometime species, needs protocol modifications, the goal remains the same – fully developed somatic embryo mimicking zygotic embryo. Desiccation as the final phase of embryonic development leads to biochemical changes approaching somatic embryos to its zygotic counterparts (e.g. Gemperlova et al. 2009) and appears to be important in the transition from embryogenic phase to germination. The objective of the presented study was to follow morphological and selected biochemical characteristics induced by various air humidity during desiccation of Norway spruce (*Picea abies* Karst.) somatic embryos.
The fully developed embryos were desiccated in three different levels of air humidity (90%, 95%, and 100%) for 10 days; the rest of desiccation (another 10 days) took place in 100% air humidity. We described changes of polyamine content as well as abscisic acid and malondialdehyde to follow the level of drought stress in embryos. Concurrently we observed the expression of two beta – 1,3, glucanase and two chitinase genes and selected ATG (autophagy related) genes, since they are assumed to play role both in development and reaction of plants to various stresses (e.g. Veluthakkal et al. 2012).
Desiccation affected polyamine levels, namely the proportions between putrescine and higher forms of polyamines (spermidine and spermine). Low humidity in the start of desiccation led to increase of putrescine level, which was lowered after subsequent rehydration in the end of desiccation. Transcriptional levels of studied genes generally followed one pattern; their expression diminished after water stress treatment. Rehydration of embryos started resumption of their expression. Although basic morphology of desiccated embryos was comparable in all three variants, germination of somatic embryos cultivated in 90% of air humidity was negatively affected. Somatic embryos were highly active during desiccation both on the level of polyamine metabolism and expression of selected genes.

Keywords: autophagy – related genes, desiccation, glucanases, chitinases, polyamines

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P49

Effects of exogenous phytosulphokine and polyamines in somatic embryogenesis of stone pine

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The stone pine (*Pinus pinea* L.) is a Mediterranean forest species considered as a fruit tree for edible pine nut production. Plant regeneration by somatic embryogenesis (SE) was achieved in this species. A general phenomenon observed in conifers is the loss of the maturation ability with increasing age of embryogenic tissue. The addition of phytosulfokine (PSK) to the proliferation medium was reported to have a stimulatory effect on the growth of embryogenic cultures and on the formation of somatic embryos. On the other hand, changes in the endogenous levels of polyamines (PAs) have been related with somatic embryo development. Exogenous spermidine and putrescine were reported to promote the elongation of suspensors and the formation of somatic embryos during maturation. In an attempt to recover the ability to produce somatic embryos of stone pine embryogenic lines that were subcultured for long time they were exposed to either PSK during proliferation or different PAs during maturation.Three embryogenic lines that showed different maturation ability were cultured on proliferation media supplemented with both 32 and 50 nM PSK. The line of better maturation ability was cultured on maturation media with 100 µM putrescine (Put), spermidine (Spd) or spermine (Spm). Results showed that the addition of PSK had a slight stimulatory effect on the formation of early somatic embryos, but complete maturation was not obtained. Moreover, exogenous PAs promoted the formation of early embryos but with abnormal morphology. Put slightly reduced culture growth and stimulated the formation of elongated embryos that developed polyembryogenesis. Spd slightly increased culture growth and stimulated the formation of clusters of abnormal somatic embryos. Spm reduced culture growth, and a small amount of malformed embryos were produced. Therefore, the loss of the ability to produce normal mature somatic embryos in the aged cultures of *P. pinea* could not be reversed with the exogenous supply of both PSK during proliferation and PAs during maturation.

Keywords: phytosulfokine, polyamines, somatic embryogenesis, stone pine.

P50

Proteome changes in elicited and infected holm oak somatic embryos

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Dieback disease, mainly caused by the oomycete *Phytophthora* spp., is responsible for forest decline in evergreen oak forest areas of the southwestern Iberian Peninsula. Among oak species, holm oak (*Quercus ilex* L.) seems to be the most sensitive to the oomycete infection. The aim of our study is to increase resilience to the pathogen by applying elicitors or oomycete extracts to holm oak somatic embryos (SEs) in order to induce epigenetic memory. To this end, we undertook a proteomic assay to elucidate whether elicited embryos increased their defense related proteins compared to the non-treated SE, after infection with active *Phytophthora cinnamomi* (strain 1630) extracts. A holm oak embryogenic line was elicited for 3 days with 0, 25 or 50µM methyl jasmonate (MeJA) in Elicitin Secretion Medium (ESM, Horta *et al.* 2008, PMPP 73:48–57). Then, SEs were transferred to proliferation medium (Martínez *et al*. 2015, Plant Cell Tiss Organ Cult DOI 10.1007/s11240-015-0722-6). After 5 days, elicited and control material was soaked for 3 hours with 40 ml ESM liquid medium containing 20% of *P. cinnamomi* exudate and then transferred to proliferation medium. After 24 hours, somatic embryos were frozen and lyophilized. Protein extraction was carried out following the TCA/Acetone method as described in Valero-Galván *et al.* (2014, Methods and Protocols vol. 1072, DOI 10.1007/978-1-62703-631-3-49), then, one-dimensional electrophoresis gels with poliacrylamide and sodium dodecyl sulfate (SDS-PAGE) were prepared in order to test the extracted samples viability. Samples were run in a single band gel and these bands were cut and analysed. Peptides and proteins were identified, characterized and quantified by nano HPLC-mass spectrometry. Twenty-four differential proteins were found, establishing four groups that coincided with the treatments. Among them, a polypeptide structurally related to the thaumatin family, the P21 protein (*Glycine max*) and the glutathione S-transferase DHAR2 *(Arabidopsis thaliana) are related to stress response. Preliminary studies suggest an increased resilience to Phytophthora cinnamomi in samples treated with 50*µM of MeJA elicitor.

Keywords: holm oak dieback, elicitation, somatic embryos, resilience, *Phytophthora* spp., proteomics.

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P51

Effect of ethylene on somatic embryogenesis of *Solanum betaceum* Cav.

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Solanum betaceum Cav., commonly known as tamarillo, is an Andean small solanaceous tree, which is cultivated for its high nutritional fruits. This species has been studied due to its particular interest in understanding aspects of *in vitro* morphogenesis, in processes such as somatic embryogenesis (SE). The Laboratory of Plant Biotechnology has been studied different aspects related with SE in tamarillo, which have shown different tissue responses to SE induction depending on the type of culture vessel used. The cause of these differences seems to be influenced by the culture atmosphere, which is constituted not only by atmospheric gases, but also by ethylene. Since ethylene has been related as a hormone that may interfere with *in vitro* morphogenesis processes, it is important to determine whether ethylene is the component of the culture atmosphere responsible for these morphological differences.

The response of tamarillo with regard to diverse stages of somatic embryogenesis was tested on different MS medium containing 50 µM silver nitrate ($AgNO_3$), 10 µM aminoethoxyvinylglycine (AVG) and 20 µM ethephon (ETH). The effect of ethylene on SE was evaluated by the induction of embryogenic callus (EC) and by the number of somatic embryos developed from EC.

The leaf explants exposed to $AgNO_3$ and AVG have produced mainly non-EC, which suggests that the inhibition of ethylene action or its biosynthesis have a negative effect in the induction of SE in tamarillo. Furthermore, the presence of ETH increased significantly the induction of EC and the formation of somatic embryos was observed after 10 weeks in the induction medium. These results suggest a positive correlation between this hormone and the induction of SE in tamarillo as well as the apparent potentiation of somatic embryos formation through a one-step process. The treatment with $AgNO_3$ and AVG has enhanced the number of somatic embryos developed from EC while the presence of ETH inhibit their formation. These results indicate that a lower action of ethylene in plant tissues seems to promote the formation of somatic embryos in a medium without auxins. Although the molecular mechanisms of ethylene action in this study remain unknown, taken together these results suggest that ethylene plays a specific role at the different stages of the SE process in tamarillo. Assays are being carried out to quantify ethylene in the culture vessels.

Keywords: $AgNO_3$, AVG, embryogenic callus, ethephon, tamarillo

P52

Cell wall remodeling by pectin esterification and AGP expression underlies somatic embryogenesis of cork oak

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Somatic embryogenesis (SE) is a feasible system for in vitro regeneration, with many biotechnological applications in woody species, but the regulating mechanisms of the process are largely unknown. Changes in cell wall mechanics controlled by the methylesterification/de-esterification status of pectins, mediated by pectin methyl esterases (PME) and pectin methyl esterase inhibitors (PMEI) underlie organogenesis initiation and embryogenesis progression in various species (Solís et al. 2016). Nevertheless, the functional meaning of pectin-related cell wall remodeling in different cell types and processes still remains unclear. Arabinogalactan proteins (AGPs) are present in cell walls, plasma membranes and extracellular secretions, playing a key role in several plant developmental processes, including different aspects of sexual reproduction and embryogenesis (El-Tantawy et al. 2013). Addition of exogenous AGPs to culture medium has been reported to promote somatic embryogenesis, however, the precise role of endogenous AGPs in the regulation of somatic embryogenesis remains poorly understood. In this study, we have investigated changes in pectin esterification and AGPs during SE in *Quercus suber*. Expression analysis of PMEI and AGP genes, immunofluorescence and confocal analysis were performed by using monoclonal antibodies to AGPs, high- and low-methylesterified pectins (LM6, LM2, LM19, LM20, JIM7, JIM5). Results allowed the characterization of the distribution patterns of AGPs and pectin esterification/de-esterification during SE progression, as well as their correlation with the expression patterns of *QsPMEI*, *QsPMEI2*, *QsLys-rich-AGP18* and *QsAGP16L1* genes. At early SE stages, cells showed high levels of esterified pectins and AGPs. At advanced SE stages, AGP expression increased and differentiating cells exhibited walls rich in de-esterified pectins. Functional analyses with catequin and Yariv reagent, inhibitors of PME and AGPs respectively, have indicated a role for both pectins and AGPs in the cell wall remodelling associated with SE of cork oak, giving new insights into the regulating mechanisms of the process for potential applications in improving SE yield in tree breeding programs.

Keywords: Cell wall, pectins, AGPs, somatic embryogenesis, cork oak

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P53

Somatic embryogenesis induction in BC1 hybrid zygotic embryos oil palm (*Elaeis oleifera* x *Elaeis guineensis*) x *Elaeis guineensis*

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The caiaué or American oil palm (*Elaeis oleifera*) is the only source currently available for tolerance to Fatal Yellowing (FA), a condition that can cause plant's death and has unknown cause and considered the major threat to this culture in Latin America. In this sense, the Program of Genetic Improvement of the Oil Palm belonging to Embrapa Western Amazon has been focused on the development of cultivars resulting from the hybridization of the African oil palm (*E. guineensis*) x caiaué. Among the applied methodologies, hybridization via backcrossing, which consists of crossing the African oil palm (recurrent parent) and the caiaué (donor) that generate backcrossing progenies (CR) of high variability and superior to those intraspecific. For the genus *Elaeis*, cloning BC1 progenies by somatic embryogenesis is considered an important tool that allows the replication of selection trials easier. Considering the specificity of protocols, this work had the objective to evaluate the induction and proliferation of embryogenic calli in zygotic embryos of CR1 of *E. guineensis* [(OxG)xG]. Zygote embryos cultured for 90 days on MS and Y3 medium with 2,4-D and (450 μM) were transferred to basal medium with 40 μM auxin combined with 2iP (0 and 10 μM). The primary calluses presented slow growth formation. At the end of 90 days of cultivation, no significant statistical difference was observed for any of the variables evaluated for culture medium and auxins and their interactions. In the medium of multiplication at 150 days, the picloram was superior to 2,4-D, favoring the proliferation of primary calli, as well as the formation of embryogenic potential structures, either in MS or Y3.

Keywords: Caiaué, callus, culture medium, plant growth regulators.

P54

Improving somatic embryogenesis in the grapevine

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Somatic embryogenesis is an excellent system for woody plant propagation. As somatic embryos are of unicellular origin in many species including grapevine, somatic embryogenesis is hence useful for several purposes, including basic developmental research, mutagenesis, genetic transformation or functional genomics. Although protocols are available for several grapevine cultivars, the application of somatic embryogenesis is still essentially empiric, with several limitations remaining. During our work on grapevine somatic embryogenesis, we have observed that the induction of the somatic embryos and their maturation are limiting stages leading to a reduction in the efficiency of regeneration of normal plants. The induction of embryogenic cultures is dependent on a number of factors, as the genotype, the type of explant or its developmental stage. Most protocols in the grapevine use floral explants for induction, what represents an important temporal limitation because these structures are available only a few days every year. Our goal was to evaluate the possibility of obtaining grapevine somatic embryos in a flowering-independent way. With this goal we tested the effect of the application of inhibitors of histone deacetylase (HDACs) enzymes on the embryogenic competence of different explants. 0.5 mM sodium butyrate allowed to significantly increase the embryogenic competence. This treatment also produced the overexpression of HDACs-encoding genes, as well as of genes related to the embryogenic competence. On the other hand, asynchrony and precocious germination were often observed during the differentiation and maturation of somatic embryos. These phenomena negatively affect to subsequent plant conversion from germinated embryos. To solve this problem, we use a semipermeable membrane placed between the culture medium and the embryogenic material during its differentiation. This system allowed us to significantly improve the maturation of somatic embryos, avoiding precocious germination and increasing plant regeneration. Using this system, we analyzed the metabolism of ABA and polyamines in the embryogenic material with the aim of studying their relationship with the water stress produced by the membrane and with their involvement in somatic embryo maturation.

Keywords: Somatic embryogenesis, grapevine, embryogenic competence, somatic embryo maturation

P55

Temperature affects somatic embryo development in maritime pine

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The adaptability of forest tree varieties to the environmental stresses induced by global warming is still largely unknown. This is a major concern for foresters because climate change could affect a crucial reproductive function of trees: seed production. In some conifers such as Norway spruce, temperature during embryogenesis has been shown to affect the development of both somatic and zygotic embryos as well as, *a posteriori*, plant phenology over several years of juvenile vegetative phase. Temperature effects on embryo development are particularly difficult to investigate *in situ*, e.g. in seed orchards. As a good *in vitro* model system of embryo development, we used the best somatic embryogenesis protocols currently available for maritime pine at FCBA and INRA (Trontin et al. 2016) to study the temperature effect (18, 23 and 28°C) when applied during the maturation phase of cotyledonary embryos. We showed for two unrelated maritime pine embryogenic lines (PN519 and AAY06006) that the temperature during embryogenesis has major impacts on the development of cotyledonary embryos (duration, yield, mass) and that these direct effects are complemented with delayed effects (estimated in up to 15-month-old emblings) on germination capacity, survival, initial growth in height and the phenology of plant development. Genotypic effects possibly related to the line's pedigree were also highlighted. Indirectly our results suggest that temperature affects the intrinsic quality of cotyledonary embryos which could be reduced at maturation temperatures lower (18°C) or higher (28°C) than the reference temperature (23°C). No significant differences in global DNA methylation could be detected among the tested maturation conditions in both immature (1 week maturation) and cotyledonary embryos (10-14 weeks maturation) from line PN519. Further biochemical, proteomic and methylome analyses are ongoing to clarify the physiological and molecular mechanisms involved in embryos' perception of temperature in maritime pine.

Keywords: *Pinus pinaster*, maturation, cotyledonary embryos, DNA methylation

Reference: Trontin J.-F., et al. (2016) Prospects for new variety deployment through somatic embryogenesis in maritime pine. In: Park Y.-S., Bonga J.M., Moon H.-K. (Eds), Vegetative Propagation of Forest Trees. KFRI/NIFoS. Seoul, Korea, pp. 572-606.

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P56

Effect of genotype, sucrose concentration and glutamine supply on the protein and lipid content in embryogenic suspension cultures of cork and holm oak

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Cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* L.) are representative species of Mediterranean forest and of the agro-silvo-pastoral system called "Dehesa" in Spain or "Montado" in Portugal. Their acorns are mainly used for hog feeding because of their high carbohydrate, protein and lipid content. Oak acorns are morphologically characterized by the absence of endosperm and the presence of an embryo with big cotyledons in which nutrients are stored. Somatic embryos produced in repetitive embryogenic cultures can therefore be used not only for the production of clonal seedlings, but also for the large scale production of nutritive compounds and other metabolites. Somatic embryogenesis was initiated from leaf tissues of adult cork oak trees and from female flowers of holm oaks. Embryogenic cultures were maintained by subculturing in SH liquid medium without PGRs. Total protein and lipid content of embryogenic tissues at the proliferation stage was determined. Five genotypes of each species were analysed following standard methods. Variability between and within species was observed. The mean protein content was 31.7%DW in cork oak, ranging from 21.4 to 38.5%DW depending on genotype. In holm oak it was 36.0%DW ranging from 29.3 to 48.4%DW. Regarding lipid content, it was 6.6%DW ranging from 3.2 to 10.5%DW in cork oak and 7.2 %DW ranging from 4.6 to 8.9%DW in holm oak.

The effects of s*ucrose concentration* and the presence of glutamine in the culture medium were also evaluated. Increasing sucrose concentration decreased the crude protein content in cork oak but did not influence that of holm oak. Sucrose concentration hardly affected the lipid content in both species. The supply of glutamine increased the crude protein levels in both species while the lipid content remained unchanged.

Keywords: glutamine, oak, protein, somatic embryogenesis, sucrose.

P57

Induction of somatic embryogenesis in *Chorisia speciosa* St. Hill

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The silk floss tree (*Ceiba speciosa*, formerly *Chorisia speciosa*), is a species of deciduous tree native to the tropical and subtropical forests of South America. It has a host of local common names, such as palo borracho (in Spanish literally "drunken stick"), samu'ũ (in Guarani) or paineira (in Brazilian Portuguese). It belongs to the same family as the baobab (*Adansonia digitata*) and the *kapok* (*Ceiba pentandra*). It is cultivated as an ornamental species because of its beautiful pink flowers and its trunk which has a barrel shape. *C. speciosa* has a tolerance of pollution and it is an important biomarker for environmental monitoring studies. The seeds are wrapped in a capsule that contains a kind of short fibre like cotton called "paina" which has the characteristic of being an excellent insulating against noise and vibrations; it is also used in the manufacturing of medicinal cotton. It has potential use for paper industries because its cellulose fibres. The wood is light and it has several applications. Not found reports on in vitro culture and induction of somatic embryogenesis in this species. The aim of this study was to establish the conditions of *in vitro* culture in order to induce somatic embryogenesis for massive propagation. Flower buttons were used as explants. They were disinfected with 96% ethanol and then flamed in a lighter. The explants were cultivated in ½ Woody Plant Medium added with 2,4-D (1,5 ppm) in darkness to induce embryogenic callus. After 15 days of cultivation, friable embryogenic calluses were formed. Then, they were sub cultivated to an embryo proliferation medium (WPM without PGR, in darkness), observing the appearance of somatic embryos in different stages. It is the first report of ES of this species.

Keywords: callus, cell proliferation, tissue culture, somatic embryos, silk floss tree, Palo borracho.

P58

Induction of somatic embryogenesis in Walnut (*Juglans venezuelensis*)

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Walnut (*Juglans venezuelensis*) is an endemic species found in Venezuela, specifically in Caracas, in some areas of the Coastal Range, including the Waraira Repano National Park (El Ávila). At the moment it is cataloged like species in critical danger of extinction, according to the Red Book of the Venezuelan Flora. The main threats facing this species are logging and the destruction of habitat for urban development. Walnut wood is very resistant, beautiful and fine, which gives it great value as a construction material. From seeds scarified with sulfuric acid, embryos were grown in culture media with different combinations of growth regulators: medium A supplemented with 0.15 mg / l of IBA and 1.0 mg / l of BA; and medium B supplemented with 0.15 mg / l of IBA and 1.5 mg / l of BA, testing lighting and dark conditions to induce the process of somatic embryogenesis. The induction of callus and somatic embryos was achieved from the explants used in medium supplemented with 0.15 mg / l of IBA and 1.5 mg / l of BA, developed in the dark. Currently, different tests are developed to achieve the germination of the embryos. The generation of plants from embryos is a key factor in a plan for the accelerated production of plants in reforestation programs of this important species.

Keywords: callus, somatic embryogenesis, endemic, embryos.

P59

Somatic embryogenesis induction from leaf explants of *Podocarpus lambertii* Klotzsch ex Endl. (Podocarpaceae)

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Podocarpus lambertii, locally known as "pinheiro bravo", is an endemic conifer from Brazil. This species occurs in many Brazilian phytogeographic domains: the Atlantic Rainforest, Caatinga, Cerrado, and Pampa. The characterization of the diversity and genetic structure of the natural populations of *P. lambertii* in southern Brazil showed low genetic diversity and high average index of fixation. The high fragmentation of this species natural population led to its inclusion on the IUCN Red List as “near-threatened”. These data reinforces the requirement of *in situ* and *ex situ* conservation strategies for *P. lambertii*. Somatic embryogenesis (SE) is an effective biotechnological tool for germplasm conservation and micropropagation. In the present study, we successfully established a protocol for embryogenic cultures induction using leaf tissue of *P. lambertii* as explant. For *in vitro* germination, the embryos were excised and inoculated in a culture medium consisting of Woody Plant Medium (WPM) basal salts supplemented with 30 g L^{-1} sucrose, 2 mL L^{-1} Plant Preservative Mixture™ (PPM), and 3 g L^{-1} gellan gum (Phytagel™). After 60 days, leaf explants from the germinated seedlings were inoculated in WPM culture medium supplemented with 1 g L^{-1} glutamine, 30 g L^{-1} sucrose, 2.5 g L^{-1} Phytagel, and different concentrations of Picloram (0, 5, 10, 20 μM) and 2,4 D (0, 5, 10, 20 μM). After 30 days, treatments supplemented with 2,4-D showed low rates of callus formation (4-8%), similar to the plant growth regulators-free treatment (8%). In contrast, treatments supplemented with Picloram showed an increased induction rate in treatments with 5 and 10 μM (33 and 45.5%, respectively), followed by a decrease in treatment with 20 μM (28.8%). Callus obtained in Picloram 10 μM treatment were maintained in multiplication cycles in the same culture medium composition used for induction. The evaluation performed by double-staining with 1% acetic carmin and 0.1% Evan's Blue showed that the callus had embryogenic features. This is the first report of embryogenic cultures induction from leaf explant for family Podocarpaceae, showing a promising strategy for both clonal mass propagation and conservation for this species.

Keywords: conifer; conservation; picloram; Podocarpaceae

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Morpho-biochemical characterization of cryopreserved embryogenic tissue of *Pinus radiata.*

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The main objective of the study was to characterize the biochemical behavior of embryogenic tissue of four *Pinus radiata* embryogenic cell lines in the cryopreservation stage. Cryopreservation of the material was performed with the slow freezing technique, using 5 cryoprotective pretreatments with 0.4 M sorbitol base. The pretreatments were: 5% (v/v) dimethyl sulfoxide (DMSO5); 10% (v/v) dimethyl sulfoxide (DMSO10); 5% (v/v) dimethyl sulfoxide and 0.09 M L-proline (DMSO5P); 10% (v/v) dimethyl sulfoxide and 0.09 M L-proline (DMSO10P); and 0.09 M L-proline (DMSO0P). Slow cooling was optimized in 120 minutes at -80 ° C in Mr Frosty containers. For thawing, the embryogenic tissue was placed in a water bath at 37 ° C with replacements of culture medium weekly. Once the tissue was recovered, measurements were made of total soluble sugars, total proteins and proline for 4 weeks after the reactivation of growth. As control, non-cryopreserved tissue was used. The protein results show that there are no significant differences between the weeks after thawing and the initial values observed before cryopreservation of the tissue. For total soluble carbohydrates, significant differences were observed only during the first week of subculture, recovering their initial values from week 2 of subculture. Finally, no significant differences were observed in the proline content in relation to the initial values. In relation to the cryoprotective pretreatments, it was observed that these did not greatly influence the behavior of the biochemical parameters in the period studied. From the above we can conclude that despite the effects caused by stress during the process of cryopreservation, it does not significantly affect the primary metabolism, since after the second subculture, once reactivated its growth, the parameters studied return to their initial values cryopreservation.

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Relationship between H_2O_2 accumulation and NO signal synthesis in osmotic stress-induced somatic embryogenesis of *Fraxinus mandshurica*

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Osmotic stress promotes somatic embryogenesis of *Fraxinus mandshurica*, which is related to reactive oxygen species (ROS) accumulation, but the underlying mechanism is still unclear. The purpose of this research is to reveal the relationship between ROS accumulation and reactive nitrogen species (RNS) in osmotic stress- promoted somatic embryogenesis. Single piece of cotyledons of *F. mandshurica* were used as explants and induced somatic embryogenesis in the osmotic stress medium supplemented with 75 $g \cdot L^{-1}$ sucrose. Meanwhile, the endogenous hydrogen oxide (H_2O_2) content of explant cell was modified by adding exogenous H_2O_2 or catalase (CAT) solution, so as to analyse the effects of exogenous H_2O_2 stimulation on somatic embryogenesis and intracellular H_2O_2 accumulation, as well as the relationship between ROS signals and RNS signals. The results showed that (1) the addition of exogenous H_2O_2 with appropriate concentration increased the number of somatic embryos; after 60 days of addition, the number of somatic embryos was the highest on explants treated with 200 $\mu mol \cdot L^{-1}$ H_2O_2, it was 17.41 per explants and 136.54% higher than that of control; (2) exogenous H_2O_2 treatment could significantly increase the intracellular H_2O_2 content and enhance the antioxidant enzymes activity (superoxidase dismutase and peroxidase); (3) H_2O_2 treatment could activate the intracellular non-enzymatic reaction pathway to facilitate NO synthesis, but the correlation between intracellular H_2O_2 and NO was not significant. These results demonstrated that the somatic embryogenesis of broad-leaved trees was closely related to the ROS accumulation and antioxidant defense reactions; both H_2O_2 and NO as signaling molecules were involved in the process of somatic embryogenesis in broad-leaved trees. In the process of exogenous hydrogen peroxide promoting somatic embryogenesis, NO signal synthesis depended on non-enzymatic reactions. The research results provide scientific basis for resolving the regulation mechanism of ROS in somatic embryogenesis of broad-leafed trees and establishing a reasonable and efficient technology system for regulating somatic embryogenesis of trees.

Keywords: *Fraxinus mandshurica*, somatic embryogenesis, hydrogen oxide, nitric oxide, osmotic stress

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Hybrid Sweetgum (*Liquidambar styraciflua* × *L. formosana*) Cultivation and Its Somatic Embryogenesis

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Sweetgum (*Liquidambar* spp.) is widely planted as an ornamental, within its natural range and elsewhere. The wood is used for furniture, interior finish, paper pulp, veneers and boards. The gum resin is medicine to cure for sciatica, weakness of nerves and to promote the movement of blood and water metabolism. American sweetgum (*L. styraciflua*) can be interfertile with Chinese sweetgum (*L. fomorsana*) and their offspring can show heterosis in robust growth. However, the somatic embryogenesis rapid propagation system of hybrid sweetgum needs to be introduced and improved.

In our study, plus trees of *L. formosana* that were selected from natural forests were used as paternal parent and *L. styraciflua* which have been successfully introduced to China were used as maternal parent to create hybrid sweetgum. Immature zygotic embryos of hybrid sweetgum were used as explants to investigate effects of somatic embryogenesis. The main results are as follows:

1. The parent materials of *Liquidambar* have been selected and conserved. 66 and 61 plus trees were selected from the natural forests of *L. formosana* in Hubei province and Henan province, respectively.

2. Hybrid sweetgum were normally acquired. 11 half sib families, nearly 8 thousand seeds were successively obtained after 3 years control pollination.

3. Somatic embryogenesis system of hybrid sweetgum was successfully introduced and improved. After induction, maintainance, and maturation, 7 cell lines developed to mature somatic embryos and some of them germinated and grew to young trees in greenhouse.

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Study on Somatic Embryogenesis in *Larix principis-rupprechtii* Mayr and *Pinus tabulaeformis* Carrière

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Somatic embryogenesis (SE) is one of the most important methods for plant regeneration in plant cell engineering, which has great application value on large scale vegetative propagation, artificial seed production, germplasm conservation, and genetic transformation, and has a wider application prospect for forest trees with long life cycles, such as conifers. From another aspect, SE is widely recognized because it is the pattern system to study the mechanism of embryogenesis. *Larix principis-rupprechtii* Mayr and *Pinus tabulaeformis* Carrière are two main afforestation species in Northern China, while their production and research are severely affected by ineffective propagation and breeding technologies. In the present study, different developmental stages of cones of *L. principis-rupprechtii* and *P. tabulaeformis* are collected for stages characterization and element determination, through which we can adjust medium component to optimize these two SE systems. For further understanding SE system, we develop transcriptomics and proteomics study on embryogenic callus (EC), non-embryogenic callus and somatic embryos in various stages. By using bioinformatics analysis, some potential key genes and proteins are identified, including 25 differentially expressed genes are embryo development related, including genes encoding late embryogenesis abundant (LEA) protein, somatic embryogenesis receptor-like kinase (SERK), embryonic flower 1 (EMF1), etc. In the same embryogenic lins, proteomic study was conducted and 92 differentially abundant proteins are identified, which are mainly related to metabolic process, signal transduction, protein biosynthesis, storage proteins, antioxidation, cytoskeleton organization, transporter, binding and so on.

Keywords: conifer; somatic embryogenesis; transcriptomics; proteomics

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Somatic embryogenesis and efficient plantlet regeneration from mature zygotic embryos of Manchurian ash (*Fraxinus mandshurica* Rupr.)

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Manchurian ash (*Fraxinus mandshurica* Rupr.) is a valuable hardwood species in Northeast China. Efficient plant regeneration through somatic embryogenesis was achieved using mature cotyledon explants of *F. mandshurica*. The results showed that the highest somatic embryogenesis percentage (67.5%) was incubated on MS½ medium (all elements are half in the MS medium) supplemented with 26.84 µM 1-naphthaleneacetic acid (NAA), 8.88 µM 6-benzyladenine (BA), 75 $g \cdot L^{-1}$ sucrose, 400 $mg \cdot L^{-1}$ casein hydrolysate (CH) and 6.5 $g \cdot L^{-1}$agar. Somatic embryos (SEs) mainly occurred directly, which was occurred mostly in the cotyledon surfaces and edges. Approximately, 81.5% of SEs occurred on the browning cotyledon explants. The SEs originated from single epidermal cells and developed to the globular, heart, torpedo, and cotyledonary stages. The highest secondary somatic embryos (SSEs) percentage was incubated on MS½ medium supplemented with 0.26 µM NAA, 400 $mg \cdot L^{-1}$ CH, 25 $g \cdot L^{-1}$ sucrose. The white somatic embryos whose length was 4 mm got the best results of secondary somatic embryogenesis, and the somatic embryogenesis percentage was the highest (up to 42.98%), proliferation quantity was much more (access to 3 fold), the somatic embryo malformation percentage was the lowest (about 20%), the synchronization percentage was higher (up to 63.05%). Furthermore, secondary somatic embryos development faster, which can develop to the cotyledonary stages within 30 d. The SSE percentage and quantity were about 2 fold greater than the direct way. However, the malformation percentage increased when embryonic callus cultured by indirect way. The increase of subculture times slowed down the somatic embryo development and increased the number of secondary somatic embryos, the synchronization embryo percentage, the browning and mortality percentage. When cultured on MS1/2 medium containing 0.2 $mg \cdot L^{-1}$BA, 20 $g \cdot L^{-1}$ sucrose, 200 $mg \cdot L^{-1}$ CH and 6 $g \cdot L^{-1}$agar, white cotyledon somatic embryos whose length was 4~8 mm had the highest germination percentage (greater than 40% when 30 d). With the increase of subculture times, the somatic embryo germination percentage reduced and the rooting percentage increased at first and then decreased. The survival percentage was 90.9% and the average height of seedling was 9.26 cm when regeneration plants were transplanted for 2 months. Plantlets developed normal phenotypes under field conditions. This study would provide feasible technology on large-scale propagation and factory production for the excellent germplasm resources of *F. mandshurica*.

Keywords: Manchurian ash, somatic embryogenesis, plant regeneration

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Avocado micropropagation: Growth in leaps and bounds for a clonal rootstock propagation solution

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Avocado is a high demand, high value tropical fruit, contributing to global economy with a world production of more than 5.5 million tonnes per year. Being planted as a grafted tree, propagation of avocado refers to propagation of rootstock cultivar, then graft it with bud-wood from a mature scion cultivar. Elite rootstock propagation is critical to maintain the quality of fruit and farm management practices. Rootstock propagation through seeds suffers high genetic variation, hence less appealing for orchard plantings. Rooting of cuttings is only possible through a 40 years old conventional technique, called 'Frolich and Platt method' which is a complex, lengthy and expensive process. This creates limitations on rapid industry expansion due to scarcity and high price of plants in many countries including Australia, specially, with the inception of high density planting. Alternative propagation methods are sought over 5 decades to ease this pressure on industry. Potential of micropropagation has been well demonstrated for wide variety of economically important plants. However, many years of research has proven avocado to be a highly recalcitrant woody perennial to in vitro culture conditions. My PhD question was to revisit the micropropagation technology for avocado clonal propagation. Over three years of research carried out in my PhD at University of Queensland, Australia has successfully established the world's first high throughput micropropagation pipeline for avocado using shoot tip culture. Extensive work has been carried out to optimise various parameters for all stages of the process; initiation, shoot induction, multiplication, in vitro hardening, root regeneration and acclimatisation. Large number of plants have been acclimatised with 97% survival under nursery conditions. Micropropagated cv. 'Reed' rootstocks grafted and ungrafted to widely used scion cv. 'Hass', are now being evaluated under field conditions in commercial orchards, to compare with conventionally propagated clonal and seedling rootstock grafted and ungrafted with cv. 'Hass'. This PhD project is highly significant in horticultural vegetative propagation arena, presenting world's first industrial technology for avocado micropropagation and first ever field evaluation of micropropagated avocado plants. This revolutionising technology has a great impingement on both Australian and global avocado industry by meeting timely supply of high demand for clonal avocado plants.

Keywords: avocado, clonal propagation, shoot tip culture, field evaluation

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In vitro propagation of UCB-1 (*Pistacia integrima* × *P. atlantica*) to unravel main limiting factors

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Statement of the Problem: Pistachio is a perennial, deciduous and subtropical. Considering the prevalence of using seedlings as rootstock, there is an irregularity in pistachio orchards and it is necessary to use vegetative methods in propagation of rootstocks. 'Badami' and 'Ghazvini' are the most common used rootstocks in Iran. Recently, UCB-1 has been also considered as an appropriate one due to its noble features. Now, because of the limitation of importing its seeds to Iran, the only way of its propagation is by tissue culture. But pistachio micropropagation is problematic due to the phenol exudation and bacterial contamination problems in establishment phase, shoot tip necrosis in proliferation, low proliferation rate and especially being hard to root. In order to *in vitro* propagation of this valuable rootstock, we carried out an experiment. Methodology & Theoretical Orientation: To overcome the contamination problem, we used different concentrations and immersion times of mercury chloride, sodium hypochlorite and chloramphenicol. Antioxidants, active charcoal, darkness and decreasing macro-nutrients concentration treatments were applied as controlling agents of phenol exudation. Modifying macro- and micro-elements' concentration was applied to IMPROVE proliferation and controlling shoot tip necrosis. In rooting stage, we used treatments of Fe-sequesteron, putrescine, modifying different nutrients' concentrations and using various types of auxins. Finally, darkening mother plant for 2 weeks with decreasing macro-nutrients' concentration and immersing explants in 150 Mm ascorbic acid for 120 min and 60 min respectively before and after sterilization process showed the highest rate (100 percent regeneration) of controlling phenol exudation (100 percent explant without phenol). Findings: The highest rooting was achieved in the half strength macro-nutrients of DKW (1/2 DKW) culture medium and 2 mg/l IBA, 150 mg/l fe-sequestron and 100 mg/l putrescine. Finally, rooted shoots (> 2 cm) were acclimatized in the mixture of perlite and peat-moss (1:1).

Keywords: pistachio, micropropagation, plant growth regulators, Iranian cultivar

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Formation of pro-embryogenic masses from mature seeds of *Plinia cauliflora*

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The jabuticabeira (*Plinia cauliflora*) is a native and endemic tree belonging to the family Myrtaceae, with distribution in the Southeastern and Southern regions of Brazil. Due to the recalcitrant nature of its seeds and the lack of efficient methods of vegetative propagation, commercial orchards have not been established yet. Somatic embryogenesis can be an alternative to obtain large numbers of plants in a short time, in good phytosanitary conditions. The objective of the present study was to initiate a protocol of somatic embryogenesis from mature seeds of *P. cauliflora*. The seeds were disinfested in 70% ethanol (1 min) and 5% sodium hypochlorite (10 min), followed by rinsing in sterile distilled water. The two cotyledons were separated and introduced individually, together with the embryonic axes, into test tubes containing Murashige and Skoog culture medium. For the induction of pro-embryogenic masses (PEM), three concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) were tested (5, 10 or 15 μM) without activated charcoal (AC) as well as different concentrations of 2,4-D (100, 200, 300 and 500 μM) combined with AC (0.5, 1 or 2 gL-1). In the AC-free culture medium, the formation of PEM started from the tenth day, while it was at the 20th day in the culture medium containing AC. The best concentration of 2,4-D used alone for PEM formation (70%) was 15 μM but oxidation of these masses was high (52.5%). Considering this high oxidation rate, AC was then tested as it can adsorb the phenolic compounds released by the seed tissues and reduce the oxidation. When 2,4-D was combined with AC, the concentration of 2,4-D was increased, since it is adsorbed by AC. In this case, the highest PEM formation rate was 82.5% and oxidation reduced to 3% in the medium containing 300 μM of 2,4 D and 1 $g.L^{-1}$ of AC. This result shows the importance of the addition of activated carbon to the induction medium of embryogenic cultures of *Plinia cauliflora*.

Key words: activated carbon, 2,4-D, Myrtaceae.

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In vitro establishment of *Bambusa oldhamii* Munro explants from field trees and molecular identification of an endophytic bacterium

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Bamboos are economically and/or ecologically important and traditionally propagated by means of seeds, division of clumps and rhizomes and stem cuttings. These methods present bottlenecks for large-scale propagation, being the micropropagation a viable alternative with great potential of application. The establishment of explants *in vitro* is the first step of *in vitro* culture. It can be hindered by the presence of microorganisms in mature tissues originating from mother trees cultivated in the field, especially for bamboos. However, many genotypes of interest are not in the juvenile vegetative state or are not cultivated in a protected environment to minimize microbial contamination during in vitro introduction. Thus, the aim of the present study was the *in vitro* establishment of *Bambusa oldhamii* primary explants from adult plants in field conditions, submitted to the action of different biocidal agents and the isolation and identification of endophytic bacteria. To control bacterial and fungal growth, the explants were subsequently exposed to 70% alcohol, 1% sodium hypochlorite (NaOCl), 0.1% mercuric chloride ($HgCl_2$), 2 g/L Cercobin®, 1% of chlorhexidine digluconate in seven treatments and then inoculated into MS medium supplemented or not with 4 mL L^{-1} of Plant Preservative Mixture (PPM^{TM}). Preliminary disinfection and complete immersion of the explants in the liquid culture medium containing 4 mL L^{-1} of PPM^{TM} visually inhibited the growth of bacteria and fungi (4 and 0% after 21 days of culture). In addition. this procedure allowed the development of shoots with an average length of 2.2 cm and posterior subcultures, being considered the best method for *in vitro* establishment of *B. oldhamii*. The presence of endophytic bacteria isolated from shoots of *B. oldhamii* and cultivated in Luria-Bertani medium was observed only after the treatment of the explants with 1% of chlorhexidine digluconate for 2 h, followed by 2 g. L^{-1} Cercobin® for 24 h, 70% alcohol for 30 sec, 0.1% $HgCl_2$ for 10 min and 1% NaOCl for 10 min. For the identification of endophytic bacteria, DNA was extracted and 16 S rDNA sequenced, followed by BLAST research of the sequences in the NCBI database. Results indicated 100% of similarity with *Ralstonia* genus. The phytogenetic analyzes using the MAFFT and MEGA7 software agrouped the isolate with *Ralstonia pickettii* type. The sensitivity of the bacterium to 4 mL L^{-1} of PPM^{TM} was confirmed by the minimal inhibitory concentration method (MIC).

Key words: Bamboo, disinfection, PPM^{TM}, *Ralstonia.*

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Somatic embryogenesis and histological analysis of jaboticabeira (*Plinia peruviana* (Poir.) Govaerts)

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Jaboticabeira (*Plinia peruviana* (Poir.) Govaerts) is a Myrtaceae tree native to Brazil. The taste and medicinal properties of its fruits, including anti-inflammatory and anti-diabetic activities, give to this species great economic importance. Among the forms of vegetative propagation, somatic embryogenesis may be an alternative to obtain a large number of uniform plants in a short time. The aim of this study was to obtain somatic embryos (SE) of jaboticabeira and to verify SE anatomy by means of histological analysis. Fruits were unpulped and seeds were cut in half before *in vitro* introduction. In order to induce SE formation, the cotyledons and embryonic axes were cultured in MS medium containing 1 $g.L^{-1}$ of glutamine and several concentrations of 2,4-D (2.5, 5, 10, 25 or 50 μM). Furthermore, different exposure periods of the explants to 2,4-D (7, 15, 30, 45 and 60 days) were tested. For SE maturation, concentrations of 30, 60 or 90 $g.L^{-1}$ of polyethylene glycol 6000 (PEG 6000) were used. For conversion into plants, SE were transferred to media containing gibberellic acid (GA_3) (1.44, 2.88, 5.77 μM). The formation of somatic embryos started from the first month of culture in induction medium and higher percentages of proembryogenic masses and SE were obtained in MS medium containing 10 μM of 2,4-D. There were no significant differences in the formation of proembryogenic masses and SE between exposure times to 2,4-D. However, histological analysis revealed that SE exposed to 2,4-D for long periods (60 d) showed morphological abnormalities and formation of a healing tissue. This tissue was no observed in SE formed after 7 or 15 days in 2,4-D, indicating the deleterious effect of this plant regulator. After 30 days of SE maturation, the supplementation of 60 $g.L^{-1}$ of PEG 6000 in the culture medium was sufficient to obtain SE in more advanced stages of development. There was no conversion of SE into plantlets in any treatment with GA_3. This difficulty of conversion may be related to the abnormalities observed in the tissues of SE. Elimination of the auxin added to the induction medium is suggested as soon as the embryogenic responses are initiated in order to avoid its harmful effects on the SE.

Key words: anatomy, 2,4-dichlorophenoxyacetic acid, PEG 6000

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