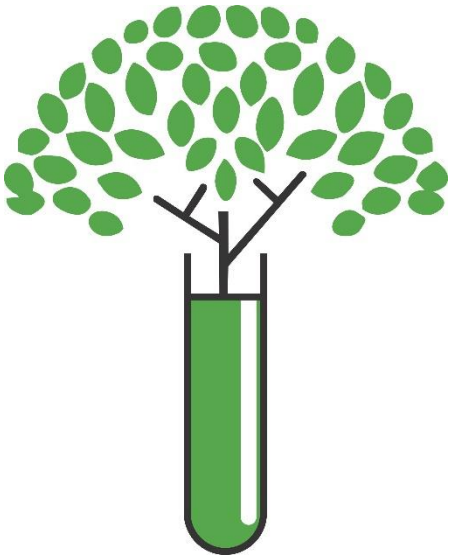
INNOVATIVE WOODY PLANT CLONING

FIRST CONFERENCE OF COST ACTION CA21157



CopyTree

17 & 18 April 2023

Faculty of Chemistry (USC)

Santiago de Compostela, SPAIN

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

MBG MISIÓN BIOLÓGICA DE GALICIA

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

FIRST CONFERENCE OF COST ACTION CA21157

European Network for Innovative Woody Plant Cloning

Book of Abstracts of the Conference Innovative Woody Plant Cloning

17 & 18 April 2023, Santiago de Compostela, Spain

Local organizer

Misión Biológica de Galicia (MBG) – Spanish National Research Council (CSIC)

Venue

Faculty of Chemistry, University of Santiago de Compostela.
Avenida das Ciencias s/n, Campus Vida
Santiago de Compostela
15705 A Coruña
Spain

CopyTree

www.copytree.eu

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
Funded by the European Union
MBG MISIÓN BIOLÓGICA DE GALICIA
CSIC CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Book of Abstracts of the Conference Innovative Woody Plant Cloning

Editors:

Stefaan Werbrouck

Nieves Vidal

Valbona Sota

Santiago de Compostela, Spain, 2023

ISBN 978-84-09-50864-8

This publication is based upon work from COST Action COPYTREE (European Network for Innovative Woody Plant Cloning) CA21157, supported by COST (European Cooperation in Science and Technology).

FIRST CONFERENCE OF COST ACTION CA21157

European Network for Innovative Woody Plant Cloning

Local Organising Committee:

Nieves Vidal
Misión Biológica de Galicia (MBG-CSIC)

María Victoria González
University of Santiago de Compostela (USC)

Conchi Sánchez
Misión Biológica de Galicia (MBG-CSIC)

Jesús Mª Vielba
Misión Biológica de Galicia (MBG-CSIC)

Purificación Covelo
Misión Biológica de Galicia (MBG-CSIC)

Saleta Rico
Misión Biológica de Galicia (MBG-CSIC)

María José Cernadas
Misión Biológica de Galicia (MBG-CSIC)

Ricardo Castro-Camba
Misión Biológica de Galicia (MBG-CSIC)

Laura López
Misión Biológica de Galicia (MBG-CSIC)

Neima Fernández
Misión Biológica de Galicia (MBG-CSIC)

Santiago Silva
CIFP Politécnico de Santiago

Cristina Buceta
CIFP Politécnico de Santiago

Bruce Christie
The Green Plant Company (NZ)

FIRST CONFERENCE OF COST ACTION CA21157

European Network for Innovative Woody Plant Cloning

Scientific Committee:

FIRST CONFERENCE OF COST ACTION CA21157

European Network for Innovative Woody Plant Cloning

Acknowledgements

We gratefully appreciate the assistance and encouragement of the following persons, institutions and companies, who understood the challenge of organising this meeting at very short notice and were always willing to help and support us:

- Mafalda Quintas and Katchamon Nimprang, from the COST headquarters in Brussels
- Jesús Sanmartín, Dean of the Chemistry Faculty at the University of Santiago de Compostela (USC)
- Carmen González from the CSIC Delegation in Galicia
- Rafael Zas, Conchi Sánchez, Chema Alfaya and Pedro Peón from the Management team of the Misión Biológica de Galicia (MBG-CSIC)
- Mila Castro, Javier Ferreiro and Gregorio López, from the Concello de Santiago de Compostela
- Turismo de Galicia, Xunta de Galicia
- Rober Ahmad Kreimech, from BE 4 Management, who made our webpage possible
- Susana Castro and Olga Gutiérrez, from the Oficina de Xestión de Eventos da USC
- Beatriz Guerra, travel agent of "Viajes el Camino"
- Lea González and Santiago Martínez from Lápices4, our preferred printing shop
- Andrés Carballo from SEREGA de Santiago SL, our favourite coffee break provider
- Lola Garcerá, designer of the Bookmark, and Dositeo Veiga, provider of the post cards "Ceos Galegos"
- Veronika Viladelová, for sharing her chestnut and walnut delicious pastries made at her "Delicias de Bohemia" shop
- Paulina Ceremuzynska, the soprano who transported us to the Medieval Galicia
- Hotels Palacio del Carmen, Monumento San Francisco, Eurostars Peregrino and Eurostars Gran Hotel
- The staff and students of the Misión Biológica de Galicia sede Santiago de Compostela

Finally, we want to thank to all those people that provided practical suggestions and helpful advice and made this Conference a success.

Welcome

Dear colleagues,

It is with great pleasure that I welcome you all to the first conference of our COST Action COPYTREE on "Innovative Woody Plant Cloning". It marks an important milestone in our efforts to promote knowledge exchange and foster innovation in the in vitro production of trees and woody plants through networking and collaboration.

This European COST action focuses on woody plants that produce timber, edible fruits, nuts, berries, saps, fodder and herbal medicines, or that have ecological, ornamental or even cultural value. Micropropagation of trees and shrubs is becoming increasingly important. In recent years, changing food patterns and climate change have increased the need for high quality planting material for fruit and nut orchards, commodity and biomass plantations, and timber forests. In Europe, researchers from universities and institutes are anticipating this evolution and trying to address a number of challenges around which our working groups are organized: (1) recalcitrance, (2) sanitation and germplasm conservation, (3) scaling up and automation, (4) proactive risk management and public acceptance, and (5) commercialization.

This conference provides a forum for researchers, industry experts and stakeholders to share their insights and findings and engage in meaningful discussions that will shape the future of the field. The leaders of WG1, WG2 and WG3, together with their deputies, have selected the oral presentations, including an exciting line-up of keynote speakers. The poster session will provide an opportunity to share expertise and network, and WG4&5 have chosen a roundtable discussion as an ideal format.

I would like to take this opportunity to express my gratitude to Nieves Vidal and her colleagues who are organizing this conference, for their tireless efforts in making this event a reality. I also wish to mention the efforts of Rober Kreimech and our Science Communication Coordinator Valbona Sota in building not only a website but also a communication platform and providing it with so much information in record time. I also want to thank our Grant Holder Scientific Representative, Lucie Fischerova, for untangling all the financial knots and assuring us of professionalism for the next four years. None of this would have been possible without the gentle support of our Cost Science Officer, Mafalda Quintas, and our Administrative Officer, Katchamon Nimprang, to whom I extend my sincere thanks for their trust.

I would also like to thank all of our speakers and participants for their contributions and for making this conference a truly collaborative and engaging experience. I wish you all a productive and enjoyable Congress and look forward to the many opportunities for learning and networking that lie ahead.

Best regards,

Stefaan Werbrouck

Chair

CONFERENCE PROGRAM

FIRST DAY - April 17, 2023	
08.30 – 09.15	Registration of participants and poster's installation
09.15 – 09.45	**CONFERENCE OPENING:** Carmen González, Rafael Zas, Mila Castro **Welcome Speech:** Stefaan Werbrouck, Chair

WORKING GROUP 1- RECALCITRANCE Moderators: Sandra Correia, Itziar A. Montalban	
09.45 – 10.30	**<u>Keynote Speaker:</u> Jorge Canhoto** Recalcitrance: do we really understand it?
10.30 – 10.45	**Coffee Break**
10.45 – 11.00	**Tobias Bruegmann** The triad of tree biotechnology
11.00 – 11.15	**Stéphane Maury** How epigenetic studies can help to characterize recalcitrance, development, and stress memory for in vitro cultures of trees?
11.15 – 11.30	**Daniela Cordeiro** Long non-coding RNAs involved in embryogenic competence acquisition in tamarillo (*Solanum betaceum* Cav.)
11.30 – 11.45	**Pilar Testillano** Innovative small molecules to improve cell reprogramming and somatic embryogenesis in crop and forest species
11.45 – 12.00	**Hajer Darouez** Dichlorprop esters as promising tools for rooting trees?
12.00 – 12.15	**Tercia Lopes** Auxin and wound-responsive mechanisms underlying micrograft union formation in almond - *Prunus dulcis* (Mill.) D. A. Webb
12.15 – 12.30	**Stefaan P.O. Werbrouck** Novel cytokinin oxidase/dehydrogenase inhibitors derived from diphenylurea for use in plant tissue culture
12.30 – 14.15	**Lunch Break**

WORKING GROUP 2 - SANITATION and CONSERVATION Moderators: Elif Aylin Ozudogru; Zhibo Hamborg; Barbara Ruffoni	
14.15 – 15.00	**<u>Keynote Speaker:</u> Manuela Nagel** Establishment and management of cryobanks, with a particular emphasis on plants *in vitro*
15.00 – 15.15	**Zhibo Hamborg** Use of high-throughput sequencing for virus diagnosis and plant tissue culture sanitation in Norway

15.15 – 15.30	**Elien Guldentops** Assessment of ONT sequencing (MinION) for plant virus detection and comparison with Illumina-based sequencing
15.30 – 15.45	**Coffee Break**
15.45 – 16.00	**Doaa Elazab** New trends in hazelnut micropropagation, organogenesis and micrografting: Ttechniques, applications and future aspects
16.00 – 16.15	**Vera Pavese** Development of biotechnological tools for *Castanea sativa* Mill breeding
16.15 – 16.30	**Mariam Gaidamashvili** Development of efficient cryopreservation protocol for a Georgian provenance of *Castanea sativa* (Mill.) embryonic axes
16.30 – 16.45	**Alois Bilavcik** The use of cryopreservation for safe storage and sanitation of fruit plant germplasm
16.45 – 17.00	**Itziar A. Montalbán** Recent advances in alternative conservation methods for radiata pine somatic embryogenesis
17.00 -	**Posters' Session**
20.00 -	**SOCIAL DINNER**

SECOND DAY - April 18, 2023	
WORKING GROUP 3 - AUTOMATION **Moderators: Nieves Vidal; Ivaylo Tsvetkov**	
09.00 – 09.45	**<u>Keynote Speaker:</u> Jana Krajňáková** Tissue culture for the 21st century forests
09.45 – 10.00	**Sakari Valimaki** Different proliferation techniques for scaling up Norway spruce somatic embryogenesis
10.00 – 10.15	**Maurizio Lambardi** Use of liquid culture with the ElecTIS bioreactor for a faster recovering of blackberry shoots (*Rubus fruticosus*) from the conservation at 4°C
10.15 – 10.30	**Coffee Break**
10.30 – 10.45	**Elsa Baltazar** Large-scale micropropagation of *Prunus* spp. rootstocks - a comparison between semi-solid and Temporary Immersion Systems
10.45 – 11.00	**Yıldız Aka Kacar** In vitro propagation of horticultural plants by TIS bioreactor systems

11.00 – 11.15	**Nuria Alburquerque** Effect of silver nanoparticles on the micropropagation of two apricot (*Prunus armeniaca* L.) cultivars
11.15 – 11.30	**Gabriella Pocsfalvi** *Ginkgo biloba*: In vitro culture as an alternative system for the production of extracellular vesicles
11.30 – 11.45	**Paweł Chmielarz** Recalcitrance of *Quercus robur* to *ex-situ* conservation: Cryopreservation and *in vitro* culture
11.45 – 12.30	**Posters' Session**
12.30 – 14.15	**Lunch Break**

WORKING GROUP 4 and 5 - RISK ASSESSMENT AND COMMUNICATION **Moderators: Yesim Yalçin Mendi; Valbona Sota**	
14.15 – 15.00	**<u>Keynote Speaker:</u> Tuija Aronen** Addressing stakeholder concerns together with EU and national legislation – somatic embryogenesis of Norway spruce in Finland
15.00 – 15.15	**Valbona Sota** Harmonized communication by active members – the foundation for CopyTree's collaborative innovation
15.15 – 15.30	**Rober Ahmad Kreimech** CopyTree's Community Platform - A Communication Tool supporting Strategic Collaborative Innovation
15.30 – 15.45	**Şule Yalçin** Increasing the Participation of Young Talents in Biotechnological Studies Through Effective Communication Language and Channels
15.45 – 16.00	**Coffee Break**
16.00 – 17.00	**ROUND TABLE** Commercial micropropagation: present situation, problematics and future perspectives Moderators: **Maurizio Lambardi; Yesim Yalçin Mendi** Participants: **Fabiano Gattabria, Jorge Canhoto, Valbona Sota, Stefaan Werbrouck, Rober Ahmad Kreimech, Tuija Aronen, Beatriz Cuenca.**
17.00 –	**Management Committee Meeting**

Contribution list

Keynote WG1

Jorge Canhoto, João Martins, Cátia Pereira, Daniela Cordeiro, André Caeiro, Mariana Neves, Sandra Correia

Recalcitrance: do we really understand it?

WG1_O1

Tobias Bruegmann, Virginia Zahn, Alexander Fendel, Alice-Jeannine Sievers, Matthias Fladung

The triad of tree biotechnology

WG1_O2

Jean-François Trontin, Celia Miguel, Caroline Teyssier, Alain Delaunay, Jörg Tost, Marie-Anne Lelu-Walter, Stéphane Maury

Epigenetic memory of sensed temperatures during somatic embryo maturation in maritime pine young trees

WG1_O3

Daniela Cordeiro, Alexandra Camelo, Ana Carolina Pedrosa, Inês Brandão, Jorge Canhoto, Christophe Espírito Santo, Sandra Correia

Long non-coding RNAs involved in embryogenic competence acquisition in tamarillo *(Solanum betaceum Cav.)*

WG1_O4

Pilar S. Testillano, Elena Carneros, Yolanda Perez-Perez, Eduardo Berenguer, Cristina Rueda-Varela, Carmen Gil, Ana Martinez

Innovative small molecules to improve cell reprogramming and somatic embryogenesis in crop and forest species

WG1_O5

Hajer Darouez, Stefaan P.O. Werbrouck

Dichlorprop esters as promising tools for rooting trees?

WG1_O6

Tércia Lopes, Ana Pedrosa, Sandra Caeiro, Mariana Correia, Elsa Baltazar, André Caeiro, Liliana Marum, Jorge Canhoto, Sandra Correia

Auxin and wound-responsive mechanisms underlying micrograft union formation in almond - *Prunus dulcis* (Mill.) D. A. Webb

WG1_O7

Nino Muvarnidze, Jaroslav Nisler, Stefaan P.O. Werbrouck

Novel cytokinin oxidase/dehydrogenase inhibitors derived from diphenylurea for use in plant tissue culture

Poster 1

Elena Carneros, Esteban M. Díaz-Luzza, Yolanda Perez-Perez, Pilar S. Testillano

Chemically-induced DNA demethylation promotes somatic embryo production of *Quercus suber* L.

Poster 2

Ricardo Castro-Camba, Mariana Neves, Sandra Correia, Jorge Canhoto, Jesús Mª Vielba, Conchi Sánchez

Ethylene inhibits adventitious rooting in chestnut trees after phase change

Poster 3

Isabel Arrillaga, Ester Sales, Paloma Moncaleán, Esther Asensio, María Carmen González-Más, Itziar A. Montalbán, María Amparo Pérez-Oliver, M. Teresa Martínez, Elena Corredoira

Multi-varietal forestry: deployment of Fagaceae and Pinaceae genotypes adapted to climate change

Poster 4

Ricardo Ferraz, Sílvia Coimbra, Sandra Correia, Jorge Canhoto

Exploring the rRNA methyltransferases signalling pathway during tamarillo (*Solanum betaceum* Cav.) somatic embryogenesis

Poster 5

Lucie Fischerová, Karel Doležal, Tomáš Moravec, Zuzana Vondráková, Kateřina Eliášová

Approaches we apply to study Norway spruce somatic embryogenesis

Poster 6

Emna Baklouti, Ameni Nasri, Ben Romdhane Amal, Ahlem Ben Ahmed, Hazar Akrimi, Sahar Baklouti, Riadh Drira, Alain Rival, Noureddine Drira, Lotfi Fki

Advances in date palm (*Phoenix dactylifera* L.) micropropagation

Poster 7

Hassiba Fraj, Stefaan P.O. Werbrouck

Intermittent contact with the volatile organic compounds of *Serendipita indica* alleviates salt stress in *in vitro Ocimum basilicum* L.

Poster 8

Teresa Hazubska-Przybył, Agata Obarska, Agata Konecka, Mikołaj Wawrzyniak, Aleksandra Staszak, Ewelina Ratajczak

Effect of exogenous ascorbic acid treatment on induction, proliferation, the hydrogen peroxide and phytohormone content during first steps of somatic embryogenesis of *Picea abies* (L.) H. Karst.

Poster 9

Ashkan Hodaei, Stefaan P.O. Werbrouck

In vitro* germination, tissue culture and transformation of *Spinacia oleraceae

Poster 10

Mariana Neves, Sandra Correia, Carlos Cavaleiro, Jorge Canhoto

Ethylene positively modulates de novo shoot organogenesis and subsequent plant development from leaf explants of *Solanum betaceum* Cav

Poster 11

Yolanda Perez-Perez, Elena Carneros, Eduardo Berenguer, Maria-Teresa Solis, Pilar S. Testillano

Remodelling of cell wall by pectin de-esterification and AGP increase is required for somatic embryo formation in cork oak

Poster 12

Elnara Sadigova, Stefaan P.O. Werbrouck

Screening new compounds for adventitious shoot regeneration

Poster 13

Jesús Mª Vielba, Nieves Vidal, Saleta Rico, Purificación Covelo, Ricardo Castro-Camba, M José Cernadas, Conchi Sánchez

Biotechnological approaches to overcome adventitious rooting recalcitrance in mature trees of Fagaceae species

Poster 14

Saba Taheri, Bogdan Parakhonskiy, Andre G. Skirtach, Stefaan P.O. Werbrouck

Slow release of plant growth regulators in *in vitro* tissue culture by nanoporous microcarriers

Poster 15

Franka Thiesen, Virginia Zahn, Ben Bubner

Establishment of *in-vitro* clones of European beech (*Fagus sylvatica* L.) as a tool for breeding programmes and resistance research

Poster 16

Jesús Mª Vielba, Saleta Rico, Nevzat Sevgin, Ricardo Castro-Camba, Purificación Covelo, Nieves Vidal, Conchi Sánchez

Rooting recalcitrance of mature chestnut *in vitro* shoots is influenced by hormone signaling and MADS-Box genes

Poster 17

Rosario Muleo, Caterina Valerio, Ivano Fogione, Francesca Luziatelli, Maurizio Ruzzi

Cross-talk among molecules excreted by *Pantoea agglomerans* and regulating system of cell fate leading to adventitious root development in the pear

Poster 18

Cristian Silvestri, Andrea Ferrucci, Michela Lupo, Giuseppe Vaia

Do we talk about in vitro tissue culture recalcitrance of olive?

Poster 19

Terézia Salaj, Bart Panis, Rony Swennen, Katarína Klubicová

Somatic embryogenesis in *Abies alba* Mill

Poster 20

Miroslav Pernis, Terezia Salaj, Maksym Danchenko, Andrej Kovac, Katarina Klubicova

Proteins secreted into the culture media of *Pinus nigra* Arn. embryogenic suspension cultures

Poster 21

Jana Šedivá, Roman Businský, Kateřina Podrábská, Hana Drahošová, Michaela Pekařová, Dagmar Řeháková

Use of plant biotechnology in research activities of Silva Tarouca Research Institute for Landscape and Ornamental Gardening

Poster 22

Baiba Krivmane, Ineta Samsone, Dainis Edgars Ruņģis

Differentially expressed conserved plant vegetative phase-change related microRNAs for assessment of juvenility during silver birch in vitro propagation

Poster 23

Marcos Viejo, Manuel Rey, Yolanda Ferradás, Javier Sampedro, Jorunn E. Olsen, Carl Gunnar Fossdal, Igor Yakovlev, Ana Lúcia Pinto-Sintra, Ma Victoria González

Epigenetic insights into somatic embryogenesis in grapevine and Norway spruce

Poster 24

Saleta Rico, Nieves Vidal, Purificación Covelo, Jesús Mª Vielba, Conchi Sánchez

***In vitro* cloning of a monumental eucalyptus tree**

Poster 25

Andrea Ferrucci, Valerio Cristofori, Vera Pavese, Cristian Silvestri

***In vitro* shoot organogenesis and somatic embryogenesis represent the main bottleneck to genetic engineering for European hazelnut**

Poster 26

Michela Lupo, Andrea Limitone, Valerio Cristofori, Cristian Silvestri

Can led light systems be a new toolbox for alleviating rooting recalcitrance problems and simplifying the acclimatization phase?

Poster 27

Maroua Grira, Romain Martin, Stefaan P.O. Werbrouck

Solving hyperhydricity in *Tabebuia guyacan* by means of lignin precursor p-coumaric acid

Poster 28

Giuseppe Vaia, Valerio Cristofori, Cristian Silvestri

"Customized medium": how to overcome the *in vitro* recalcitrance of Sea buckthorn (*Hippophae rhamnoides* L.)

Poster 29

Hojjat Ataee, Mahdi Alizadeh, Kourosh Vahdati, Saadat Sarikhani

Identification and evaluation of natural germplasm to solve the problem of drought stress

Poster 30

Vladislava Galović, Marko Kebert, Saša Orlović

Recalcitrance – a challenge in woody plants

Keynote WG2

Manuela Nagel

Establishment and management of cryobanks, with a particular emphasis on plants *in vitro*

WG2_O1

Zhibo Hamborg, Dag-Ragnar Blystad

Use of high-throughput sequencing for virus diagnosis and plant tissue culture sanitation in Norway

WG2_O2

Elien Guldentops, Maaike Heyneman, Kris De Jonghe, Stefaan P.O. Werbrouck

Assessment of ONT sequencing (MinION) for plant virus detection and comparison with Illumina-based sequencing

WG2_O3

Doaa Elazab, Maurizio Lambardi

New trends in hazelnut micropropagation, organogenesis and micrografting: techniques, applications and future aspects

WG2_O4

Vera Pavese, Andrea Moglia, Paolo Gonthier, Elena Corredoira, Mª Teresa Martínez, Daniela Torello Marinoni, Roberto Botta

Development of biotechnological tools for *Castanea sativa* Mill. breeding

WG2_O5

Mariam Gaidamashvili, Tamari Kutchava, Eka Khurtsidze

Development of an efficient cryopreservation protocol for a Georgian provenance of *Castanea sativa* (Mill.) embryonic axes

WG2_O6

Alois Bilavcik, Stacy Denise Hammond Hammond, Olena Bobrova, Milos Faltus, Jiri Zamecník, Igor Koloniuk, Jana Franova

The use of cryopreservation for safe storage and sanitation of fruit plant germplasm

WG2_O7

Itziar A. Montalbán, Ander Castander-Olarieta, Paloma Moncaleán

Recent advances in alternative conservation methods for radiata pine somatic embryogenesis

Poster 31

Sanja Bogunović, Miran Lanšćak, Zvonimir Vujnović, Nevenka Ćelepirovć, Mladen Ivanković

Micropropagation and *in vitro* conservation of narrow-leaved ash in Croatia

Poster 32

Efigjeni Kongjika, Valbona Sota

The need for establishing the long-term conservation strategies of important autochthonous plant germplasm in Albania, helped by CopyTree experience

Poster 33

Oksana V. Pasat, Volodymyr I. Lushchak

Potassium dichromate improves morphometric and biochemical characteristics of paulownia plantlets due to induction of mild oxidative stress

Poster 34

Bart Panis

Application of cryopreservation for the conservation of plant genetic resources and stock cultures, virus eradication and tool for breeding

Poster 35

Eva Pokorná, Martina Komárková, Pavlína Máchová, Veronika Zemanová, Jozef Lacek, Miloš Faltus

The effects of diverse pre-treatment conditions on metabolism of grey poplar explants used for cryopreservation

Poster 36

Amal Rabaaoui, Radhouane Gdoura, Stefaan P.O. Werbrouck

Use of cryopreservation for conservation and eradication of pathogens of in vitro cultures of date palm (*Phoenix dactylifera* L.)

Poster 37

Jorge Sofia, Jorge Cunha, Margarida Teixeira Santos

Phytosanitary approach to unique genotypes held by the national ampelographic collection of Portugal with the aim of providing clean materials

Poster 38

Tatjana Vujovic, Tatjana Andjelic, Darko Jevremovic, Milena Djordjevic, Sanja Radicevic

Cryopreservation of stone, pome and small fruit species in Serbia using vitrification-based techniques

Poster 39

Martina Komárková, Eva Pokorná, Helena Cvrčková, Pavlína Máchová

Effective increase of *in vitro* multiplication of different forest tree species for the purposes of cryopreservation

Poster 40

Iveta Megrelishvili, Maia Kukhaleishvili, Zurab Khidesheli, Levan Ujmajurideze, Nino Maziashvili

Study of viral, bacterial and phytoplasma diseases in Georgia

Poster 41

Daniela Duarte, Alberto Cardoso, Ana Pedrosa, Elsa Baltazar, Tércia Lopes, Jorge Canhoto, Sandra Correia

***In vitro* establishment and multiplication of *Cydonia oblonga* Mill. selected germplasm**

Poster 42

Ana Pedrosa, Alberto Caeiro, Tércia Lopes, Elsa Baltazar, Miguel Teixeira, Cláudia Rato, Jorge Canhoto, Sandra Correia

SSR marker-based analysis of clonally propagated quince tree (*Cydonia oblonga* Mill.) varieties

Poster 43

Marzieh Shamshiri, Hojjat Ataee

Virus resistance gene transfer from tolerant walnut genotypes to virus-susceptible superior commercial walnut cultivars

Poster 44

João Martins, Joana Costa, Jorge Canhoto

Microbiome diversity and composition of *Arbutus unedo* L. (strawberry tree) during *in vitro* and *ex vitro* growth: implications for clonal plant production

Poster 45

Ben Bubner, Franziska Past

***In vitro* cultures of common ash (*Fraxinus excelsior* L.) in research on resistance against ash dieback: problems and possible solutions**

Keynote WG3

Jana Krajnakova, Cathie Reeves, Taryn Saggese, Cuong Kim Lee, Ulrika Egertsdotter, Cyrus Aidun, Sam Davidson, Tancred Frickey, Celine Mercier, Mikko Tikkinen, Tuija Aronen, Russell Burton

Tissue culture for the 21st century forests

WG3_O1

Sakari Välimäki, Mikko Tikkinen, Teresa Hazubska-Przybył, Laura Paavilainen, Frida Salonen, Ewelina Ratajczak, Saila Varis, Tuija Aronen

Different proliferation techniques for scaling up Norway spruce somatic embryogenesis

WG3_O2

Doaa Elazab, Maurizio Lambardi

Use of liquid culture with the ElecTIS bioreactor for a faster recovering of blackberry shoots (*Rubus fruticosus*) from the conservation at 4°C

WG3_O3

Elsa Baltazar, Tércia Lopes, Mariana Correia, Ana Pedrosa, Daniela Duarte, Jorge Canhoto, Sandra Correia

Large-scale micropropagation of *Prunus* spp. rootstocks - a comparison between semi-solid and Temporary Immersion Systems

WG3_O4

Yildiz Aka Kacar

***In vitro* propagation of horticultural plants by TIS bioreactor systems**

WG3_O5

Cristian Pérez-Caselles, Lorenzo Burgos, Nuria Alburquerque

Effect of silver nanoparticles on the micropropagation of two apricot (*Prunus armeniaca* L.) cultivars

WG3_O6

Maneea Moubarak, Immacolata Fiume, Ani Barbulova, Gabriella Pocsfalvi

***Ginkgo biloba*: *in vitro* culture as an alternative system for the production of extracellular vesicles**

WG3_O7

Paweł Chmielarz, Szymon Kotlarski, Małgorzata Pałucka, Urszula Wasileńczyk, Paulina Kosek, João Paulo Rodrigues Martins, Juan Manuel Ley López, Mikołaj Krzysztof Wawrzyniak, Marcin Michalak

Recalcitrance of *Quercus robur* to *ex-situ* conservation: Cryopreservation and *in vitro* culture

Poster 46

Carmen Salinero, Pilar Vela, Noemí Rial, Olga Aguín

Applications of *in vitro* techniques in the study of phytopathogenic fungi and oomycetes

Poster 47

Nourhene Jouini, Maurizio Lambardi, Carla Benelli, Waed Tarraf, Tolga Izgu, Maria Antonietta Germanà

An innovative protocol to propagate and preserve the threatened Sicilian Fir through somatic embryogenesis technique

Poster 48

Caroline Teyssier, Armelle Delile, Céline Ridelle, Nathalie Boizot, Marie-Anne Lelu-Walter

Analysis of environmental effects on the quality of conifer somatic embryos

Poster 49

María Teresa Martínez, Fátima Mosteiro, Sol Campañó, Pablo Piñeiro, Elena Corredoira

Somatic embryogenesis in holm oak: optimization of maturation and germination steps

Poster 50

Yildiz Aka Kakar, Hakan Erol, Dicle Dönmez, Belgin Biçen, Özhan Şimşek

Modern aproaches in *in vitro* clonal banana micropropagation: next generation tissue culture systems

Poster 51

Essia Sebai, Amel Abidi, Akkari Hafidh

Micropropagation of Tunisian *Thymus capitatus*: a step towards production of polyphenol-rich extracts showing *in vitro* anthelmintic activities

Poster 52

Roberto Berni, Sylvain Legay, Samuel Jourdan, Diego Rios-Salgado, Sébastien Planchon, Kjell Sergeant, Jenny Renaut, Jean-Francois Hausman, Gea Guerriero

Establishment of an embryogenic culture of *Hylocereus undatus*: cultivation in bioreactors and analyses via -omics

Poster 53

Mariana Correia, Tatiana Soares, Tércia Lopes, Elsa Baltazar, Maria Celeste Dias, Jorge Canhoto, Mónica Zuzarte, Sandra Correia

Physiological and biochemical responses of clonally propagated *Prunus* spp. GF677 rootstocks to water-deficit and stress recovery

Poster 54

Itziar A. Montalbán, Ander Castander-Olarieta, Paloma Moncaleán.

Optimizing late stages of *Pinus radiata* somatic embryogenesis: a previous stage to scaling up

Poster 55

Radisras Nkurunziza, Joanna Jankowicz-Cieslak, Ivan L. Ingelbrecht, Stefaan Werbrouck

In vitro* tissue culture and genotyping innovations to accelerate mutation-assisted breeding of *Coffea arabica

Poster 56

Noemi Tel-Zur

Embryo rescue as a tool in Pitaya (*Hylocereus*, Cactaceae) breeding programs

Poster 57

Marga Fraga, Beatriz Vidal, Cristina Vigo, Adriana Romero, Aránzazu Rodriguez, Minia Bralo, Gloria Saccatoma

Cultigar, a project for the conservation, production, and improvement of plant species

Poster 58

Hajer Ben Ghozlen, Stefaan Werbrouck, Sven Mangelinckx

Potential of metabolic engineering for resveratrol production in peanut cell cultures

Poster 59

Juan Luis Fernandez-Lorenzo, Ana Couso

New approaches for *in vitro* micrografting of chestnut

Poster 60: *Not presented*

Poster 61

Claudia Ruta, Giuseppe Basile, Patrick Guarini, Giuseppe De Mastro

Direct somatic embryogenesis from leaves of coffee plants in different phenological stages

Poster 62

Mirni Ulfa Bustami, Stefaan Werbrouck

Somatic embryo induction in the Indonesian elite *Theobroma cacao* clone

Poster 63

Lilyana Nacheva, Nataliya Dimitrova, Ivaylo N. Tsvetkov

Micropropagation of woody plants: common oak (*Quercus robur* L.) and pear (*Pyrus communis* L.) rootstock OHF 333 as examples

Poster 64

Barbara Ruffoni, Marco Savona

Competences and skills of the team of Research Centre for Vegetable and Ornamental Crops in Sanremo

Poster 65

Irum Saadia Khan, Stefaan P.O. Werbrouck, Stephen Page

Maintaining leaf variegation during *Polemonium caeruleum* micropropagation

Poster 66

Lilyana Nacheva, Nataliya Dimitrova

Effect of LED light on the *in vitro* micropropagation of fruit trees

Poster 67

Fabiano Gattabria, Massimiliano Meneghini, Alice Patella, Giuliano Dradi, Gianluca Magnani, Romano Roncasaglia

Improving hazelnut micropropagation: a comparison of *in vitro* and *ex vitro* rooting

Poster 68

Saleta Rico, Diego Gago, Conchi Sánchez, Anxela Aldrey, Mª Ángeles Bernal, Beatriz Cuenca, Bruce Christie, Nieves Vidal

Photoautotrophic micropropagation of trees cultured in bioreactors with forced ventilation

Poster 69

Santiago Silva, Juan Carlos Codesido

Development of an in-house bioreactor system to micropropagate ornamental plants

Keynote WG4&5

Tuija Aronen, Terhi Latvala, and Mikko Tikkinen

Addressing stakeholder concerns together with EU and national legislation – somatic embryogenesis of Norway spruce in Finland

WG4&5_O1

Valbona Sota

Harmonized communication by active members – the foundation for CopyTree's collaborative innovation

WG4&5_O2

Rober Ahmad Kreimech

CopyTree's Community Platform- A communication tool supporting strategic collaborative innovation

WG4&5_O3

Şule Yalçin

Increasing the participation of young talents in biotechnological studies through effective communication language and channels

Poster 70

Francisco J. Lario, Beatriz Cuenca

Micropropagation of forest trees: a tool for breeding programmes and production of new Forest Reproductive Material (FRM)

Poster 71

Bekim Gashi, Valbona Sota, Mirsade Osmani, Efigjeni Kongjika

Applications of *in vitro* tissue culture technologies in Kosovo: Past and future perspective

Poster 72

Nieves Vidal, Conchi Sánchez, Tomé Mouriño, Marta Soto de Paz, Lidia Mouriño, Purificación Covelo

"Sound of the forest", using science and music for an interactive experience about trees

Poster 73

Liva Purmale, Rafaels Joffe, Anna Korica, Anta Sparinska

Transmission of research results in the field of micropropagation to students of technical schools - case study

Poster 74

Yeşim Yalçın Mendi, Yildiz Aka Kakar

Production of ornamental plants commercially in biotechnological companies in Adana

Poster 75

Yeşim Yalçın Mendi, Metin Koçak, Aycan Alp, Başar Sevindik, Mehmet Tütüncü, Tolga İzgü, Senem Uğur

Biotechnological studies in *Cyclamen* sp

ABSTRACTS

Keynote Conference WG1

Recalcitrance: do we really understand it?

Jorge Canhoto[1], João Martins[1], Cátia Pereira[1], Daniela Cordeiro[1], André Caeiro[1], Mariana Neves[1], Sandra Correia[1,2]

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2]InnovPlantProtect CoLab, 7350-478 Elvas, Portugal

jorgecan@ci.uc.pt

When applied to micropropagation, recalcitrance is the inability of explants to regenerate plants. In this sense, it is the opposite of totipotency. It can also be applied in other contexts, for example when genetic transformation is unsuccessful or when microspores are unable to embark into an embryogenic pathway. The literature shows that some groups of plants are more associated with recalcitrance, including woody plants, making it difficult to CopyTree. In many situations, recalcitrance may simply reflect a poor composition of the culture media or inappropriate culture conditions that are incapable of stimulating somatic embryogenesis, organogenesis or axillary shoot development. However, recalcitrance is also related to deeper reasons related to the genotype of the explants, their physiological state or structural organization. Thus, it seems that genetic, epigenetic, physiological and anatomical factors, by themselves or acting together, can be the basis of recalcitrance. In fact, it is well known that some genes are involved on somatic embryo formation whereas others have an inhibitory role. Data also show that DNA methylation and other epigenetic processes are closely related to organogenesis and somatic embryogenesis induction. In this work these aspects will be discussed in the light of the processes of organogenesis and somatic embryogenesis that we have been studying in several woody species, such as *Acca sellowiana*, *Arbutus unedo*, *Solanum betaceum* and several pines.

Oral WG1-1

The triad of tree biotechnology

Tobias Bruegmann, Virginia Zahn*, Alexander Fendel*, Alice-Jeannine Sievers, Matthias Fladung

Thuenen Institute of Forest Genetics, Grosshansdorf, Germany
* Equal contribution

tobias.bruegmann@thuenen.de

Breeding forest trees is slow due to the special characteristics of forest trees such as slow growth and long generation cycles. The use of biotechnological methods for gene characterisation could significantly accelerate tree breeding, which could become necessary for climate change adaptation. However, biotechnological methods are not available for the vast majority of forest tree species.
The junior research group "Genetic Technologies" established at the Thuenen Institute of Forest Genetics in 2021 focuses on a thematic triad and seeks to transfer biotechnological methods such as tissue culture, genetic transformation and genome editing approaches to recalcitrant tree species. Based on the working technologies in the tree genus *Populus* (poplar), the currently severely impaired European beech (*Fagus sylvatica*) in particular is in the focus for expanding the molecular toolbox.
The goals of tissue culture include establishing sterile cultures from wild populations and testing protoplast isolation and regeneration. Genome editing involves testing different transformation methods such as *Agrobacterium*-mediated transformation, protoplast transformation and ballistic transformation with a particle gun. The aim is to knock out selected candidate genes using CRISPR/Cas editing vectors in order to characterise the involvement of individual genes in drought stress tolerance in trees. With additional knowledge, breeding research for climate change adaptation of trees can be accelerated.

Oral WG1-2

How epigenetic studies can help to characterize recalcitrance, development, and stress memory for *in vitro* cultures of trees?

Jean-François Trontin[1,2], Celia Miguel[3], Caroline Teyssier[4], Alain Delaunay[2], Jörg Tost[5], Marie-Anne Lelu-Walter[4], Stéphane Maury[2]

[1]FCBA BioForBois, Pôle Industrie Bois & Construction, Campus Forêt-Bois de Pierroton, 71 Route d'Arcachon, Cestas, 33610, France.
[2]LBLGC, INRAE, Université d'Orléans, EA 1207 USC 1328, 45067 Orléans, France
[3]BioISI-Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, 1749-016 Lisboa, Portugal.
[4]BioForA, INRAE, ONF, UMR 0588, 45075 Orléans, France.
[5]Laboratoire Epigénétique et Environnement, LEE, CNRGH CEA, Evry, France.

stephane.maury@univ-orleans.fr

Forest tree species have long reproductive cycles and therefore their adaptability to climate change is a major concern for foresters. For example, global warming already influences tree flowering and seed production. *In vitro* plant technologies such as somatic embryogenesis is a promising high-performance clonal propagation system to scale up production of genetically adapted varieties but also to study under controlled conditions the effect of environmental factors on embryo development. While these techniques are available since decades, there are still concerns to improve their efficiency (notably recalcitrance) or diversify their uses for example with stress memory and priming. Among the mechanisms involved in the regulation of plant development, epigenetic and particularly DNA methylation are key processes for the control of gene expression and transposable element activity. However, little is known about the epigenetic component for *in vitro* cultures of woody species. In this presentation, we will explain the rationale concerning epigenetics and *in vitro* cultures, we will present methods in epigenomics and illustrate how epigenetic studies can help to better characterize recalcitrance, development, and stress memory with some of our results.

Oral WG1-3

Long non-coding RNAs involved in embryogenic competence acquisition in tamarillo (*Solanum betaceum* Cav.)

Daniela Cordeiro[1], Alexandra Camelo[2], Ana Carolina Pedrosa[1], Inês Brandão[1,2], Jorge Canhoto[1], Christophe Espírito Santo[1,2] , Sandra Correia[1,3]

[1] Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[2] Centro de Apoio Tecnológico Agro-Alimentar (CATAA) de Castelo Branco, 6000-459 Castelo Branco, Portugal
[3] InnovPlantProtect CoLab, Estrada de Gil Vaz, 7350-478 Elvas, Portugal

danielacordeiro@outlook.pt

Somatic embryogenesis (SE) is a process by which somatic cells reprogram, acquire totipotency and embark on embryo formation. Although this is a useful tool for the micropropagation of numerous crops, its use is still limited in woody species due to bottlenecks, such as embryogenic competence loss during subcultures and the often-low embryo development rates. Hence, great interest exists in analysing the regulatory networks involved. As transcription modulators, long non-coding RNAs (lncRNAs) were found essential in a wide range of biological processes. Thus, this work aimed to identify lncRNAs related to the embryogenic competence in *Solanum betaceum* Cav. For that, Nanopore® long-read sequencing was conducted in cell lines with distinct cell fates. Based on their coding potential, 60 transcripts were selected as lncRNA candidates and similar expression patterns were found among embryogenic cell lines (EC) and cells that lost their embryogenic potential (LTC). In turn, non-EC (NEC) showed differentially expressed lncRNAs when compared to EC and LTC. Whereas lncRNAs up-regulated in EC and LTC were predicted to target embryogenesis-related genes, such as *AGAMOUS-like 15* and *WUSCHEL-related HOMEOBOX 2*, lncRNAs upregulated in NEC were predicted to target genes involved in auxin and ethylene signalling and carbohydrate metabolism. Altogether, these results contribute to the understanding of the regulatory networks involved in cell reprogramming during totipotency acquisition.

Oral WG1-4

Innovative small molecules to improve cell reprogramming and somatic embryogenesis in crop and forest species

Pilar S. Testillano[1], Elena Carneros[1], Yolanda Perez-Perez[1], Eduardo Berenguer[1,a], Cristina Rueda-Varela[1], Carmen Gil[2], Ana Martinez[2]

[1]Pollen Biotechnology of Crop Plants group.
[2]Translational Medicinal and Biological Chemistry group.
Center of Biological Research Margarita Salas (CIB), CSIC. Ramiro de Maeztu 9, 28040 Madrid, Spain.
[a] Present address: Plant Reproduction and Development Lab. ENS de Lyon, CNRS, INRAE, UCBL, F-69342 Lyon France

testillano@cib.csic.es

Despite decades of application of in vitro culture, plant regeneration from somatic cells, either microspores (for DH production), protoplasts/calli (for gene-editing/transformation) or vegetative tissues (for clonal propagation) is still highly inefficient in many species. Our group investigates the cellular and molecular basis of stress-induced cell reprogramming and totipotency to identify new targets and efectors for efficient manipulation of in vitro regeneration systems. We have studied, among other factors, the role of: (a) autophagy, cystein proteases, cell death, (b) auxin, cytokinins, BRs, (c) epigenetic marks (DNA methylation, histone PTMs), and (d) cell wall remodelling. In recent years, we have initiated an innovative research line, in collaboration with chemical biology experts to screen novel small molecules from chemical libraries of the pharmaceutical field, never used before in plants, to improve in vitro plant regeneration. Several chemical hits, inhibitors of kinase activities (GSK3, LRRK2), have been identified as promoters of cell reprogramming and somatic embryogenesis, while they enhanced expression of embryogenesis markers and activated key signalling pathways, in crop and forest species, as rapeseed, barley and cork oak. Promising results were obtained with several types of small molecules with different chemical and physiological activities. These findings represent a completely new technological innovation with high potential of transfer to forest nursery sector.

Berenguer et al. 2021. J. Exp. Bot. 72, 7808-7825.

Supported by PID2020-113018RB-I00 (funded by MCIN/AEI /10.13039/501100011033), TED2021-129633B-I00; CPP2021-008750 (funded by MCIN/AEI /10.13039/501100011033 and NextGenerationEU/ PRTR). C.R.V. funded by Comunidad de Madrid and EU/FEDER (PEJ-2020-AI/BIO-17772).

Oral WG1-5

Dichlorprop esters as promising tools for rooting trees?

Hajer Darouez, Stefaan P.O. Werbrouck

Laboratory of Applied In Vitro Plant Biotechnology, Dept. Plants & Crops, Faculty of Bioscience, University of Ghent, Ghent, Belgium.

hajer.darouez@ugent.be

Dichlorprop-P 2-ethylhexyl ester (DCPE) is a synthetic auxin-like plant growth regulator for improving the size of citrus fruits. When 1 µM was added to the in vitro medium of poplar, it induced epinasty combined with excessive adventitious rooting throughout the shoot, including the leaves. Because DCPE was too potent, pulsed treatments were carried out and the ability to root was again tested. Even a pulsed treatment of 0.1 µM for 4 hours induced roots on the whole shoot. To understand this reaction, transport of DCPE and DCP and their effects on endogenous auxins were analyzed.
Robinia Pseudoaccacia, after application of conventional auxins such as IAA, IBA and NAA, generally produces too much callus at the base of the shoot during micropropagation. Due to the poor shoot-root connection, this is not very favourable for acclimatisation. DCPE was added to the rooting medium and its effect on nodule segments was compared with that of the related substances dichloroprop (DCP) and 2,4-D. As expected, their continuous presence stimulated excessive callus formation at the base of the stems. Therefore, node segments were pulsed with 0, 2.5, 5 and 10 µM of each compound for 1 h. 2,4-D produced only callus, DCP produced some roots, but optimal rooting was obtained with the 5 µM DCPE pulse. To conclude, compound auxins are promising compounds for solving rooting problems in woody plants.

Oral WG1-6

Auxin and wound-responsive mechanisms underlying micrograft union formation in almond - *Prunus dulcis* (Mill.) D. A. Webb

Tércia Lopes[1], Ana Pedrosa[1], Sandra Caeiro[2], Mariana Correia[1], Elsa Baltazar[1], André Caeiro[1], Liliana Marum[3,4], Jorge Canhoto[1], Sandra Correia[1,2]

[1] Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2] InnovPlantProtect CoLAB, 7350-999 Elvas, Portugal
[3]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal
[4]MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, CEBAL, 7801-908 Beja, Portugal

tercia.lopes.95@gmail.com

Micrografting is the functional union of a scion and a rootstock carried out *in vitro*. Molecular mechanisms involved in scion-rootstock interactions, like wound-responsive and auxin-related factors, have been referred as key factors for the successful establishment. However, their specific role in woody species remains unclear. The aim of this work was to evaluate wound-responsive and auxin-related mechanisms involved in micrograft union established from micropropagated almond shoots. Homografts of bitter almond (BA) and heterografts composed by BA or GF677 (GF) rootstocks and "Lauranne" (L) or "Ferraduel" (F) varieties as scions, were performed. Samplesg for IAA quantification by HPLC and qPCR analysis were taken before grafting (t0) and 7 (t1) and 21 (t3) days after grafting. Regarding GFxL micrografts, gene expression analysis of *WIND1* transcription factor (TF) appeared to be down-regulated, decreasing from t0 to t2. This was also observed for GFxF. On the contrary, *ESRI* (TF) was up-regulated. Total IAA quantification showed no significant differences between genotypes at t0. However, in GFxL micrografts an increase of IAA was observed from t0 to t1 followed by a significant decrease at t2. These results were in accordance with *TIR* (TF) which expression decreased from t0 to t2. Down-regulation of *IAA26* (TF) was also observed in BAxF. So far, the results have shown that wound responses and auxin levels affect gene expression during almond micrografting.

Oral WG1-7

Novel cytokinin oxidase/dehydrogenase inhibitors derived from diphenylurea for use in plant tissue culture

Nino Muvarnidze[1], Jaroslav Nisler[2], Stefaan P.O. Werbrouck[1]

[1] Laboratory for Applied In Vitro Plant Biotechnology, Dept. Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium (nina.murvanidze@gmail.com)
[2] Isotope Laboratory, Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czechiaotopeaboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Medicinal Research, Institute of Experimental Botany AS CR & Palacký University, Slechtitelů 11, Olomouc 783 71, Czech Republic

stefaan.werbrouck@ugent.be

Cytokinin oxidase/dehydrogenase (CKX) degradation is one of the mechanisms regulating local cytokinin homeostasis in plants in vitro. Recently, a new class of potent inhibitors of these enzymes has been developed. They are mainly derived from diphenylurea. The range of derivatives synthesised has been very broad. Before being used in micropropagation systems, they were first tested on CKX isoforms from maize and Arabidopsis. The best compounds showed IC50 values in the concentration range of 10-8 M. We were able to show that these compounds, unlike TDZ or CPPU, have no intrinsic cytokinin activity, but only protect isoprenoid cytokinins from oxidation. The success of their application in vitro depends on the compound and the plant species. A number of early results are presented, ranging from shoot meristem induction to somatic embryogenesis. They appear to offer promising new tools to be used in plant tissue culture and biotechnology.

Chemically-induced DNA demethylation promotes somatic embryo production of *Quercus suber* L.

Elena Carneros, Esteban M. Díaz-Luzza, Yolanda Perez-Perez, Pilar S. Testillano

Pollen Biotechnology of Crop Plants group, Biological Research Center Margarita Salas (CIB), CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain

ecarneros@cib.csic.es

Cell reprogramming and somatic embryogenesis (SE) initiation involve changes in global genome organization in which DNA methylation plays a key role. Several studies have reported changes in DNA methylation during *in vitro* morphogenic processes in some herbaceous species, but little is known about DNA methylation dynamics during SE in trees. This work analyses the changes in global DNA methylation levels, nuclear distribution of methylated DNA and expression of DNA methyltransferase genes, as well as the effect of the DNA demethylating agent 5'-Azacytidine (AzaC) in cork oak SE. Results showed a reduction of global DNA methylation levels after SE induction and early stages of SE, in proembryogenic masses, followed by an increase of methylation during embryo differentiation. This pattern correlated with expression profiles of several DNA methyltransferase genes. AzaC treatment reduced global DNA methylation, while enhanced SE induction rate, promoted proembryogenic masses proliferation, and induced *QsSERK1* expression. However, continuous AzaC treatment prevented further embryo development. AzaC removal from culture medium restored embryo development and led to the formation of a higher number of embryos compared to control cultures. These findings open the way for new strategies using small molecule epigenetic modulators to enhance SE yield in forestry breeding programs.

Supported by: PID2020-113018RB-I00 (funded by MCIN/AEI /10.13039/501100011033), TED2021-129633B-I00; CPP2021-008750 (funded by MCIN/AEI /10.13039/501100011033 and NextGenerationEU/ PRTR).

Ethylene inhibits adventitious rooting in chestnut trees after phase change

Ricardo Castro-Camba[1], Mariana Neves[2], Sandra Correia[2,3], Jorge Canhoto[2], Jesús Mª Vielba[1], Conchi Sánchez[1]

[1] Dept. of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
[2] Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[3] InnovPlantProtect CoLab, Estrada de Gil Vaz, 7350-478 Elvas, Portugal

ricardo.castro@mbg.csic.es

Phase change refers to the process of maturation and transition from the juvenile to the adult stage. During this process certain species like chestnut lose the ability to form adventitious roots, thereby hindering the successful micropropagation of adult plants. While auxin is the primary hormone involved in adventitious root formation, other hormones, such as ethylene, are also thought to play a role in its development.
In this study, experiments were carried out to determine the effects ethylene on the development of adventitious roots. The analysis was performed in two types of microshoots derived from the same tree; a juvenile-like line derived from basal shoots (P2Rb) and a line derived from crown branches (P2C). An exogenous ethylene precursor ACC (30 µM) and its inhibitor AgNO3 (30 µM) were applied alongside with the auxin IBA (25 µM). Treatments were applied to the explants for 5 days under darkness conditions before their transfer to fresh hormone-free rooting medium during 25 days under a normal photoperiod. Ethylene and auxin-related gene expression analysis by qPCR was performed. Our results indicate that ethylene inhibits adventitious root induction in mature shoots (P2C), confirmed by the overexpression of *ACO, ACS* and *EIN2* and *ERF3*, genes related to ethylene biosynthesis and signalling pathways. Additionally, the overexpression of *JMJ30* suggests that this inhibition may also be mediated through epigenetic changes.

Funding: This work was funded by Xunta de Galicia (Spain) through the projects IN607A/2021 and PIE 202140E015.

Multi-varietal forestry: deployment of Fagaceae and Pinaceae genotypes adapted to climate change

Isabel Arrillaga[1], Ester Sales[2], Paloma Moncaleán[3], Esther Asensio[4], María Carmen González-Más[1], Itziar A. Montalbán[3], María Amparo Pérez-Oliver[1], M. Teresa Martínez[5], Elena Corredoira[5]

[1] BiotecMed Institute and Plant Biology Department, University of Valencia, Vicent A. Estellés s/n, 46100 Burjassot, Valencia, Spain
[2] Dept. Agricultural and Environmental Sciences, Higher Polytechnic School, University of Zaragoza, Ctra. Cuarte s/n, 22197 Huesca, Spain
[3] NEIKER-BRTA, Centro de Arkaute N-104 km 355, 01192 Vitoria-Gasteiz, Spain
[4]Dept. Analytical Chemistry, Veterinary Faculty, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain
[5] Galicia Biological Mission (MBG-CSIC), Santiago de Compostela, Avda. Vigo s/n, 15705 Santiago de Compostela, Spain

Maria.A.Perez-Oliver@uv.es

Global climate change menaces forest plantations, that will depend on trees capacity to cope with abiotic stresses such as high temperatures and water scarcity, and with biotic factors such as soil-borne pathogens. Conventional breeding schemes of these long-lived plant species are slow, therefore to avoid dependence on seed-based forestry, efficient strategies of vegetative propagation are needed. The collaborative project VARIFOR, funded by the EU and Spanish Government (PID2020-112627RB-C3), aims to produce, characterize, conserve and propagate improved genotypes of forest species threatened in Spain: *Quercus ilex, Q. suber*, *Castanea sativa, Pinus radiata* and *P. pinaster*. Genotypes with superior performance will be generated using biotechnologies including somatic embryogenesis, marker assisted selection, genome editing, and the induction of epigenetic adaptations (priming). These genotypes could be the basis for the first Spanish multi-varietal forestry program, which implies the deployment, through vegetative propagation, of a range of genetically tested varieties. The project will also address the bottlenecks of *in vitro* propagation by scaling-up multiplication in temporary immersion systems, by optimizing steps of the embryogenesis process and acclimatization, and by developing inexpensive and simple alternatives to cryopreservation. The project also aims to study physiological, epigenetic and molecular changes associated to plant resilience using omics tools.

Exploring the rRNA methyltransferases signalling pathway during tamarillo (*Solanum betaceum* Cav.) somatic embryogenesis

Ricardo Ferraz[1,3], Sílvia Coimbra[2,3], Sandra Correia[1,4], Jorge Canhoto[1]

[1] Universidade de Coimbra, Centro de Ecologia Funcional, Laboratório Associado Terra, Departamento de Ciências da Vida, Coimbra, Portugal
[2] Faculdade de Ciências da Universidade do Porto, Departamento de Biologia, Universidade do Porto, rua do Campo Alegre, 4169-007 Porto, Portugal
[3] LAQV Requimte, Sustainable Chemistry, Universidade do Porto, 4169-007 Porto, Portugal
[4] InnovPlantProtect CoLAb, Estrada de Gil Vaz, 7350-478 Elvas, Portugal

rikayferr@hotmail.com

Somatic embryogenesis (SE) is a valuable tool for plant breeding, with applications ranging from plant cloning to conservation. Genetic transformation and gene editing are also dependent on reliable *in vitro* cloning protocols such as SE. Tamarillo, is a solanaceous tree with well-established *in vitro* culture protocols that make it an appropriate experimental system to induce and study SE in woody species. In previous work, an rRNA methyltransferase (MTase), consistently expressed in non-embryogenic calli of tamarillo (NEP-TC, Non-Embryogenic Protein from Tamarillo Callus) was identified as being putatively involved in the inhibition of SE. Despite being related to organelle development, rRNA MTases functional roles in plant regeneration processes are still unknown. Based on this, the main objective of this work is to functionally characterise the rRNA MTases signalling pathway in *Solanum betaceum* SE. For that different functional genomics and proteomics tools are being developed for tamarillo, including cross-linking, followed by analysis of cDNA and interacting proteins identification. Further functional characterisation of enzymes from the NEP-TC family will also be conducted with *Arabidopsis thaliana*. With this set of tools, it is expected to characterise all NEP-TC's interactome and mode of action during SE. Such results will contribute with information on a regulatory network not yet explored but with possible important applications for several woody species.

Approaches we apply to study Norway spruce somatic embryogenesis

Lucie Fischerová[1], Karel Doležal[2], Tomáš Moravec[3], Zuzana Vondráková[1], Kateřina Eliášová[1]

[1]Laboratory of Biologically Active Compounds, Institute of Experimental Botany of the Czech Acadamy of Sciences (IEB CAS), Rozvojová 263, Prague, Czech Republic
[2]Laboratory of Growth Regulators, IEB CAS, Šlechtitelů 27, 78371 Olomouc, CZ
[3]Laboratory of Virology, IEB CAS, Rozvojová 263, Prague, CZ

fischerova@ueb.cas.cz

One of the main objectives of COPYTREE is to share our expertise to tackle the challenges of *in vitro* cloning of woody plants. Here we present the methodological approaches we have implemented in studying *in vitro* mass propagation systems, mainly in Norway spruce somatic embryogenesis. According to the goal of knowledge transfer, we offer to share them in the COPYTREE network.
Our expertise cover methods of anatomy and histochemical detections (in combination with light and fluorescent microscopy), determination of the content of plant hormones (using UHPLC-MS/MS methods), and expression analysis (qPCR). To control the developmental processes of *in-vitro* cultures, we use a broad spectrum of newly synthesized bioactive molecules as well as modulators of plant hormone metabolism and perception (e.g. anti-auxins, anti-gibberellins, new cytokinin derivatives, inhibitors of cytokinin oxidase/dehydrogenase). Newly we are also implementing methods of virus detection (RT-PCR, RT-LAMP).

Advances in date palm (*Phoenix dactylifera* L.) micropropagation

Emna Baklouti [1], Ameni Nasri[1], Ben Romdhane Amal[1], Ahlem Ben Ahmed[1], Hazar Akrimi[1], Sahar Baklouti[1], Riadh Drira[1], Alain Rival[2], Noureddine Drira[1], Lotfi Fki[1]

[1] Dept. of Biology, Faculty of Sciences of Sfax, Sokra street km 3.5, Tunisia
[2] Cirad - DGDRS, Jakarta, Indonesia.

Lotfifki@yahoo.fr

During recent years, different approaches have been designed for date palm micropropagation. Considerable progress has been made in the development and optimization of diverse regeneration pathways. However, several problems still need to be solved and are currently under study, such as the proliferation of endophytic bacteria within *in vitro* cultured material and the occurrence of somaclonal variants in regenerated offspring. Protocols for large scale micropropagation of date palm (*Phœnix dactylifera* L.) via somatic emryogenesis and caulogenesis have been described.
Thousands of somatic embryos derived from highly proliferating suspension cultures were produced. Friable embryogenic calli were initiated from both leaf and inflorescence explants. Suspension cultures consisting of proembryonic masses were established from calli showing a high competency for somatic embryogenesis. The subculture of suspensions in liquid medium enriched with low amounts of plant growth regulators (1 mg/L 2,4-D with 300 mg/L charcoal) resulted in the differentiation of large numbers of somatic embryos.
Adventitious bud clusters of date palm were successfully established from juvenile leaves taken from offshoots using MS basal medium supplemented with small amounts of 2,4-D (0.2 mg/L). Explants bearing buds were transferred to MS medium supplemented with 70 g/l sucrose to obtain multiple bud clusters. The positive effects of temporary immersion on shoots proliferation were significant since compared to the cultivation on solid media, multiplication rate increased from 2 to 3. Elongation of shoots was achieved on thin-film liquid medium without PGRs and rooting was induced in liquid MS basal medium supplemented with 4 mg/L IBA.

Intermittent contact with the volatile organic compounds of *Serendipita indica* alleviates salt stress in *in vitro Ocimum basilicum* L.

Hassiba Fraj, Stefaan P.O. Werbrouck

Laboratory for Applied In Vitro Plant Biotechnology, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium

hassibaing@live.fr

Serendipita indica is a plant growth-promoting fungus. It is a natural soil inhabitant that can colonize the roots of a variety of plants, including cultivated crops. *S. indica* is known to enhance nutrient uptake and improve stress tolerance when inoculated into soil. This study was undertaken to investigate the effect of its volatile organic compounds (VOCs) on salt-stressed *Ocimum basilicum* in vitro, via a customized temporary immersion bioreactor system (SETIS). For all salt concentrations, VOCs of *S. indica* significantly improved plant growth. This resulted in heavier and taller plants, more shoots per plant, and longer roots. This was observed even for the control without salt. These findings encourage the application of *S. indica* as a new tool, not only for salt stress reduction but also for general 'in vitro' stress. Moreover, the proposed original adaptation of a temporary immersion system will be instrumental to investigate the effects associated with volatile compounds and better understand their mechanism of action.

Effect of exogenous ascorbic acid treatment on induction, proliferation, the hydrogen peroxide and phytohormone content during first steps of somatic embryogenesis of *Picea abies* (L.) H. Karst.

Teresa Hazubska-Przybył[1], Agata Obarska[1], Agata Konecka[2], Mikołaj Wawrzyniak[1], Aleksandra Staszak[3], Ewelina Ratajczak[1]

[1] Dept. of Developmental Biology, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kornik, Poland
[2] Dept. of Silviculture, Institute of Forest Sciences, Warsaw University of Life Science, Warsaw, Poland
[3] Laboratory of Plant Physiology, Department of Plant Biology and Ecology, Faculty of Biology, University of Bialystok, Ciolkowskiego 1J, 15-245 Bialystok, Poland

hazubska@man.poznan.pl

Ascorbate (ASA) is a natural plant compound that is involved in the regulation of basic cellular processes. It is a potent, water-soluble antioxidant that prevents oxidative damage to cells and may be involved in embryogenesis. ASA may also act as a cofactor for many enzymes catalysing important biochemical reactions. So far, it has been shown that the addition of ASA to the culture medium improved the development of somatic *Picea glauca* embryos during the maturation phase. Our aim was to test whether the concentration of this antioxidant would affect the level of embryonic tissue (ET) initiation of *P. abies* (as a control species) and its rate of proliferation, as well as the levels of H_2O_2 (which may be related to oxidative stress) and phytohormones in the treated plant material. Mature zygotic embryos (explants) followed by embryogenic tissue of one ET line (IC 94) were treated with exogenous ascorbic acid, which was added to the medium at concentrations of 0, 25, 50, 100 and 200 mg l^{-1}. The results show that ASA concentration determined the frequency of embryogenic tissue induction and the H_2O_2 content of the plant material analysed (explants, 8-week-old non-embryonic callus and proliferating ET). The ASA concentration influenced the content of some phytohormones in the plant material, e.g. indolyl-3-acetic acid (IAA), abscisic acid (ABA) or brasinolide (BL), which are/may be related to the SE process.

In vitro germination, tissue culture and transformation of *Spinacia oleraceae*

Ashkan Hodaei, Stefaan P.O. Werbrouck

Laboratory for Applied In Vitro Plant Biotechnology, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium

ashkan.hodaei@ugent.be

Spinach will be in flower quite soon after sowing in spring. This is because long days induce flowering. Because advanced spinach is worth nothing, this comes at the expense of yield. The ultimate goal of this research is to use CRISPR-Cas to knock out one of the key genes involved in the induction of flowering. This work develops shoot regeneration and transformation using the GFP reporter gene. Spinach was initiated by seed and we examined if callus could be induced on the seedling explants and if adventitious shoots could be induced. Also the reaction of the seedlings to daylength and light wavelength was tested as well as agrobacterium mediated transformation.
On the whole, spinach is considered to be a difficult plant to grow in tissue culture. However, spinach does have some positive characteristics such as easy induction of callus and easy transformation using the Agrobacterium-mediated transformation method. Spinach is very sensitive to day length. Under long days, it immediately starts flowering. Under short days, however, vegetative growth continues. Different light colours have no significant effect on its growth or bolting behaviour. Seedlings grown under blue & red, blue & far red, blue and normal light had similar growth behaviour. Agrobacterium-mediated transformation is efficient in leaves and roots, but more stable in roots than in leaves. The transformation rate in cotyledons is low.

Ethylene positively modulates de novo shoot organogenesis and subsequent plant development from leaf explants of *Solanum betaceum* Cav

Mariana Neves[1], Sandra Correia[1,2], Carlos Cavaleiro[3,4], Jorge Canhoto[1]

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2]InnovPlantProtect CoLab, 7350-478 Elvas, Portugal
[3]CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, University of Coimbra, Portugal
[4]Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal

mneves@student.uc.pt

We evaluated the effect of ethylene on *de novo* shoot organogenesis (DNSO) of the woody species *Solanum betaceum* Cav. (tamarillo), by culturing leaf explants in presence of different ethylene modulators. Exogenous application of ethylene precursor ACC and ethephon (ETH) increased almost two-fold the number of shoots per explant compared with control conditions. Moreover, inhibiting ethylene action with $AgNO_3$ or its biosynthesis with aminoethoxyvinylglycine (AVG) reduced the capacity of plant tissues to regenerate, accompanied by a reduced number of shoots per explant. Shoots regenerated from $AgNO_3$ and AVG treatments had their subsequent development negatively affected, with visible inhibition of shoot development and root formation. Differences in gene expression of ethylene biosynthetic enzymes, *ACS* and *ACO1,* ethylene transcription factor *ERF061* and auxin efflux carrier *PIN1,* were assessed at 3- and 8- weeks of culture. *ACS* was markedly upregulated in ETH treatments for both time points and downregulated in AVG presence. *ERF061* was upregulated in AVG and ETH treatments at 3 weeks of culture, but at the end of culture period, *ERF061* seems to be only upregulated in presence of ethylene precursors. Furthermore, we found an upregulation of *PIN1* at 3 weeks of culture for both ACC and ETH treatments along with a downregulation in $AgNO_3$ and AVG treatments. These results suggest a positive effect of ethylene on DNSO of tamarillo with a possible modulation in auxin distribution.

Remodelling of cell wall by pectin de-esterification and AGP increase is required for somatic embryo formation in cork oak

Yolanda Perez-Perez [1], Elena Carneros[1], Eduardo Berenguer[1,a], Maria-Teresa Solis [2], Pilar S. Testillano[1]

[1]Pollen Biotechnology of Crop Plants Group, Biological Research Centre Margarita Salas (CIB), CSIC, Ramíro de Maeztu, 9, 28040, Madrid, Spain
[2]Dep. Genetics, Microbiology and Physiology, Fac. Biology, UCM, Ciudad Universitaria, 28040, Madrid, Spain
[a] Present address: Plant Reproduction and Development Lab. ENS de Lyon, CNRS, INRAE, UCBL, F-69342 Lyon France

yperez@cib.csic.es

Changes in cell wall mechanics controlled by methylesterification of pectins, mediated by pectin methyl esterases (PMEs) and pectin methyl esterase inhibitors (PMEIs) underlie organogenesis initiation and embryogenesis progression. Arabinogalactan proteins (AGPs) are highly glycosylated proteins located at the surface of plasma membranes, in cell walls and in extracellular secretions, with key roles in a range of plant developmental processes. In this study, we investigated changes in pectin esterification and AGPs during somatic embryogenesis (SE) in *Quercus suber*, a forest tree with economic and ecologic value, by a multidisciplinary approach that included expression analysis of PME, PMEI and AGP genes; PME activity assays; immuno dot blot assays; immunofluorescence and confocal analysis with monoclonal antibodies to AGPs, high- and low-methylesterified pectins (LM20, JIM7, LM19, JIM5, LM6, LM2). Functional analyses were also carried out by treatments with catechin (PME inhibitor) and Yariv reagents AGP blocking agents).
Results indicate a key role for pectins and AGPs in the cell wall remodeling, that is required for SE progression, in cork oak. These findings open up new possibilities to improve *in vitro* embryo production and breeding in a woody species, in which information on cellular processes underlying SE is still scarce.

Pérez-Pérez Y, Carneros E, Berenguer E, Solís MT, Bárány I, Pintos B, Gómez-Garay A, Risueño MC, Testillano PS (2019) Front. Plant. Sci. 9: 1915

Supported by: PID2020-113018RB-I00 (funded by MCIN/AEI /10.13039/501100011033), TED2021-129633B-I00; CPP2021-008750 (funded by MCIN/AEI /10.13039/501100011033 and NextGenerationEU/ PRTR).

Screening new compounds for adventitious shoot regeneration

Elnara Sadigova, Stefaan P.O. Werbrouck

Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

elnara.e.sadigova@gmail.com

Nisler et al (2018) described a group of urea-derived compounds with exceptionally high anti-senescence activity. Among these, 1-(2-methoxyethyl)-3-(1,2,3-thiadiazol-5yl)urea (MTU, formerly ASES) proved to be the most effective at retarding chlorophyll degradation in detached wheat leaves placed in darkness, surpassing also the activity of known cytokinins.
This aim of this research was to examine anti-senescence effects in vitro, particular during adventitious shoot induction. Senescence might play a role during this process, especially when it takes a long time. ASES was combined with cytokinin oxidation inhibitors and 2iPR in model plants such as *Nicotiana tabacum* and *Lobelia erinus*.
The experiments showed that all inhibitors have an impact with or without combined, on various plant species differently. In almost all cases ASES had a positive effect on chlorophyll degradation and shoot regeneration, especially in *Lobelia erinus*. although in low concentrations we couldn't see any significant effect on the survival rate of explants in both species.

Biotechnological approaches to overcome adventitious rooting recalcitrance in mature trees of Fagaceae species

Jesus Mª Vielba, Nieves Vidal, Saleta Rico, Purificación Covelo, Ricardo Castro-Camba, M José Cernadas, Conchi Sánchez

[1] Dept. of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain

conchi@mbg.csic.es

Recalcitrance to *de novo* root formation is a major limitation for propagation of elite genotypes of many tree species. We are working on micropropagation of chestnut, pedunculate oak, cork oak, which are recalcitrant to *in vitro* culture and adventitious rooting (AR), particularly at the adult stage. To study the maturation-related decline of AR we developed an *in vitro* system of shoots with the same genotype but different ontogenetic stage. AR of juvenile-like shoots is about 90%, while mature shoots rarely form roots, showing that maturation imposes rooting recalcitrance. Rejuvenation treatments improved in vitro response of explants but not the AR. When juvenile material was not available, acceptable rooting rates were achieved in some oak and cork oak genotypes using crown derived shoots. To improve the rooting response of adult material we are testing epigenetic- and hormone -modulators. The molecular basis of maturation-related loss of AR is being analysed by comparing gene expression profiles of rooting- competent and -incompetent shoots. Expression analysis showed that *SCL1*, *GH3-1*, *Rap2.12 like.1*genes are related to wounding and/or AR in a tissue-specific manner. Functional analysis of genes is being performed by *Agrobacterium*-mediated genetic transformation. A recent transcriptomic analysis has shown that the activity of MADS-Box genes seems to be related to the rooting-recalcitrant behaviour of mature material, by impairing the acquisition of root founder cell fate.

Funding: This work was funded by Xunta de Galicia (Spain) through the projects IN607A and "Contrato Programa" 2021 (AGI/CSIC I + D + I 2021, Ref-ACAM 20210200033), by the CSIC program I-COOP + 2020 (RefCOOPB20584) and PIE 202140E015.

Slow release of plant growth regulators in *in vitro* tissue culture by nanoporous microcarriers

Saba Taheri[1], Bogdan Parakhonskiy[2], Andre G. Skirtach[2], Stefaan P.O. Werbrouck[1]

[1] Department of Plant and Crops and
[2] Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium

seyedehsaba.taheri@ugent.be

Plant growth regulators (PGRs) are essential in the tissue culture of plants. So far, they are dissolved in the medium and the tissues that come into contact with the medium receive a large influx. However, in many biological developmental stages of plants, gradients in space and time of hormones and signaling molecules play an important role. The problem is that this is very difficult or impossible to achieve by simply dissolving these substances in the medium. The solution could be to develop slow release systems. One of the most promising candidate carriers is based on mesoporous micro $CaCO_3$ particles, which can release PGRs over time to cause a spatial/temporal gradient in plant tissue. The aim of this research which combines plant in vitro technology with nano-biotechnology expertise, is to develop $CaCO_3$ particles with different physical properties that can influence the absorption and release profile. The effect of encapsulation and aggregation into bigger so called milliparticles is assessed. These particles are tested in bioassays with model species and are applied to in vitro regeneration of meristems of a diverse range of plant species. Their handy size makes it possible to apply differently charged milliparticles to the explant with tweezers. The combinations are endless and can be considered a fascinating advantage of this technology, which has the potential to become a unique tool for plant biotechnology.

Establishment of *in vitro* clones of European beech (*Fagus sylvatica* L.) as a tool for breeding programmes and resistance research

Franka Thiesen[1], Virginia Zahn[2], Ben Bubner[1]

[1] Thuenen-Institute of Forest Genetics, 15377 Waldsieversdorf, Eberswalder Chaussee 3a, Germany
[2] Thuenen-Institute of Forest Genetics, 22927 Großhansdorf, Sieker Landstrasse 2, Germany
franka.thiesen@thuenen.de

franka.thiesen@thuenen.de

The future of the European beech, as a key species in European forests, is at stake due to drought and biotic stress. To improve the trees resilience and resistance, extensive research is needed. This implies the need for reliable in-vitro micropropagation. However, the recalcitrance of beech has so far hindered the successful propagation. In our approach, we rely on a paradigm shift: instead of selecting plus-trees as explant source, we use seedlings from a wide range of trees from different provenances and trial sites in Germany. The screening of large seedling numbers and the variability of the origins increase the probability of obtaining suitable genotypes for extensive micro-cutting propagation. Later, established genotypes will be assessed for growth parameters and resistance.
We acquired beech seeds from 10 trial sites with information on origin, climatic conditions and vitality of the trees. Additionally, buds were collected from a provenance trial in Northern Germany. For in-vitro establishment, a protocol has been developed and 78 genotypes could be established. Further, the establishment via seedlings from 12000 seeds is planned. By optimising the in-vitro growing conditions we aim for high micro-cutting multiplication- and rooting rates. To assess the genetic diversification of genotypes, a marker set for microsatellite analysis of the in-vitro material is being tested. Additionally, well propagating in-vitro genotypes can be stored in our cryopreservation unit.

Rooting recalcitrance of mature chestnut *in vitro* shoots is influenced by hormone signaling and MADS-Box genes

Jesús Mª Vielba[1], Saleta Rico[1], Nevzat Sevgin[1,2], Ricardo Castro-Camba[1], Purificación Covelo[1], Nieves Vidal[1], Conchi Sánchez[1]

[1] Dept. of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
[2] Department of Horticulture, University of Sirnak, 73100 Sirnak, Turkey

jmvielba@mbg.csic.es

In recalcitrant woody species, like chestnut (*Castanea sativa* Mill.), one of the major maturation-related shifts is the loss of the ability to form adventitious roots in response to auxin treatments. To analyze the molecular mechanisms underlying this phenomenon, an *in vitro* model system of two different lines of microshoots derived from the same field-grown tree was used. Juvenile shoots root readily when treated with exogenous auxin, while mature microshoots rarely form roots. A transcriptomic analysis was developed to compare the gene expression patterns in both types of shoots 24 hours after hormone and wounding treatment. Our results suggest that the inability of adult tissues to respond to the inductive treatment relies in a deep change of gene expression imposed by maturation that results in a significant transcriptome modification. Differences in phytohormone signaling seem to be the main cause for the recalcitrant behavior of mature shoots, with abscisic acid and ethylene negatively influencing the rooting ability of the chestnut plants. We have identified a set of related MADS-box genes whose expression is modified but not suppressed by the inductive treatment in mature shoots, suggesting a putative link of their activity with the rooting-recalcitrant behavior. Overall, distinct maturation-derived auxin sensibility and homeostasis, and the related modifications in the balance with other phytohormones, seem to govern the outcome of the process in each type of shoots.

Funding: This work was funded by Xunta de Galicia (Spain) through the projects IN607A and "Contrato Programa" 2021 (AGI/CSIC I + D + I 2021, Ref-ACAM 20210200033), by the CSIC program I-COOP + 2020 (RefCOOPB20584) and PIE 202140E015.

Cross-talk among molecules excreted by *Pantoea agglomerans* and regulating system of cell fate leading to adventitious root development in the pear

Rosario Muleo[1], Caterina Valerio[1] Ivano Fogione[1], Francesca Luziatelli[2], Maurizio Ruzzi[2]

[1] Dept. of Agricultural and Forestry Sciences, University of Tuscia, Via San C. De Lellis, snc, 01100 Viterbo, Italy
[2] Department for Innovation in Biological, Agro-Food and Forest Systems, University of Tuscia, Via San C. De Lellis, snc, 01100 Viterbo, Italy

muleo@unitus.it

Plants, more than animals, have the plastic capacity to reprogram the fate of a destined cell. The adventitious roots (AR) generated from vegetative plant organs (stem or leaves) play a relevant function in the plant's survival. AR formation is regulated by a plethora of factors (physiological, anatomical, and biochemical), and gene expression regulation paves the way for achievement. Auxins play a central role and activate a signalling system for molecular and morphological changes. The research activity in our laboratory is run to evaluate the effect of metabolites excreted by *Pantoea agglomerans* on AR's induction and initiation phase in microcuttings of woody fruit species, using transgenic and wild type *Pyrus communis* genotypes. *P. agglomerans* release auxins in the culture medium, whose activity can be compared against IBA. Explants treated with bacterial metabolites showed an early adventitious root appearance, different regulation of physiological and phenotypic, like root origin directly from the stem tissue, and absence of callus development, with decreased root number formation. qRT-PCR results of auxin signaling, WOX, IAA/Aux, ARFs genes, callus development, cell division, and tissue identity, tighter with miRNA156, 160, 167, indicated that the bacterial metabolites act differently from exogenous auxin in the induction and development of ARs.

Do we talk about *in vitro* tissue culture recalcitrance of olive?

Cristian Silvestri, Andrea Ferrucci, Michela Lupo, Giuseppe Vaia

Department of Agriculture and Forest Science (DAFNE), University of Tuscia, Via San Camillo De Lellis, s.n.c., 01100 Viterbo, Italy

silvestri.c@unitus.it

The application of plant tissue culture and micropropagation in olive not yet been sufficiently exploited and used, although it can be considered a very valid tool for olive micropropagation, but also for the application of the next generation breeding technique. The term 'recalcitrance' is often used in the scientific literature concerning *in vitro* propagation and plant tissue culture of olive, referring to that condition in which cells, tissues and organs to respond to *in vitro* manipulation.
Forty years after the formulation of the medium “OM” (Olive Medium), dozens of scientific papers have been published for olive micropropagation, taking into account many different cultivars and with many different media and growth regulators, starting from the phase of *in vitro* establishment phase to rooting and acclimatisation. Does it make sense to still identify the olive as a 'recalcitrant species for tissue culture and micropropagation'? Here we discussed the state of the art and the recent advances on olive micropropagation and, in general, tissue culture, with the aim to summarise what has been achieved and what is still difficult to implement for this important species.

Somatic embryogenesis in *Abies alba* Mill

Terézia Salaj[1], Bart Panis[2,3], Rony Swennen[2], Katarína Klubicová[1]

[1] Plant Science and Biodiversity Center, Institute of Plant Genetics and Biotechnology, Akademicka 2, 950 07 Nitra, Slovakia
[2] Laboratory of Tropical Crop Improvement, Faculty of Bioscience Engineering, KU Leuven, Willem de Croylaan 42, 3001 Leuven, Belgium
[3]Alliance of Bioversity International and CIAT, c/o KU Leuven, Willem de Croylaan 42, 3001 Leuven, Belgium

katarina.klubicova@savba.sk

The aim of our study was to initiate embryogenic tissue of *A. alba* Mill and characterise them by physiological and morphological parameters. As a starting material, we used immature zygotic embryos. We tested the effect of five different cytokinins supplemented into the DCR medium on the initiation frequency and did not observe any difference among their effect. Here, we include the results obtained during three consecutive years. Overall, 61 cell lines were initiated out of 1308 explants, with initiation frequencies ranging between 0.83 and 13.33%. Microscopic observations showed the presence of embryo-like structures in all initiated cell lines. Besides these structures, we could observe huge polyembryonic complexes or "twin" embryos when two or several meristematic "heads" joined, sharing a common suspensor. We selected four different cell lines for maturation experiments. All of them produced cotyledonary somatic embryos. However, the maturation capacity ranged from 16 to 252 embryos per g of fresh weight. The initiated tissue we maintained on solid media or by cryopreservation. For cryopreservation, we applied the two-step slow-freezing technique.

Proteins secreted into the culture media of *Pinus nigra* Arn. embryogenic suspension cultures

Miroslav Pernis[1], Terezia Salaj[1], Maksym Danchenko[1], Andrej Kovac[2], Katarina Klubicova[1]

[1] Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Akademická 2, P.O. Box 39A, 95007 Nitra, Slovakia
[2] Institute of Neuroimmunology, Dúbravská cesta 9, 845 10 Bratislava 45, Slovakia

miroslav.pernis@savba.sk

The plant secretome encompasses the set of proteins that are freely secreted out of the plant cell into extracellular space and can also include loosely bound cell wall proteins. These proteins are likely to be involved in the regulatory mechanisms during somatic embryogenesis. Therefore we analysed the secretome of four embryogenic cell lines of black pine (*Pinus nigra* Arn.). After extraction from the cell suspension culture media, proteins were separated and identified by GeLC-MS/MS (denaturing gel electrophoresis followed by liquid chromatography and tandem mass spectrometry). Out of 212 identified proteins, 131 were predicted by TargetP-2.0 and SignalP-5.0 servers to contain an N-terminal signal sequence. Subcellular localisation prediction tools predicted 54 of the remaining proteins to have extracellular localisation (possible non-classical secretory pathway) and 27 to have intracellular localisation**.** From our data, we cannot assess if these are moonlight proteins or intracellular contamination. Using the InterProScan software, we annotated the proteins with functional domains. This allowed us to group identified proteins into several functional classes. The three most represented classes were: proteins acting on cell wall carbohydrates, oxidoreductases and proteases. Several other functional classes were assigned. Our study offers a general view of the black pine embryogenic cell culture secretome and provides a basis for more complex comparative studies.

Use of plant biotechnology in research activities of Silva Tarouca Research Institute for Landscape and Ornamental Gardening

Jana Šedivá, Roman Businský, Kateřina Podrábská, Hana Drahošová, Michaela Pekařová, Dagmar Řeháková

Dept. of Plant Biotechnology, Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Publ. Res. Inst. (VÚKOZ), Květnové náměstí 391, 252 43 Průhonice, Czech Republic

sediva@vukoz.cz

Plant biotechnology comprise a group of biological techniques which have found a broad use in agriculture, horticulture, and forestry. The Department of Plant Biotechnology of VÚKOZ includes two laboratories, the Laboratory of Explant Cultures, and the Laboratory of DNA Analysis. Research activities are focused on three basic areas: plant production, breeding and identification using DNA markers.
Main research topics:
1) Optimizing of micropropagation protocols for valuable genotypes (e.g. disease, pest and cold resistant) of woody and herbaceous species (e.g. *Sorbus* sp., *Rhododendron* sp., *Buxus* sp., *Aesculus hippocastanum*).
2) Increasing of genetic variability in selected species by induced polyploidization (*Moringa oleifera, Anemone sylvestris, Vavilovia formosa*).
3) Maintaining *in vitro* collections of valuable plant genotypes (e.g. *Rhododendron* sp., *Dahlia pinnata*).
4) Preservation and repatriation of selected endangered species via *in vitro* technologies (e.g. *Daphne cneorum, Sorbus* sp., *Pulsatilla* sp.).
5) Study of genetic diversity and population structure of the selected forest and ornamental woody plants with molecular markers (e.g. *Quercus petraea, Larix decidua, Populus nigra, Betula sp.*).
For the regeneration of plants from *in vitro* cultures, mainly organogenesis, marginally somatic embryogenesis is used.

Differentially expressed conserved plant vegetative phase-change related microRNAs for assessment of juvenility during silver birch *in vitro* propagation

Baiba Krivmane, Ineta Samsone, Dainis Edgars Ruņģis

Latvian State Forest Research Institute "Silava", 111 Rīgas st, Salaspils, LV-2169, Latvia

baiba.krivmane@silava.lv

In plants, phase change from the juvenile stage to maturity involves physiological and anatomical changes, which are initiated and controlled by evolutionary highly conserved microRNAs. This process is of particular significance for the *in vitro* propagation of woody plant species, as individuals or tissues that have undergone the transition to vegetative maturity are recalcitrant to propagation. However, mature explants can be rejuvenated by manipulation of *in vitro* culture conditions. Conserved miRNAs that were differentially expressed between juvenile (including rejuvenated) and mature silver birch tissues were identified using high-throughput sequencing of small RNA libraries. Expression of some miR156 isoforms was high in juvenile tissues and has been found to regulate phase transitions, as previously reported in a range of species. Additional miRNAs, such as miR394 and miR396, that were previously reported to be highly expressed in juvenile woody plant tissues were also identified in this study. However, expression of miR172 was low in all sample types in this study. Expression of miR172 has been widely reported to increase as plants mature, particularly in studies of annual and model species. Possibly miR172 is involved in specific maturation processes in silver birch, such as flowering, which were not investigated in this study.

Epigenetic insights into somatic embryogenesis in grapevine and Norway spruce

Marcos Viejo [1,2], Manuel Rey[3], Yolanda Ferradás[1], Javier Sampedro[1], Jorunn E. Olsen[4], Carl Gunnar Fossdal[2], Igor Yakovlev[2], Ana Lúcia Pinto-Sintra[5], Mª Victoria González[1]

[1] Dept. of Functional Biology, Faculty of Biology, University of Santiago de Compostela, 15782, Spain
[2] Dept. of Molecular Plant Biology, Norwegian Institute of Bioeconomy Research (NIBIO), Ås, 1433, Norway
[3] Dept. of Plant Biology and Soil Sciences, Faculty of Biology, University of Vigo, 36310, Spain
[4] Dept. of Plant sciences, Faculty of Biosciences, Norwegian University of Life Sciences (NMBU), 36310, Spain
[5] Dept. of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801, Portugal

marcos.viejo@usc.es

Our group is devoted to the development of protocols and applications of somatic embryogenesis (SE) in two woody species, grapevine (*Vitis vinifera*) and Norway spruce (*Picea abies*). In grapevine we have available SE protocols for several cultivars native to Galicia and we are currently studying the genetic, epigenetic and molecular determinants that connect the developmental stage of floral explants to their embryogenic competence. The aim is to develop efficient ways to induce SE in a genotype and tissue-independent manner to avoid the seasonality of floral explants. The information gained will permit to isolate natural somatic mutants, which is the main source of variability in this species. Besides, the optimisation of SE is an essential prerequisite for the application of new breeding techniques such as genome editing. This technology will make it possible to obtain new varieties and help to overcome current challenges such as disease, pest resistance or climate change.
In Norway spruce we study the epigenetic memory mechanism that impacts the timing of bud burst and bud set in a life-lasting manner in plants generated at contrasting temperatures (epitypes) during SE. We have recently found out that the epigenetic memory is laid down in the embryos as specific DNA methylation patterns in genes related to the plant's bud phenology. This novel knowledge, potentially shared by other species, will be of application in breeding programs involving plant production through SE.

In vitro cloning of a monumental eucalyptus tree

Saleta Rico, Nieves Vidal, Purificación Covelo, Jesús Mª Vielba, Conchi Sánchez

Dept. of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain

saleta@mbg.csic.es

Eucalyptus species are widely used in agroforestry systems as they have economic and commercial value due to great demand of eucalyptus-derived products. Micropropagation techniques have an enormous potential for mass cloning as well as for *ex situ* preservation of elite genotypes. However, eucalyptus are recalcitrant to *in vitro* culture, especially for regeneration and root induction, with a morphogenetic response highly dependent on the genotype and age of the donor plant.
The aim of this study was to develop a protocol for micropropagation and *ex situ* conservation of standing adult eucalyptus trees. Nodal segments from crown branches of a monumental *Eucalyptus globulus* tree* were used as initial explants and cultured in Murashige and Skoog medium (MS).
At the initial step, there was a 20% of contamination and 10% of non-contaminated explants were successfully established *in vitro*. Established shoot cultures exhibited good proliferation rates in MS medium supplemented with 0.4 mg/L of metatopoline. Adventitious roots were induced with indole-3-butyric acid (IBA) by combining different IBA concentrations and application periods. The highest rooting percentage was 54% and root number ranged from 1-2 roots per shoot.
Our protocol allowed us to obtain a moderate percentage of rooted plantlets by using two steps of IBA treatment, by decreasing the IBA concentration in the second step.

*https://www.monumentaltrees.com/en/esp/galicia/lalin/8945_soutolongo/

Funding: This work was funded by Xunta de Galicia (Spain) through the projects IN607A/2021 and PIE 202140E015.

In vitro shoot organogenesis and somatic embryogenesis represent the main bottleneck to genetic engineering for European hazelnut

Andrea Ferrucci[1], Valerio Cristofori[1], Vera Pavese[2], Cristian Silvestri[1]

[1] Department of Agriculture and Forest Science (DAFNE), University of Tuscia, Via San Camillo De Lellis, s.n.c., 01100 Viterbo, Italy
[2] Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini, 2, Grugliasco, 10095 Torino, Italy

andrea.ferrucci@unitus.it

In the last few years European hazelnut (*Corylus avellana* L.) cultivation area has soared reaching regions which were traditionally characterized by a sporadic presence of this species, posing the need to obtain new varieties with an adaptive attitude to a wide range of environments. Hazelnut breeding programs were based on crossings and selection, a technique that shows several limits when applied to this highly heterozygous species, also characterized by a long juvenile phase. New plant breeding techniques, based on genetic engineering, could represent a valid alternative for the rapid obtainment of new varieties, enabling the introduction of only desired traits. Although the application on hazelnut of these methodologies is feasible, the definition of suitable protocols for the adventitious shoot organogenesis and somatic embryogenesis is essential. To date, somatic embryogenesis in hazelnut has only been reported from seed-derived tissues, while only one protocol has been published for adventitious organogenesis from *in vitro* rejuvenated mature tissues. The possibility to genetically engineer and then regenerate somatic tissues would allow the retainment of genetic basis of a valuable cultivar. This contribution aims to shed light on what has been done by now and what we are doing to overcome this bottleneck to the application of new plant breeding techniques on this species.

Can led light systems be a new toolbox for alleviating rooting recalcitrance problems and simplifying the acclimatization phase?

Michela Lupo, Andrea Limitone, Valerio Cristofori, Cristian Silvestri

[1] Department of Agriculture and Forest Science (DAFNE), University of Tuscia, Via San Camillo De Lellis, s.n.c., 01100 Viterbo, Italy

michela.lupo@unitus.it

Rooting and acclimatization phases are a bottleneck for micropropagation of many woody trees, in particular for the recalcitrant ones. LED light systems became very popular in plant tissue culture both for their economicity, and for the possibility of modulating their light spectra. Moreover, the spreading of epigenetic is trying to answer the increasing interest in studying how different light spectra affect the activation or repression of key genes involved in different process, including rooting. Several studies show that blue light and red light stimulates rapid root induction, increasing the expression levels of auxins synthesis-related genes. Considering the important activity of LED light in activating auxins signaling and production, is it possible to study protocols for easy- and difficult-to-root species and, furthermore, the rooting and acclimatization phases can be combined into a single process. Here we report some examples of our experiences of different woody species like mulberry, pear, blueberry, hazelnut, wolfberry and kiwifruit and olive. Among these different species we encountered many differences in the response to the different LED light systems that is strictly dependent on species and genotype. Future studies will be conducted to gain a deeper understanding of the different molecular and physiological mechanisms that are triggered by the use of LED light systems so that the wavelengths can be modulated in a species-specific manner and in precise relation to the *ex vitro* rooting phase.

Solving hyperhydricity in *Tabebuia guyacan* by means of lignin precursor p-coumaric acid

Maroua Grira[1], Romain Martin[2], Stefaan P.O. Werbrouck[1]

[1] Laboratory for Applied In Vitro Plant Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[2] Green Lab SARL, Panama

grira.maroua@gmail.com

Precious wood trees are highly sought after for their high-quality wood. This has been made from *Tabebuia guyacan*, an endangered species. In vitro propagation can help to mass propagate this tree, but problems during in vitro culture, such as hyperhydricity, are responsible for a large percentage of loss during acclimatization. P-coumaric acid, a lignin precursor, has been reported to resolve hyperhydricity in some cultures by increasing lignin concentrations and decreasing intercellular water. Exogenous p-coumaric acid was applied at various concentrations during hyperhydric culture conditions. The addition of this lignin precursor had a positive effect: leaves and stems were able to recover and regain a normal shape similar to that of non-hyperhydric plants. As hypothesized, the lignin concentration was also affected by the addition of p-coumaric acid.

"Customized medium": how to overcome the in vitro recalcitrance of Sea buckthorn (*Hippophae rhamnoides* L.)

Giuseppe Vaia, Valerio Cristofori, Cristian Silvestri

[1] Department of Agriculture and Forest Science (DAFNE), University of Tuscia, Via San Camillo De Lellis, s.n.c., 01100 Viterbo, Italy

giuseppe.vaia@unitus.it

The inability of plant cells and tissues to respond to tissue culture is known as recalcitrance. Plant tissues with high phenolic content or able to release phenols, early *in vitro* recalcitrance is frequently caused by the oxidation of the explant on culture media. Especially woody plants contain large quantities of phenols related to the secondary thickening and lignification and, for this reason, micropropagation of these species is still problematic because sub-optimal culture media can accentuate these recalcitrance phenomena, resulting in stunted growth and necrosis. Anyway, the design of plant tissue culture media is a complicated task due to the interaction of many factors. An example of a species recalcitrant to micropropagation and in general, tissue cultures *in vitro*, is Sea buckthorn (*Hippophae rhamnoides* L.), a spiny deciduous shrub widely cultivated in Asia and recently in Europe for its berries, very rich in bio-active compounds with biological and therapeutic activities including antioxidant, anti-inflammatory, antibacterial and antineoplastic properties. The aim of our activities is to develop an accurate, efficient and reliable multiplication medium, trying to compare data from analysis of the main macro- and microelements found in leaves and seeds of sea buckthorn, with those of other species well adapted to well-known media such as MS, DKW and WPM. In addition, our experiments take into account other important aspects such as the types of cytokinins and agars used. These tests are accompanied by the quantification of the phenols content, used as a stress marker, in order to determine the growth conditions able to reduce the recalcitrance of these species to adapt to the in vitro conditions.

Identification and evaluation of natural germplasm to solve the problem of drought stress

Hojjat Ataee [1,2]*, Mahdi Alizadeh[1], Kourosh Vahdati[2], Saadat Sarikhani[2]

[1]Department of Horticulture, Gorgan University of Agricultural Sciences & Natural Resources, Iran
[2]Department of Horticulture, College of Abouraihan, University of Tehran, Tehran, Iran

hojjatataee89@gmail.com

Walnut with the scientific name *Juglans regia* L. is one of the most important fruits of temperate regions. Iran is one of the most important centers of walnut production and diversity in the world. Despite the great importance of walnuts, in most countries they have faced an important challenge in terms of producing this important and valuable product. One of the biggest challenges is drought and dehydration. In many regions of Iran, drought is the biggest factor limiting the production of agricultural products, especially walnuts, and it directly causes heavy damage to the country's walnut production every year. In addition to the direct damage of drought on walnut production, the secondary damages of this stress, including the spread of pests and diseases, in turn increase the severity of drought damage and have caused this stress to be considered as one of the important problems of the world's fruit industry. Identifying, protecting and using genetic resources as one of the most valuable national treasures of any country is of particular importance and considering the existence of a very large and diverse walnut germplasm in the country, the first necessary step in breeding programs is to identify superior genotypes. The evaluation of the available walnut germplasm in the country, while providing the possibility of obtaining a number of superior walnut genotypes in terms of important breeding traits as well as resistance to abiotic stresses, including tolerance to drought stress, provides the conditions for preparing a gene bank of desirable genes in walnut breeding programs. It will clear the way for the next reform programs.

Recalcitrance – a challenge in woody plants

Vladislava Galović, Marko Kebert, Saša Orlović

University of Novi Sad, Institute of Lowland Forestry and Environment, Novi Sad, Serbia

vladislava.galovic@gmail.com

Specificity of woody plant species are perennial nature implicating long generation time, the presence of seasonal dormancy, often huge genomes, dioecy and ploidy. In dioecious plants, the sex-determining mechanism may also be disrupted by polyploidization, with the potential evolution of hermaphroditism, highly heterozygous has its limitations for conventional breeding. Mitigating various ecological influences in the era of escalating climate changes urges plant scientists to implement state of the art, easy to handle and efficient plant transformation methods with reliable *in vitro* regeneration protocol at hand.

Unlike many other trees, species within genus *Populus* (including aspen and cottonwood) can be regenerated *in vitro* via direct organogenesis (as opposed to embryogenesis), its small genome and fully sequenced make poplar genome a model plant in molecular research. Although species within the genus *Populus* are, in general, easier to transform and regenerate *in vitro* than most other trees, many poplar species are still recalcitrant. Many protocols that previously have been reported were developed for a specific genotype or species. Thus, it has often been necessary to re-optimize a protocol each time research is initiated with a new genotype.

One of the biggest bottlenecks in the creation of transgenic plants is tissue culture. It could be performed in handful of species and the regeneration should last up to several months and often the side effects are unpredictable changes to genomes. Most of the forest species including poplar are recalcitrant and hard to transform.

New solutions to overcome tissue culture bottleneck in forest trees have been performed. Maher et al. 2019 suggests developmental regulators (DRs) in *A. thaliana* like Wuschel (WUS), Shoot meristemless (STM) and Monopteros (MP). DRs works in conjugation with plant regulators (cytokinin and auxin) to establish and maintain meristem identity. Expression of DRs and gene editing reagent creates transgenic and GE shoots by de novo meristem induction where these shoots produce flowers and seeds and is inherited. This system is used in some dicots and also monocots but never tried in forest species.

Being involved in COST Action CA21157 COPYTREE it will be possible networking between scientists to improve knowledge in implementation of new plant production methods, especially in forestry, where recalcitrance is expressed in different areas of production and conservation of plant material.

Key words: poplar, tissue culture bottleneck, recalcitrance

The author acknowledges the opportunity to be financed by COST Action CA21157 COPYTREE and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No: 451-03-68/2022-14/ 200197).

Keynote Conference WG2

Establishment and management of cryobanks, with a particular emphasis on plants *in vitro*

Manuela Nagel

Genebank department, Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, OT Gatersleben 06466 Seeland, Germany

nagel@ipk-gatersleben.de

To protect plant genetic resources (PGR), conservation of original habitats has been complemented by *ex situ* conservation of PGR. In the case of clonal plants, conservation of plants in field genebanks, slow-growth *in vitro* storage or cryopreservation enables the conservation of specific genetic backgrounds.
For the long-term preservation, more than 17 cryobanks have been established since the 1990s, storing more than 15,000 accessions worldwide, mainly potatoes, bananas and apples. Depending on the material, different cryopreservation protocols are used. For elms, mulberries or apples, dormant buds are usually preserved, often collected as twigs from the field in the middle of winter. Then the twigs are dehydrated, slowly cooled to -30°C and immersed in liquid nitrogen for further storage. For shoot tips of potatoes, garlic, cassava, strawberries, but also citrus fruits, effective vitrification methods have been developed using Plant Vitrification Solution 2 (PVS2) or PVS3 as cryoprotectors. This often involves excising *in vitro* plants, pre-culturing them on a sucrose medium and treating them with concentrated liquids and cryoprotectants, resulting in vitrification of the cells during immersion in liquid nitrogen. The shoot tips are then stored in liquid nitrogen. However, depending on the purpose of the cryobank and the plant material, different cryopreservation approaches with different pre-treatments of *in vitro* plants can be chosen and have an influence on the management of the cryobank.

Oral WG2-1

Use of high-throughput sequencing for virus diagnosis and plant tissue culture sanitation in Norway

Zhibo Hamborg, Dag-Ragnar Blystad

Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research, Ås, Norway

zhibo.hamborg@nibio.no

High throughput sequencing (HTS), also called next-generation sequencing (NGS), has dramatically raising interest in the field of plant tissue culture sanitation, virus diagnostics and plant health in Norway. 16S ribosomal RNA sequencing with Illuminia MiSeq has been applied to study bacterial endophytes' communities on begonia tissue culture by culture-independent methods. Our findings indicated that the 'axenic' concept cannot be applied to begonia tissue cultures due to a high diversity of bacterial endophytes identified. In addition, the diversity of the bacterial communities inside the plant tissues were highly genotype dependent on the hosts. Different samples of important or developing economical crops, such as potato, shallot, garlic, strawberry, and raspberry have been tested by HTS during the last years. Either symptomatic plants or nuclear mother stocks have been deep sequenced with Illumina HiSeq 2500 with outsourcing service for different project purposes. All these results have been implemented with virus diagnostic, virus elimination process and plant health certification in Norway.

Oral WG2-2

Assessment of ONT sequencing (MinION) for plant virus detection and comparison with Illumina-based sequencing

Elien Guldentops[1,2], Maaike Heyneman[1], Kris De Jonghe[1], Stefaan P.O. Werbrouck[2]

[1] Flanders Research Institute for Agricultural Fisheries and Food (ILVO), Plant Sciences Unit, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium
[2] Laboratory of Applied In Vitro Plant Biotechnology, Dept. Plant & Crops, Faculty of Bioscience Engineering, University Ghent, Belgium

elien.guldentops@ilvo.vlaanderen.be

To minimize the risks associated with virus transmission through vegetative propagation the use of virus-tested propagation materials is strongly encouraged. HTS-based untargeted detection replacing specific molecular methods, or even biological indexing, is extremely promising for this purpose: the complete virome can be assessed without the need for prior knowledge. Illumina sequencing has already proven to be a reliable and sensitive method for virus detection, yet also the ONT technology has gained interest as a promising tool over the last years. Nanopore sequencing generates long reads, can be performed in every lab, decreases the costs, and improves accuracy. For MinION sequencing a protocol based on Liefting et al. (2021) was used. In short, total RNA extraction was followed by rRNA depletion and random primed double stranded cDNA synthesis. Barcodes and adapters were added and samples were sequenced for 72 hours on a MinION Flow Cell. Reads were classified by comparing against the Genbank non-redundant nucleotide database with Kraken2 and mapped to virus reference genomes with Minimap2. Five plant samples previously screened with Illumina sequencing, infected with multiple viruses/viroids from different genera, were selected for this study. Overall, most of the viruses could be detected and were identified correctly with this protocol. Viruses detected below 50 rpm with Illumina sequencing could only be detected with poor genome coverage or failed to be detected. It can be concluded that correct virus detection and identification is possible with this MinION protocol but sensitivity is still lower.

Oral WG2-3

New trends in hazelnut micropropagation, organogenesis and micrografting: techniques, applications and future aspects

Doaa Elazab [1,2], Maurizio Lambardi[1]

[1] IBE/Institute of BioEconomy, National Research Council (CNR), 50019 Sesto Fiorentino, Florence, Italy
[2] Department of Pomology, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

doaa.elkassas@agr.au.edu.eg

Hazelnut is the fourth most valuable nut crop in terms of economic importance after cashew, almond, and walnut. Hazelnut, one of the tree nuts, is important for human nutrition and health. *Corylus colurna* is today considered an interesting rootstock for hazelnut productive orchards. The optimization of the various *in vitro* and *ex vitro* steps of *C. colurna*, from the mother plant selection for the collection of initial explants to the multiplication *in vitro*, rooting and acclimatization in greenhouse, up to the evaluation of their good adaptation to the graft and valuable characteristics induced on *C. avellana* cultivars, can be pursued to micropropagate selected rootstocks of *C. colurna*. To achieve this, we explored firstly various methods of *in vitro* introduction stage, which is the most difficult stage on hazelnut due to the huge amounts of phenols and contamination. Among them, the very innovative use of silicon to cover the cut ends of uni-nodal segments had a very positive effect in blocking phenol release. Moreover, a regeneration procedure from organogenesis, which is innovative for *C. colurna*, should be considered very interesting and promising to overcome the high problematics of contamination when introducing *in vitro* explants from mother plants in the field, as a minimum part of tissue is required to initiate the development of shoots. Different culture factors from *C. colurna in vitro* shoot culture lines were tested for inducing organogenesis, i.e., (1) entire or portion of leaves were put in culture in adaxial or abaxial position, (2) basal portion of the leaf, attached to a stem, and (3) different media (Simola and MS**)** and combinations of growth regulators. Moreover, micrografting methods were applied for the first time in hazelnut to assess the compatibility between *C. avellana* and *C. colurna* and produce disease-free plants. The micropropagated selected lines are presently under testing for conservation in slow growth storage and cryopreservation.

Oral WG2-4

Development of biotechnological tools for *Castanea sativa* Mill. breeding

Vera Pavese[1], Andrea Moglia[1], Paolo Gonthier[1], Elena Corredoira[2], Mª Teresa Martínez[2], Daniela Torello Marinoni[1], Roberto Botta[1]

1 Dipartimento di Scienze Agrarie, Forestali e Alimentari—DISAFA, Università degli Studi di Torino, Largo Paolo Braccini 2, Grugliasco, 10095 Torino, Italia
2 Misión Biológica de Galicia, Sede en Santiago, Consejo Superior de Investigaciones Científicas, Avd. Vigo, s/n, 15705 Santiago de Compostela, Spain

vera.pavese@unito.it

European chestnut (*Castanea sativa* Mill.) is a tree species of high economic interest, cultivated for its excellent fruit and timber quality. This species is affected by two diseases that affect its survival: ink disease, caused by the oomycete *Phytophthora* spp. and chestnut blight caused by the fungus *Cryphonectria parasitica.*
For woody species, traditional breeding is not a viable strategy for obtaining improved plants in a short time due to the long juvenile stage and the high heterozygosity. For these reasons, among new breeding technologies, CRISPR/Cas9 represents an innovative technique that allows target mutations, enabling the complete deactivation of the gene of interest. Since nowadays, the main focus is to improve plant tolerance to pathogens, here we present the study of Susceptibility (S) genes, which are genes responsible for host-pathogen recognition and whose silencing causes disruption of compatibility and ensures greater plant tolerance. Therefore in *C. sativa* (susceptible) and *C. crenata* (tolerant) species were evaluated the expression of S genes after pathogen inoculation. Among the S genes studied, the *pmr4* gene was found to be highly expressed in the susceptible species, confirming its potential in increasing chestnut tolerance. Since biotechnological resources on chestnut were limited, the CRISPR/Cas9 technology was successfully applied for the first time in chestnut, using the *phytoene desaturase* (*pds*) gene, a marker gene involved in chlorophyll biosynthesis, whose mutation causes the appearance of an albino phenotype. Subsequently, the CRISPR/Cas9:*pds* system was also transferred as a ribonucleoprotein to chestnut protoplasts in order to obtain transgene-free plants.
The CRISPR/Cas9 transformation protocol will be further applied for the knock-out of the *pmr4* candidate gene.

Oral WG2-5

Development of an efficient cryopreservation protocol for a Georgian provenance of *Castanea sativa* (Mill.) embryonic axes

Mariam Gaidamashvili, Tamari Kutchava, Eka Khurtsidze

Dept. of Biology, Faculty of Exact and Natural Sciences, Iv. Javakhishvili Tbilisi State University, 1, Chavchavadze Ave., 0179 Tbilisi, Georgia

mariam.gaidamashvili@tsu.ge

Sweet chestnut (*Castanea sativa* Mill.) is the dominant species in the mountainous forests of Western Georgia occupying most areas covered with forests. *Castanea sativa* is included in the 'Red List' of Georgia under state Vulnerable (VU) and has been subjected to conservation measures in response to the Global Strategy for Plant Conservation. Rapidly developing *in vitro* techniques open new possibilities for the safe *ex-situ* conservation of chestnut germplasm. In the present study optimized cryopreservation protocols based on dehydration, PVS2-vitrification, and the encapsulation–vitrification techniques followed by 'one-step freezing' in liquid nitrogen have been developed for embryonic axes (EAs) of *Castanea sativa*. Survival and plantlet regrowth in post-cryopreservation of EAs were evaluated. Our research demonstrated the feasibility of the long-term preservation of embryonic axes using different techniques. All techniques resulted in inducing specimen tolerance to ultra-rapid freezing, although to a different extent. The germination rate of the cryo-stored embryonic axes was 66.7% after 5h of specimen dehydration (reducing the initial moisture content to 21%). Pre-treatment of embryonic axes in PVS2 vitrification solution for 30 min produced 55.6% fully developed plantlets in post-cryopreservation. An improved cryopreservation protocol has been developed by incorporating 0.3% (w/v) activated charcoal into alginate beads in the 'encapsulation–vitrification' technique. Cryopreserved embryonic axes showed 70% embryo survival and 64% recovery into the whole plants which was sufficiently higher than those without activated charcoal. The valid protocol developed for the Georgian provenance of *Castanea sativa* can now be tested on a wide range of chestnut cultivars and hybrid clones to achieve the practical long-term cryopreservation of *Castanea* genus germplasm. The advantages of the utilization of zygotic embryos for safe and effective long-term conservation of threatened hardwoods concerning the post-cryopreservation recovery of explants will be discussed.

Oral WG2-6

The use of cryopreservation for safe storage and sanitation of fruit plant germplasm

Alois Bilavcik[1], Stacy Denise Hammond Hammond[1], Olena Bobrova[1,2], Milos Faltus[1], Jiri Zamecník[1], Igor Koloniuk[3], Jana Franova[3]

[1] Plant Physiology and Cryobiology, Crop Research Institute, Drnovska 507, 16106 Prague 6, Czech Republic
[2] Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine
[3] Biology Centre CAS, Ceske Budejovice, Czech Republic

bilavcik@vurv.cz

In the context of global climate change, there are currently changes in the economic ties and needs of society, as well as changes in the approach to producing and protecting economically important tree species. Thus, the safe conservation of a broad spectrum of fruit tree species is becoming very important. Different techniques for *in vitro* and dormant bud cryoconservation will be presented. The vitrification and encapsulation-dehydration techniques for *in vitro* cultures and two-step cryopreservation for dormant buds are the main procedures used for the long-term conservation of important vegetatively propagated plants in the Crop Research Institute, Prague. In collaboration with the Biology Centre, Czech Academy of Science, Ceske Budejovice, cryopreservation began to be used as a "cryo-knife", a tool for sanitizing raspberry from viruses. The use of a temporary immersion system in the *in vitro* cultivation of fruit trees will also be presented. Newly developed procedures in the field of biotechnology for propagation and safe preservation using cryopreservation techniques will contribute to solving the challenges arising from the COPYTREE project.

This work was partly supported by the project NOBERRYVIRUSCZ funded by grant from Iceland, Liechtenstein and Norway through the EEA Grants and the Technology Agency of the Czech Republic (TO01000295).

Oral WG2-7

Recent advances in alternative conservation methods for radiata pine somatic embryogenesis

Itziar A. Montalbán, Ander Castander-Olarieta, Paloma Moncaleán

Neiker-BRTA, Centro de Arkaute, Apdo 46, 01080 Vitoria-Gasteiz, Spain

imontalban@neiker.eus

Cryopreservation of embryogenic tissues is an essential requirement to maintain the competence of embryogenic cell lines and to obtain plants from them years later. This method can be used to conserve especially valuable genetic resources and together with efficient somatic embryogenesis protocols, it has allowed the implementation of multi-varietal forestry. However, cryopreservation may not be an option for small research groups or developing countries due to liquid nitrogen cost. So, we have sought for alternative options for different materials for the somatic embryogenesis process in *Pinus radiata*.

In this sense, we successfully conserved at 4°C green cones for embryogenic tissue induction for up to four months without detriment in induction rates. We have also stored somatic embryos at 4°C in different media for 3, 6 and 9 months and successfully obtained plant conversion.

As an alternative to cryopreservation of embryogenic cell lines, we have developed a method to preserve them at -80°C; although this methodology still needs further research, it has been possible to regenerate plants from cell lines stored at -80°C for four years. Currently, we are studying the preservation of somatic embryos at -80°C combining somatic embryogenesis to regenerate these materials, with organogenesis protocols to increase the efficiency of the propagation process.

Micropropagation and *in vitro* conservation of narrow-leaved ash in Croatia

Sanja Bogunović, Miran Lanšćak, Zvonimir Vujnović, Nevenka Ćelepirovć, Mladen Ivanković

Croatian Forest Research Institute, Cvjetno naselje 41, Jastrebarsko, Croatia

sanjam@sumins.hr

Narrow-leaved ash (*Fraxinus angustifolia* Vahl.) is one of the most important tree species in the lowland floodplain forests of the Republic of Croatia. In recent years, increased dieback of narrow-leaved ash has been noticed in Europe, but also in the entire territory of the Republic of Croatia. Tree dieback in forest stands of all ages in some localities creates major environmental and economic problems and classifies this species as one of the most endangered at the moment. Climate change and groundwater and flood disturbance have led to physiological weakening of narrow-leaved ash. After the appearance of the pathogenic fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya in interaction with other pathogens, rapid dieback of this tree species has occurred. The aim of this research was to develop protocol for micropropagation of narrow-leaved ash and conservation of potentially resistant individuals *in vitro*. This would enable fast and successful propagation of the most resistant and high-quality narrow-leaved ash trees, which would contribute to the conservation of this species due to climate change and attacks of pathogens and pests. Some potentially resistant trees have been found in forest stands and they served as a source of plant material for micropropagation. Several protocols have been tested and some of them have shown promising results. The resistance of cloned seedlings will be tested in the continuation of the research. The developed technology may be used for mass propagation of common ash superior trees.

The need for establishing the long-term conservation strategies of important autochthonous plant germplasm in Albania, helped by CopyTree experience

Efigjeni Kongjika[1], ValbonaSota[2]

[1] Biotechnology & Genetics Scientific Research Unit, Section of Natural and Technical Sciences, Academy of Sciences of Albania
[2] Department of Biotechnology, Faculty of Natural Sciences, University of Tirana

kongjikaef@yahoo.com

The Albanian researchers working on *in vitro* cultures have 25 years of experience in micropropagation and *in vitro* conservation of autochthonous of some spontaneous species (endemic, endangered, and with economic values) using slow growth technologies for short- or mid-term conservation. Albania is considered a rich area in fruit species, with about 70 species and subspecies, with over 1200 forms and populations of native fruit trees and shrubs. However, a biodiversity regression is observed due to climate and socio-economic changes, which results in a risk of losing wild gene pools with beneficial characteristics of resistance to biotic and abiotic stresses. Preserving plant genetic resources under *in vitro* conditions is among the priority issues. Cryopreservation is a reality that effectively overcomes the complications presented by traditional preservation in seed banks and field collections through minimal growth *in vitro* techniques. The Academy of Sciences of Albania (ASA) has granted "CRYOFRUIT" project intending to create the first Albanian Plant Cryobank (ASA's Cryobank), which will act as a service for public and private institutions to preserve and use a modern approach to the propagation of fruit tree species with high dietary and therapeutic characteristics for farmers. The genetic material is stored as synthetic seeds for the sanitized plum using meristem culture and certified by phytosanitary and genetic testing through the "VITROCERT" project. ASA has founded national networks working on Plant Biotechnology. The Plant Tissue Culture Laboratory in the Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, plays the central role in realizing this project. The experience of the Institute of BioEconomy-CNR, Florence, Italy, for cryopreservation has helped the conception of the new project. Including Albanian researchers in the CopyTree Program will create conditions for strongly cooperating internationally with scientific research centers and universities working on plant tissue conservation by modern techniques.

Potassium dichromate improves morphometric and biochemical characteristics of paulownia plantlets due to induction of mild oxidative stress

Oksana V. Pasat[1,2], Volodymyr I. Lushchak[1,2]

[1] Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenko Str, Ivano-Frankivsk, 76018, Ukraine
[2] Research and Development University, 13a Shota Rustaveli Str, Ivano-Frankivsk, 76018, Ukraine

volodymyr.lushchak@pnu.edu.ua

Paulownia is a deciduous tree with high growth potential that can grow on polluted soils. Ukraine has a lot of highly polluted lands of technogenic and war origin. We set up a laboratory for plant micropropagation, collected several Paulownia genotypes available in Ukraine, grew them in our home garden and selected the most frost-resistant clones. They were micropropagated and planted on more than 5 hectares of black soil fields. Most of the trees are growing well, but obtaining logs is problematic due to freezing of the tops and winter scarring. Chromium is one of the most common pollutants. Due to its ability to change its valence state, it participates in redox processes and may be involved in the generation of reactive oxygen species. The toxicity of chromium compounds depends on their valence state. In the Ivano-Frankivsk region of Western Ukraine, there are several sites with high chromium contamination. Therefore, we selected potassium dichromate as a model pollutant to test its effects on the growth, morphometric and biochemical characteristics of selected Paulownia plantlets. The plantlets were incubated hydroponically for 3, 7 and 14 days in the presence of potassium dichromate at different concentrations. Dichromate improved morphometric and biochemical characteristics such as increased levels of starch, chlorophylls, carotenoids, and anthocyanins. It also induced a mild oxidative stress, which could have hormetic effects and improve the growth of the plantlets.

Application of cryopreservation for the conservation of plant genetic resources and stock cultures, virus eradication and tool for breeding

Bart Panis [1,2]

[1] Alliance of Bioversity International and CIAT, Willem de Croylaan 42 bus 2455, 3001 Leuven, Belgium
[2] Dept. Biosystems, KU Leuven, Willem de Croylaan 42 bus 2455, 3001 Leuven, Belgium

b.panis@cgiar.org

Plant cryopreservation is increasingly becoming a widely accepted and applied method for the long-term conservation of plant genetic resources that cannot be conserved through seeds. This is caused by the fact that efficient and reliable cryopreservation protocols, applicable to a wide variety of species and cultivars such as droplet vitrification and plate cryopreservation methods became available. Currently, between 20,000 and 25,000 accessions are safely preserved in liquid nitrogen and more initiatives to increase these numbers are in the pipeline. Crops with more than 1000 accessions cryopreserved are apple, banana, mulberry, cassava, garlic and potato. It is estimated that worldwide between 100,000 and 150,000 unique accessions of vegetatively propagated and recalcitrant seed crops are currently held in field and *in vitro* and genebanks. A global initiative is thus needed to make sure that all these accessions are safely maintained for next generations. Like the Svalbard global seed vault that is storing almost one million seed samples as backup for national and international seed banks, a safety cryopreservation back up facility should be established.

The effects of diverse pre-treatment conditions on metabolism of grey poplar explants used for cryopreservation

Eva Pokorná[1], Martina Komárková[1], Pavlína Máchová[1], Veronika Zemanová[2], Jozef Lacek[3], Miloš Faltus[4]

1 Dept. of Forest Tree Species Biology and Breeding, Forestry and Game Management Research Institute, Strnady 136, 252 02 Jíloviště, Czech Republic
2 Dept. of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Prague 6, Czech Republic
3 Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 165 02 Prague 6, Czech Republic
4 Dept. of Genetics and Plant Breeding, Crop Research Institute, Drnovská 507, 161 06 Prague, Czech Republic

pokorna@vulhm.cz

Successful cryopreservation method of forest tree species depends on many factors including good physiological state and health of plants as well. In our study, we selected a grey poplar (*Populus* × *canescens* Aiton Sm.) explants as a model tree species for its fast growth and easily micropropagation. To characterize metabolism of grey poplar explants during diverse pretreatment conditions (*Treatment A – C*) we followed an endogenous levels of plant hormones and free amino acids with relative gene expression patterns. Grey poplar explants of *Treatment A* were cultivated in control conditions (24ºC, 16 h photoperiod and an irradiance of 30 µmol m^{-2} s^{-1}), explants of *Treatment B* and *Treatment C* were cold hardened at 6ºC for four weeks under the same conditions whereas last two weeks of cultivation was added 0.7 M sucrose into the explants (*Treatment C*) to reach 0.3 M concentration of sucrose in both, medium and solution.
Our results showed that *Treatment C* strongly reduced in grey poplar explants auxin and cytokinin levels, phytohormones with a key role during plant growth and development in comparison to other *Treatments (A* and *B)*. Similarly, we observed increase in total content of free amino acid levels in grey poplar explants cultivated as *Treatment C*. In addition, relative gene expression profiles of selected genes revealed that *Treatment C* downregulates genes involved in cell division cycle and phytosynthesis in contrast to upregulation of genes related with plant stress. Taken together, we showed that pretreatment conditions substantially affect metabolism in grey poplar explants whereas deciphering these changes in plant physiological processes enable us to improve cryopreservation process of forest tree species.

Use of cryopreservation for conservation and eradication of pathogens of *in vitro* cultures of date palm (*Phoenix dactylifera* L.)

Amal Rabaaoui[1], Radhouane Gdoura [2], Stefaan P.O. Werbrouck[1]

[1] Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[2] Laboratory of Toxicology-Microbiology and Environmental Health, Department of Biology, University Sfax, Sfax, Tunisia

amal.rabaaoui@ugent.be

The date palm (*Phoenix dactylifera* L.) is widely cultivated throughout North Africa. The preservation of date palm genetic resources has become an important topic. Safe storage of this germplasm and the eradication of pathogen can be achieved through the application of a wide variety of tissue culture techniques, among them cryopreservation. Juvenile leaves and immature inflorescences of date palm are characterized by an important reactivity under *in vitro* conditions. Indeed, tissue proliferation can be observed after only two weeks of *in vitro* cultivation. Using low concentration of 2,4-D, however, bud neo-formation and PEMs (proembryogenic masses) differentiation is very slow and might require more than one year. We studied the cryopreservation of these highly proliferating meristem cultures and proembryos using the ultra-rapid droplet freezing and the encapsulation-vitrification methods and three parameters have been studied: Survival rates, morphogenetic capacity and eradication of pathogens (*bacillus*).
These techniques, based on a high-speed cooling of the dehydrated meristems and proembryos, proved to be efficient. Survival rates of about 67% could be obtained for 'Deglet Nour' PEMs that were treated for 30 min with the PVS2 solution. Moreover, we observed that the cryogenic treatment does not affect the morphogenetic capacity of the tissues. They also proved to be effective for the eradication of bacteria already isolated from *in vitro* cultures of date palms and present eradication rates of about 37% of 'Deglet Nour' cultivar treated during the first 3 months to 65% after 6 months.

Phytosanitary approach to unique genotypes held by the national ampelographic collection of Portugal with the aim of providing clean materials

Jorge Sofia[1,2], Jorge Cunha[1], Margarida Teixeira Santos[3]

[1] Instituto Nacional de Investigação Agrária e Veterinária, I.P./INIAV-Dois Portos, 2565-191 Torres Vedras, Portugal
[2] Centro de Investigação CERNAS-IPV, Instituto Politécnico de Viseu, Campus Politécnico, Repeses, 3504-510 Viseu, Portugal
[3] Instituto Nacional de Investigação Agrária e Veterinária, I.P./INIAV-SAFSV, 2780-157 Oeiras, Portugal

jorge.sofia@iniav.pt

Minor grapevine cultivars (MGCs), also known as "underutilized" or "neglected" cultivars, are grape cultivars that have received relatively little attention in terms of research, production, and marketing. However, there is a growing interest in these MGCs among different stakeholders in the grape and wine industry.
The Portuguese ampelographic collection (Coleção Ampelográfica Nacional-CAN) is a *Vitis* germplasm bank, identified by the international code PRT051, with all the grapevine varieties authorized for Portuguese viticulture, including MGCs. It also serves as an important genetic resource for grapevine identity, breeding research and improvement, and it is the only place where many of the MCGs are preserved. However, the incidence of viruses, specially the regulated non quarantine pests, in some of these unique genotypes poses a threat to their genetic integrity, affecting their agronomic performance and commercial value and also their chance of being planted. Therefore, there is an interest in cleaning viruses from some MGCs' genotypes to recover the quality of these grapevine cultivars and to have them available to nurseries.
As viruses, Grapevine Trunk Diseases (GTDs) are considered a problem of installed, mature grapevines, but, it is our perception, after 30 years of research on the GTDs' subject, that this problem starts in the nursery, mainly due to poor quality of the propagation materials, its poor handling and above all, to the lack of awareness and information on new propagation technics and vegetative materials' maintenance.
It is our expectation, within this COPYTREE COST action, to come into contact with the latest techniques for cleaning viruses of grapevine propagating material and with the latest advances in the production process of woody propagating materials in order to transmit and try to implement it with the Portuguese nursery sector.

Cryopreservation of stone, pome and small fruit species in Serbia using vitrification-based techniques

Tatjana Vujovic, Tatjana Andjelic, Darko Jevremovic, Milena Djordjevic, Sanja Radicevic

Department of Fruit Physiology, Fruit Research Institute, 32000 Cacak, Serbia

tvujovic@institut-cacak.org

The use of vitrification-based cryopreservation techniques has extended the application of cryopreservation to a large number of plant species, including various fruit tree species. The present work describes the use of these techniques within the Department of Fruit Physiology of FRI, Čačak as part of the conservation strategy for temperate fruit genetic resources. Utilisation of vitrification technique for conservation of *Prunus* and *Malus* germplasm has shown that significant increase in regrowth of cryopreserved shoot tips can be achieved by altering the duration of dehydration and/or type of vitrification solution employed. Optimization of the droplet-vitrification protocol in representatives of *Prunus*, *Malus* and *Rubus* genera was conducted by evaluation of the effect of different vitrification solutions, treatment durations, temperature of dehydration and duration of unloading on recovery of cryopreserved explants. Vitrification methods using aluminium cryo-plates (both V and D cryo-plate methods) were also applied for cryopreservation of different autochthonous plums, cherry plum, cherry rootstocks, strawberry, blueberry and saskatoon berry. Additionally, these methods were evaluated for plum pox virus eradication from autochthonous plums (*P. domestica* L.) widely present under different local names on the Balkan Peninsula.

Effective increase of *in vitro* multiplication of different forest tree species for the purposes of cryopreservation

Martina Komárková, Eva Pokorná, Helena Cvrčková, Pavlína Máchová

Dept. of Forest Tree Species Biology and Breeding, Forestry and Game Management Research Institute, Strnady 136, Jíloviště, 25202, Czech Republic

komarkova@vulhm.cz

At the beginning of each experiment, it is necessary to have a large number of input samples. According to plant *in vitro* tests, mass multiplication of sufficient number of explants in short time is quite easy thanks to the ability to change the ratio of phytohormones in culture media. Such a need of rapid multiplication is important for example for the cryopreservation method. During this procedure, the explants are exposed to pregrowth, cryoprotection, freezing, thawing, and plant regeneration.
The aim of our work was to determine the most effective concentration of phytohormones to increase the multiplication efficiency of some Czech native forest tree species, *Populus canescens* (grey poplar), *Sorbus torminalis* (wild service tree), *Betula pubescens* (white birch), and *Tilia cordata* (linden). For this purpose, different culture media (MS medium, N6 medium) with the addition of cytokinins (BAP-benzylaminopurine) and auxins (IBA-Indole-3-Acetic Acid; NAA-α-Naphtaleneacetic Acid) were tested. Considerable clonal differences and changes in reproductive ability in response to different concentrations of cytokinins emerged during testing of *S. torminalis* and *B. pubescens*. For higher multiplication rate of *P. canescens* and *T. cordata*, lower concentrations of cytokinins were needed, compared to wild service tree and white birch.

Study of viral, bacterial and phytoplasma diseases in Georgia

Iveta Megrelishvili[1,2], Maia Kukhaleishvili[2], Zurab Khidesheli[1], Levan Ujmajurideze[1,] Nino Maziashvili [1]

[1] Scientific Research Center of Agriculture, Marshal Gelovani Ave. 6, 0159, Tbilisi, Georgia
[2] Georgian Technical University, Biotechnology Center, Kostava Str. 77, 0177, Tbilisi, Georgia

ivetameg@yahoo.com

The Scientific Research Center of Agriculture is a multi-disciplinary agronomy organization in Georgia, which works in horticulture, viticulture, winemaking, agroforestry and other filed. The main objective of this work is to study viral, phytoplasma, and bacterial diseases of plants in different regions of Georgia. The causative agents of these diseases are tested using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), the rapid one-step AgriStrip assay, molecular-TaqMan® triplex real-time PCR, and Loop-Mediated Isothermal Amplification (LAMP) methods.

Among the grapevine viral infections has determined that the highest prevalence is characterized by Grapevine Fleck Virus-19.26%,following Grapevine Fanleaf Virus-12.9% and Grapevine Leaf Roll Viruses. We have investigated of Grapevine flavescence dorée (FD) and Grapevine Bois Noir (BN) on 16 grapevine cultivars, including Georgian and foreign varieties. The high infection rate were revealed in grapevine cultivar "Chardonnay". First time in Georgia FD was identified using the TaqMan real-time qPCR method.

Plant virology laboratory works the production of healthy, virus free planting materials using apical meristem method in collaboration with Georgian Technical University, Biotechnology Center.

The Biotechnology Center has successfully developed the in vitro propagation of grapes, cherry rootstocks Gisela, walnuts and berries. The Biotechnology center is regularly working on modification of MS medium, improvement of in vitro plants growth and development conditions and development of modern seed production methods.

Thus, the scientific research of Scientific Research Center of Agriculture promotes to the production of healthy, virus-free planting material in Georgia, the integrated management of plant pests and, finally, the improvement of the phytosanitary situation in the country.

In vitro establishment and multiplication of *Cydonia oblonga* Mill. selected germplasm

Daniela Duarte[1], Alberto Cardoso[1], Ana Pedrosa[1], Elsa Baltazar[1], Tércia Lopes[1], Jorge Canhoto[1], Sandra Correia[1,2]

[1] Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2] InnovPlantProtect CoLAB, Estrada de Gil Vaz, 7350-999 Elvas, Portugal

daniela.d@sapo.pt

Quince (*Cydonia oblonga* Mill.) is mainly cultivated for fruit production in Portugal but is also used as rootstock for pear and apple. As climate changes are a concerning threat to agricultural systems and to biodiversity, it is important to find alternatives, and underutilized species such as C. *oblonga* have resilience traits important for crop breeding. Our work aimed to conserve selected germplasm of this species through *in vitro* establishment and micropropagation. Winter branches were collected from georeferenced trees in Cova da Beira region. Branch disinfection started with a commercial detergent wash followed by 70% ethanol. The branch segments were forced to sprout under controlled conditions (16h light photoperiod). Shoot tips were disinfected with fungicide, commercial bleach and a wash with 1% w/v ascorbic acid. The explants were then placed in MS basal medium with 0.2 mg/L of BAP (16h photoperiod, 24ºC) for one month. After 4 weeks, high contamination percentages were observed in most genotypes. However, 'Galega' variety genotypes were established *in vitro* with a survival rate of 4%. Surviving shoots were transferred to multiplication medium with the addition of GA, IBA and BAP. The results showed multiplication percentages of 53%. This approach allowed to successfully establish and multiply selected quince tree germplasm, relevant for ongoing conservation and breeding programs.

SSR marker-based analysis of clonally propagated quince tree (Cydonia oblonga Mill.) varieties

Ana Pedrosa[1], Alberto Caeiro[1], Tércia Lopes[1], Elsa Baltazar[1], Miguel Teixeira[2], Cláudia Rato[2], Jorge Canhoto[1], Sandra Correia [1,2]

[1] Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2] InnovPlantProtect CoLAB, Estrada de Gil Vaz, 7350-999 Elvas, Portugal

anasimoespedrosa@gmail.com

Quince tree (*Cydonia oblonga* Mill.) is a relevant medium-sized fruit tree of the Rosaceae family, produced mostly due to its fruits use in processed jams. Quince grafts resistance and durability are also significant traits that led to the species wide use as rootstock in closely related pome trees. Cultivar breeding by intraspecific crossing has been very scarce, and germplasm resources are barely phenotypically described. Associated with efficient clonal propagation systems, phenotypic and molecular analysis of *C. oblonga* germplasm is crucial for breeding and conservation purposes. Insights about the existent genetic diversity and its quality are crucial for an effective use of selected genetic resources in breeding programs. The main aim of this work was to evaluate the genetic diversity within clonally propagated quince cultivars using six SSR markers. A total of forty-three accessions collected from georeferenced orchards in Portugal Centre Region were analysed. Hierarchical and non-hierarchical analysis of polymorphic SSRs allowed evaluate genetic similarity between accessions. With low genetic diversity, the cultivars clustered into four major groups, with 'Galega' accessions being the most closely related and ‘Portugal’ the ones with higher variability. The results observed here provide a eliable methodology for further studies involving SSR-based analysis of clonally propagated quince trees with the goal of preserving and/or valuing selected genotypes.

Virus resistance gene transfer from tolerant walnut genotypes to virus-susceptible superior commercial walnut cultivars

Marzieh Shamshiri[1], Hojjat Ataee[2]

[1] Plant Pathology Department, Faculty of Agriculture, Tarbiat Modares University (TMU), Tehran, Iran
[2] Department of Horticulture, Gorgan University of Agricultural Sciences & Natural Resources, Iran

m.shamshiri.1992@gmail.com

Walnut is one of the most important nuts in the world. Several viruses including *Cherry leaf curl virus*, *Prunus necrotic ring spot virus* and *Tomato ring spot virus* have been reported from walnut trees in the world. Despite virus infection of different genotypes of Iranian walnut trees, the effect appears to be minimal compared with the serious virus damage reported by other investigators outside Iran. The purpose of our study is to evaluate the extent of virus infection in virus-tolerant Iranian walnut genotypes compared to the commercial walnut cultivars in different parts of the world, using total RNA analysis. We will use Chandler, Franquette, Fernor, Pedro, Hartley, sourced from overseas as they have been confirmed to be infected with the viruses, and investigate possible resistance pathways. We aim to identify genes involved in the resistance of walnut trees and to transfer them to susceptible cultivars.
The proposed research will include an initial evaluation and sampling of trees from known walnut cultivars that are symptomless and others showing virus symptoms. We will confirm the viral infection using serological and molecular methods, total RNA extraction from different samples and total RNA sequencing. Gene expression will be evaluated in resistant cultivars and the resistance genes in Iranian walnuts will be transferred to sensitive trees using cloning. Optimizing the tissue culture for the establishment and propagation of walnut will be required. The transgenic walnut plantlets with the resistance genes will be compared with virus tolerant Iranian walnuts. After being confirmed as more tolerant, the plants will be transferred in vitro to the pots for proliferation and rooting followed by transfer to the greenhouse for adaptation.

Keywords: walnut, virus, tolerance, total RNA sequencing

Microbiome diversity and composition of *Arbutus unedo* L. (strawberry tree) during *in vitro* and *ex vitro* growth: implications for clonal plant production

João Martins[1], Joana Costa[1,2], Jorge Canhoto[1]

[1] Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2] Laboratory for Phytopathology, Instituto Pedro Nunes, 3030-199 Coimbra, Portugal

joao.martins@uc.pt

Strawberry tree (*Arbutus unedo* L.) is a versatile tree belonging to the Ericaceae family. It has a widespread distribution throughout the Mediterranean region and can tolerate both biotic and abiotic stresses. The tree is economically valuable due to its bioactive compounds and edible berries, which has created a high demand for true-to-type plants. Micropropagation techniques are commonly used to produce strawberry tree clonal plants, making it crucial to characterize the tree's microbiome to ensure successful propagation and acclimatization.

This study aims to identify the endophytic communities present on the strawberry tree's *in vitro* plant tissues and investigate their prevalence and/or alteration upon plant acclimatization. A culture-independent method was used to identify the microbiome of two genotypes under micropropagation and *ex vitro* conditions. The bacterial OTUs were assigned to 7 phyla and 79 genera, with only one Archaea genus identified. The most abundant and diverse bacterial phylum was Actinobacteriota (48%), followed by Proteobacteria (43%), Firmicutes (6%), and Bacteroidota (3%).

Significant differences were observed in the composition and diversity of the microbiome when comparing *in vitro* genotypes. However, *ex vitro* samples had similar microbiome compositions. A higher diversity was found in both genotypes *ex vitro* compared to their respective *in vitro* plants. The results of this study are relevant to micropropagation as the genotype was found to be a crucial factor in shaping the microbiota structure and some bacteria that might influence propagation rates and recalcitrance were identified.

In vitro cultures of common ash (*Fraxinus excelsior* L.) in research on resistance against ash dieback: problems and possible solutions

Ben Bubner, Franziska Past

Thuenen-Institute of Forest Genetics, 15377 Waldsieversdorf, Eberswalder Chaussee 3a, Germany

ben.bubner@thuenen.de

Ash dieback caused by the ascomycete *Hymenoscyphus fraxineus* threatens the state of *Fraxinus excelsior* as a forest tree. Several research projects try to save ashes by selecting plus trees with tolerance against the pathogen that can be planted in seed plantations. Since resistance is heritable it is assumed that seedlings from plus trees will express increased resistance. In our laboratory we use *in vitro* cultures to propagate sterile micro cuttings. The aim is to produce ramets for use in infection experiments and as rootstocks for grafting scions from tolerant plus trees.
We show initial successes of *in vitro* propagation but also problems that are caused by poor internodal extension of the micro cuttings. Several methods that related to the sort of explant source have been tested to improve the performance of micro cuttings. For instance, shoots have been used that regenerated from source plants attacked by caterpillars of *Sphinx ligustri*. Despite unorthodox methods it remains difficult to establish ash *in vitro* cultures from old trees with known status of ash dieback tolerance. In our laboratory it was more promising to use *in-vitro* cultures established from seedlings that show recently satisfying reproduction rates. In this approach the resistance has to be tested after establishing the *in vitro* culture.

Keynote Conference WG3

Tissue culture for the 21st century forests

Jana Krajnakova[1], Cathie Reeves[1], Taryn Saggese[1], Cuong Kim Lee[2], Ulrika Egertsdotter[2], Cyrus Aidun[2], Sam Davidson[1], Tancred Frickey[1], Celine Mercier[1], Mikko Tikkinen[3], Tuija Aronen[3], Russell Burton[4]

[1] Scion, Titokorangi Drive, Private Bag 3020, Rotorua 3046, New Zealand
[2] Renewable Bioproducts Institute, Georgia Institute of Technology, Atlanta, GA 30332, USA
[3] Production systems, Natural Resources Institute Finland (Luke), Savonlinna, Finland
[4] Russell Burton, Forest Growers Research, Ltd., Rotorua, New Zealand

jana.krajnakova@scionresearch.com

The New Zealand Forest industry has made substantial advances over the last century in improvements to the growth rate, wood quality and resilience of radiata pine. Improvements through breeding are continuing and are of increasing importance in the face of climate change, new pests and pathogens, and rising expectations from growers and timber users. However, bottlenecks exist in deploying the best radiata pine genetics for all forest growers, mainly because of the lengthy process of trialling and releasing new genotypes.
The New Zealand Forest industry, through their research entity, Forest Growers Research Ltd., has been successful in obtaining funding for a 7 year, multi-million-dollar, sector-led partnership programme (Tissue culture techniques for 21st century forests). This programme aims to remove barriers and deliver a reliable and cost-effective tissue culture process through the automated multiplication and rapid production of small rooted plantlets, fully harnessing the genetic improvement from the radiata pine breeding and genomics programmes and making mainstream varietal forestry a reality.
The foundation of the project is *Pinus radiata* somatic embryogenesis. Scion, with involvement of collaborators from Georgia Institute of Technology (USA) and Natural Resources Institute (Finland), are focussing on these aims. Research progress using temporary immersion bioreactors, improvements of existing protocols, automated embryo management systems, and machine learning are discussed.

Oral WG3-1

Different proliferation techniques for scaling up Norway spruce somatic embryogenesis

Sakari Välimäki[1], Mikko Tikkinen[1], Teresa Hazubska-Przybył[2], Laura Paavilainen[1], Frida Salonen[1], Ewelina Ratajczak[2], Saila Varis[1], Tuija Aronen[1]

[1]Production Systems, Natural Resources Institute Finland (Luke), Vipusenkuja 5, 57200 Savonlinna, Finland
[2]Institute of Dendrology, Polish Academy of Sciences, Kórnik, Poland

sakari.valimaki@luke.fi

Somatic embryogenesis (SE) is the most efficient method for vegetative propagation of conifers, including Norway spruce. It can be used to propagate uniform regeneration material from elite parent trees. However, it is labor-intensive and requires scaling up for increased plant lots and decreased production costs. In this study, various SE proliferation techniques using semisolid or liquid media, Petri plates, and bioreactors were compared. The results are discussed in the context of the Finnish Norway spruce SE program, which involves testing thousands of genotypes and pilot propagations with 120 SE lines. Any propagation method needs evaluation using enough lines to enable selection based on field rather than laboratory performance. Semisolid media is commonly used for culturing embryogenic tissue, which is subcultured manually as clumps. Spreading the tissue on filter discs on semisolid media enhanced growth rate and slightly increased embryo yield. Suspension cultures provide a means to increase culture volume without increasing workload, but the switch from semisolid to liquid media required rinsing the tissue before maturation for satisfactory embryo yield. Maturation must still be carried on semisolid media to sustain embryo yield and quality. Although TIS bioreactors promise the benefits of both liquid media and solid support for maturation, they were laborious to handle, and the embryo yield or quality did not surpass that from semisolid plates.

Oral WG3-2

Use of liquid culture with the ElecTIS bioreactor for a faster recovering of blackberry shoots (*Rubus fruticosus*) from the conservation at 4°C

Doaa Elazab[1,2], Maurizio Lambardi[1]

[1]IBE/Institute of BioEconomy, National Research Council (CNR), 50019 Sesto Fiorentino, Firenze, Italy
[2]Department of Pomology, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

maurizio.lambardi@ibe.cnr.it

The liquid culture in temporary immersion system (TIS) is today one of the most promising innovation in the panorama of micropropagation. ElecTIS is a new single container bioreactor (patent n° 2617282 by Claudio Depaoli) which does not require forced air blowing, instead making the culture material mobile and the liquid medium stationary. The timed up-and-down movement of the basket containing the shoot culture ensures periodic contact with the liquid medium, positioned at the base of the container. In this study we tested, for the first time, its use in the recovery of blackberry shoot cultures (*Rubus fruticosus*, cvs Thornfree and Chester), coming from 5 months of slow growth storage (SGS), at 4°C and in the dark. The shoot recovery at standard culture conditions was performed on 2 different ElecTIS, i.e., one with a smaller basket ('ElecTIS$_S$', 18x13 cm, equal to 234 cm^2), and one with a large basket ('ElecTIS$_L$', 21x16 cm, equal to 336 cm^2), comparing the culture in TIS (cycle of 8 min every 6 h, equal to 32 min/day) with the traditional one in gelled medium in glass jars (500 cc). Media were prepared with DKW, added of 0.5 mg/L of BA and 0.01 mg/L IBA. After the 1st and the 2nd subcultures of 5 weeks, the shoot growth parameters and the relative growth rate highlighted a clear superiority of ElecTIS in promoting the recovery of shoot cultures from SGS, compared to the culture in traditional gelled medium. The analyses of chlorophyll content and stoma functionality confirmed the high quality of shoots from ElecTIS bioreactor.

Oral WG3-3

Large-scale micropropagation of *Prunus* spp. *r*ootstocks - a comparison between semi-solid and Temporary Immersion Systems

Elsa Baltazar[1*], Tércia Lopes[1], Mariana Correia[1], Ana Pedrosa[1], Daniela Duarte[1], Jorge Canhoto[1], Sandra Correia[1,2]

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2]InovPlantProtect CoLab, Estrada de Gil Vaz, 7350-999 Elvas, Portugal

e.c.s.baltazar@gmail.com

Micropropagation using semi-solid media has been the most common method for plant propagation. However, for large-scale cloning the costs are high and the technique can be time consuming. Temporary immersion systems (TIS), appear as a solution to improve propagation rates and to decrease costs. *Prunus* such as sweet cherry, peach or almond, are worldwide economic relevant crops, whose propagation depends on the availability of high-quality rootstocks. Among the rootstocks with highest market demands are Gisela 6 (*P.cerasus* x *P.canescens*), and GF677 (*P. persica* x *P.dulcis)*. The main objective of this work was to optimize a micropropagation system for the upscaled production of Gisela 6 and GF677. Thus, both rootstocks were firstly established *in vitro* and multiplication rates were compared for semi-solid media and TIS (SETIS^{TM} bioreactors). For each multiplication condition, different variables were evaluated (after 4 weeks), including the type of culture containers and media composition. With *in vitro* establishment success rates higher than 60% for both rootstocks, micropropagation in semi-solid media allowed for a multiplication percentage of 57.9% for GF677, after 2 subcultures (8weeks). For TIS, similar multiplication percentages (56.9%) were achieved, after the first subculture (4weeks). Results from Gisela 6 are still being analysed. These preliminary results show that TIS may be a suitable method for an upscaled production of *Prunus* spp. rootstocks.

Oral WG3-4

In vitro propagation of horticultural plants by TIS bioreactor systems

Yildiz Aka Kacar

University of Cukurova, Faculty of Agriculture, Horticulture Department, Adana, Turkey

ykacar@cu.edu.tr; yildizakakacar01@gmail.com

In vitro micropropagation methods can be an alternative to traditional clonal propagation of many different plants and the conservation of genetic resources. However, micropropagation protocols for most in vitro plants are labor-intensive, costly, and difficult to automate.
The production system and automation unit operations can be scaled up to achieve large-scale propagation with tissue culture methods. Bioreactor systems have many advantages. It provides an increase in multiplication and rooting in plantlets. It increases the plant quality and reduces the cost as no solidifying agent is used in the nutrient medium. High-capacity culture vessels are available for mass production. Large amounts of plant transfers can be made in a short time. Roots develop in a liquid environment; the strong root is obtained and there is no need for cleaning from agar.
Although there are many different bioreactor systems, TIS (Temporary Immersion System) is the most widely used. TIS usually involves two different cycles; one of them is wetting, and the other is the drying cycle occurring periodically. Several different bioreactor systems have been developed, such as RITA, RALM, PLANTIMA, ELECTIS, SETIS, MATIS, PlantForm, etc.
There are many studies on TIS bioreactor systems in horticultural species. In this presentation, examples of studies and projects related to bioreactors that we have done in horticultural species (banana, citrus, carob, blackberry, some ornamental plants, fruit rootstocks, myrtus, etc.) will be presented and discussed.

Keywords: Bioreactors, *in vitro*, plant tissue culture, Temporary Immersion System.

Oral WG3-5

Effect of silver nanoparticles on the micropropagation of two apricot (*Prunus armeniaca* L.) cultivars

Cristian Pérez-Caselles[1], Lorenzo Burgos[1], Nuria Alburquerque[1]

[1] Fruit Biotechnology Group. Department of Plant Breeding, CEBAS-CSIC, Campus Universitario de Espinardo, Edificio Nº 25, 30100 Murcia, Spain

nalbur@cebas.csic.es

Silver nanoparticles (AgNPs) have been described as antimicrobial agents but frequently they have a dose-dependent phytotoxic effect. Therefore, it is necessary to investigate the effects of AgNPs on micropropagation and plant quality when they are added to the culture medium. Apricot is one of the main species of the genus *Prunus*. Effective protocols for *in vitro* propagation of apricot in semisolid medium and Temporary Immersion Systems (TIS) have been reported. This work aims to study the effect of silver nanoparticles on the micropropagation of two apricot cultivars in two *in vitro* cultivation systems (semisolid medium and TIS). AgNPs have been added to the shoot multiplication medium at different concentrations (0, 25, 50, 75, and 100 mg/L) in both culture systems. After four weeks of culture, proliferation, shoot length, productivity, leaf surface, and fresh and dry weight were measured. AgNPs had a detrimental effect when added to the semisolid medium, reducing micropropagation and biomass production. However, AgNPs had a beneficial effect on plants grown in TIS improving proliferation and increasing biomass. This improvement depended on the cultivar, being much more pronounced in 'Canino' than in 'Mirlo Rojo'.

Oral WG3-6

Ginkgo biloba: *in vitro* culture as an alternative system for the production of extracellular vesicles

Maneea Moubarak[1,*], Immacolata Fiume[1], Ani Barbulova[1], Gabriella Pocsfalvi[1]

[1] EVs-MS Research Group, Institute of Biosciences and BioResources, National Research Council of Italy, via P. Castellino 111, 80131 Napoli, Italy. https://evs-ms.com/
*sabbatical leave from Damanhour University, Egypt

gabriella.pocsfalvi@ibbr.cnr.it

Extracellular vesicles (EVs) are biomembrane-enclosed structures that are ubiquitously secreted by living cells. By transferring proteins, lipids, nucleic acids and other molecules to close or distant cells, EVs contribute to tissue homeostasis and have roles in many physiological and pathological processes such as immune regulation, cancer and infection. Moreover, mammalian cell-derived EVs are considered as one of the most promising nanovectors in biomedicine. Plants also secrete vesicles, morphologically similar to mammalian EVs. Recently we have shown that plant EVs released by root show antifungal activities in vitro [1]. Moreover, plant is a valuable resource for the high-yield production of nanovesicles resembling to EVs [2]. In this context, we aimed to exploit the cell suspension culture of *Ginkgo biloba*.

Ginkgo biloba, the last living species in the order Ginkgoales is dioecious tree characterized by extreme longevity and exceptional resistance. Ginkgo has a strong connection with people by inspiring art and spirituality over the centuries. The leaf extract of ginkgo represents one of the most studied and commonly used herbal remedy in the western world, while ginkgo seeds are widely used in Asian cuisines and medicine. Ginkgo is a valuable street tree in urban landscapes; however, they are underutilized in horticulture.

We have established and optimized fine suspension cultures from ginkgo seed (embryo and female gametophyte) and isolated EVs by gradient density ultracentrifugation (GDUC). Physical, morphological and molecular characteristics of the ginkgo EVs were analysed by nanoparticle tracing analysis, interferometric light microscopy, Qubit assay, SDS-PAGE and cryo-TEM. Batch suspension culture yielded to EVs characterized by average diameter 168.9 +/- 8.2 nm and 6.3E+07 particles per μg of protein. GDUC resulted in a single visible band at the density 1.113 g/mL. Ginkgo EVs show a complex protein profile. The EVs biocargo may provide further insights into their role in tissue culture plant regeneration mechanism that can be further exploited in COPYTREE.

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant, agreement No 895579, acronym "greenEV".

References

[1] M. De Palma *et al.*, "Plant roots release small extracellular vesicles with antifungal activity," *Plants*, vol. 9, no. 12, pp. 1–14, Dec. 2020.

[2] R. Mammadova *et al.*, "Protein Biocargo and Anti-Inflammatory Effect of Tomato Fruit-Derived Nanovesicles Separated by Density Gradient Ultracentrifugation and Loaded with Curcumin," *Pharmaceutics*, vol. 15, no. 2, p. 333, Jan. 2023.

Oral WG3-7

Recalcitrance of *Quercus robur* to *ex-situ* conservation: Cryopreservation and *in vitro* culture

Paweł Chmielarz[1], Szymon Kotlarski[2], Małgorzata Pałucka[3], Urszula Wasileńczyk[3], Paulina Kosek[3], João Paulo Rodrigues Martins[1], Juan Manuel Ley-López[1], Mikołaj Krzysztof Wawrzyniak[1], Marcin Michalak[4]

[1] Institute of Dendrology, Polish Academy of Sciences, 62-035 Kórnik, Poland
[2] Bukowy Las 20, 63-014 Dominowo-Murzynowo Kościelne, Poland
[3] Kostrzyca Forest Gene Bank, 58-535 Miłków, Poland
[4] Department of Plant Physiology, Genetics and Biotechnology, University of Warmia and Mazury, in Olsztyn, M. Oczapowskiego 1A, 10-721 Olsztyn, Poland

pach@man.poznan.pl

500-800-year-old-oaks (*Quercus robur* L.) have many valuable traits of resistance, which allowed them to survive for many years. In recent years a decline of the oldest *Q. robur* growing in Poland, often monumental trees, has been observed. *Q. robur* is a recalcitrant species, which is difficult for *ex-situ* conservation. Seeds are difficult to store them, because they are desiccation and freezing sensitive. Additionally, in recent years, the disappearance of distinct mast seeding years has been observed. Vegetative propagation of individual oaks using traditional methods is not possible because they cannot be easily propagated by cuttings, especially very old oaks. In our research, we attempt to answer the question: of how *Q. robur in vitro* cultures and cryopreservation can be used to conserve the genetic diversity of this species. Embryogenic tissue obtained from immature zygotic embryos, after osmotic desiccation, was cryopreserved. Plumules (an apical meristem of an embryonic axis), 1 mm in size, isolated from mature acorns, were cooled in liquid nitrogen (–196°C) after cryoprotection. To initiate pedunculate oak *in vitro* culture, lignified shoots were collected from mother trees at the end of April, and the next adventitious shoots were induced from them in the vase culture at 25°C. As a result of our research, properly growing plants from cryopreserved embryogenic tissue and plumules were obtained. Using micropropagation, some 500-800-year-old *Q. robur* trees could be introduced to the sterile cultures and successfully multiplied. However, some very old trees were resistant to micropropagation, and after several months of culturing, the shoots of specific individuals died. We presume that this was due to the genetic factors and/or the levels of plant hormones, different for old trees.

Applications of *in vitro* techniques in the study of phytopathogenic fungi and oomycetes

Carmen Salinero, Pilar Vela, Noemí Rial, Olga Aguín

Estación Fitopatolóxica Areeiro, Deputación Pontevedra, 36153 Pontevedra, Spain

carmen.salinero@depo.es

In vitro techniques have various applications in plant pathology research, among which are the study of the mechanisms of virulence and pathogenicity of different organisms, and the search of tools for their control. This is because, with this methodology, a large number of healthy plants free of diseases or pests can be available in a short time at any time of the year, a frequent limiting factor in this type of studies. At the Estación Fitopatolóxica Areeiro (EFA), several in vitro culture methods have been developed to obtain: 1) grapevine plants for the study of phytopathogenic species of the genera *Phytophthora* and *Armillaria*, both of which cause root rot; 2) plants of different chestnut clones to investigate the pathogenicity of *Cryphonectria parasitica*, responsible for chestnut blight disease; 3) callus cultures of *Camellia* species to determine alterations caused by *Ciborinia camelliae*, the causal agent of camellia flower blight, in the necrotrophic phase of infection; 4) somatic embryo lines of *Camellia* species, with the aim of implementing a method for editing genes involved in the plant response to infection by *C. camelliae*. In vitro culture techniques for grapevine were developed in liquid and solid media, in the case of chestnut plants in liquid media, and for *Camellia* studies in solid media.

An innovative protocol to propagate and preserve the threatened Sicilian Fir through somatic embryogenesis technique

Nourhene Jouini[1], Maurizio Lambardi[2], Carla Benelli[2], Waed Tarraf[2], Tolga Izgu[2], Maria Antonietta Germanà[1]

[1] Departement of Agricultural, Food and Forestry Sciences, University of Palermo, Palermo, Italy
[2] IBE- Institute of the BioEconomy, National Research Council (CNR), Florence, Italy

jn.nourhen@gmail.com

Somatic embryogenesis (SE) is an innovative technique for plant cloning. This revolutionary biotechnological tool is applied to clone, propagate, and even to conserve different plant species. *Abies nebrodensis* is an endangered species located exclusively in the Madonie Parc, Sicily, Italy. This species was always the target of different projects and plans for conservation and protection, as only 30 adult individuals are existing all over the world. Thus, LIFE European program establish the Life4Fir project which aims to preserve those trees with a new strategy based on the application of new biotechnological tools. This threatened species has several difficulties such as the high percentage of seeds devoid of embryos which is the main cause of the poor germination rates. Therefore, the development of highly reproducible *in vitro* approaches, particularly somatic embryogenesis, cryo-conservation, and encapsulation technology, could be a key step for large-scale propagation and long-term preservation of this risked Nebrodi Fir. The present work illustrates an exclusive protocol for SE from the Sicilian Fir '*Abies nebrodensis'.* Cones from *Abies* adult trees with specific identification number (IN) were collected and different experiments were carried out for callus initiation from both zygotic immature and mature embryos, testing diverse culture media and different combinations of plant growth regulators (PGR). Callus initiation was reached from mature and immature zygotic embryos cultivated in SH medium supplemented with 1 mg/L-1 of 6-benzylaminopurine (BAP). Cell lines were transferred onto SH basal salt medium, supplemented with 4,27 μM abscisic acid (ABA) and 8% polyethylene glycol (PEG-4000) for the maturation and the development of the somatic embryos. Our findings revealed that the SE was influenced by culture medium, PGR type, the origin of donor trees and the storage period of seeds.

Analysis of environmental effects on the quality of conifer somatic embryos

Caroline Teyssier[1], Armelle Delile, Céline Ridelle, Nathalie Boizot, Marie-Anne Lelu-Walter

BioForA, INRAE (National Research Institute for Agriculture, Food and the Environment), Orléans, France.

caroline.teyssier@inrae.fr

Our group has been working in the field of somatic embryogenesis for many years. This research has allowed the development of somatic embryogenesis in many conifers (hybrid larch, Scots pine, maritime pine, Douglas-fir). For more than 10 years, we have focused on the determination of the physiological stage and the evaluation of the quality of embryos during maturation, to improve somatic embryo maturation protocols in relation to environmental conditions and to identify the determinism embryonic (Teyssier et al., 2011; Morel et al., 2014; Gautier et al., 2018; Lelu-Walter, et al.2018). The species are hybrid larch, Douglas fir and maritime pine. This work is supervised by Marie-Anne Lelu-Walter who has expertise in cell culture and Caroline Teyssier who specializes in molecular analysis (proteomic studies and assays of carbohydrates, proteins and lipids in somatic embryos).

In recent years, our studies have also focused on the sexual reproduction of these same conifers. The research follows two axes: seed quality for more vigorous sowing and the effects of climate change on seed maturation. Should we change the time of harvest in seed orchards? What would be the best post-harvest treatment for seeds?

Gautier F, Eliášová K, Leplé J-C, Vondráková Z, Lomenech A-M, Le Metté C, Label P, Costa G, Trontin J-F, Teyssier C, Lelu-Walter M-A (2018) Repetitive somatic embryogenesis induced cytological and proteomic changes in embryogenic lines of *Pseudotsuga menziesii* [Mirb.]. BMC Plant Biology 18:164-188.

Lelu-Walter M-A, Gautier F, Eliášová K, Sanchez L, Teyssier C, Lomenech A-M, Le Metté C, Hargreaves C, Trontin J-F, Reeves C (2018) High gellan gum concentration and secondary somatic embryogenesis: two key factors to improve somatic embryo development in *Pseudotsuga menziesii* [Mirb.]. Plant Cell Tiss Organ Cult 132:137-155.

Morel A, Trontin J-F, Corbineau F, Lomenech A-M, Beaufour M, Reymond I, Le Metté C, Ader K, Harvengt L, Cadene M, Label P, Teyssier C, Lelu-Walter M-A (2014) Cotyledonary somatic embryos of *Pinus pinaster* Ait. most closely resemble fresh, maturing cotyledonary zygotic embryos: biological, carbohydrate and proteomic analyses. Planta 240:1075-1095.

Teyssier C, Grondin C, Bonhomme L, Lomenech A-M, Vallance M, Morabito D, Label P, Lelu-Walter M-A (2011) Increased gelling agent concentration promotes somatic embryo maturation in hybrid larch (*Larix* × *eurolepsis*): a 2-DE proteomic analysis. Physiologia Plantarum 141:152-165.

Somatic embryogenesis in holm oak: optimization of maturation and germination steps

María Teresa Martínez, Fátima Mosteiro, Sol Campañó, Pablo Piñeiro, Elena Corredoira

U.T. Biotecnología y Mejora Forestal, Misión Biológica de Galicia (MBG), CSIC, Sede de Santiago de Compostela, Adv Vigo s/n, 15705 Santiago de Compostela, Spain

elenac@mbg.csic.es

The holm oak *Quercus ilex*, a widely distributed forest species in the Mediterranean basin, provides a great variety of products and ecosystem services. In recent decades, populations of the species have been decimated, mainly by *oak decline* syndrome. Somatic embryogenesis (SE) is a biotechnological tool of great potential value for clonal propagation and germplasm conservation. However, in *Q. ilex*, as in other woody species, the lack of an efficient germination protocol limits the application of SE. In this context, the objectives of this study were first to increase the plant conversion rate and second to improve the quality of the plants obtained. For this purpose, we evaluated the effects of a maturation treatment with an osmotic agent prior to cold storage for 2 months and of fast desiccation after cold storage. We also studied the effects of the composition of the germination medium, evaluating different types and concentrations of cytokinins and carbon sources and also an ethylene inhibitor (silver thiosulphate, STS). Neither the maturation treatment or desiccation improved the plant conversion rate. However, the conversion rate was affected by the type of sugar and cytokinin included in the germination medium, with respectively sucrose (3%) and meta-topolin (1 mg/L) producing the best results. Likewise, the inclusion of STS significantly increased the number of embryos that developed into plants and also improved the quality of the plants (longer shoots and greater number of leaves).

Acknowledgements
This study was partly funded by the MICINN (Spain) within project PID2020-112627RB-C33.

Modern approaches in *in vitro* clonal banana micropropagation: next generation tissue culture systems

Yildiz Aka Kakar[1], Hakan Erol[2], Dicle Dönmez[2], Belgin Biçen[3], Özhan Şimşek[4]

[1] Horticulture Department, Agriculture Faculty, Çukurova University, Adana 01330, Turkey
[2] Biotechnology Research and Application Center, Çukurova University, Adana 01330, Turkey
[3] YK Teknopolis, Çukurova University, Adana 01330, Turkey
[4] Horticulture Department, Agriculture Faculty, Erciyes University, Kayseri 38280, Turkey

ykacar@cu.edu.tr

Banana (*Musa* spp AAA), an important fruit crop of the Musaceae family, is widely grown in developing countries and is the second largest fruit crop in the world, after citrus. Banana production in Turkey is very important in the Mediterranean coastal region, which has a mild winter climate. The trend has continued rapidly in different locations in recent years but the growers have experienced problems such as nematodes, viruses, and fungal diseases. Since diseases are often spread through vegetative propagation, there has been a great deal of effort to create disease-free planting material on a large scale through tissue culture, which also has the advantages of speed and effectiveness. Recently, temporary immersion bioreactor systems have been used as an alternative to conventional tissue culture to achieve low cost and high success in mass production. A new TIS bioreactor system named PlantForm and SETIS ™ has been recently developed.

In the present study, optimum conditions were determined for mass micropropagation, rooting, and acclimatization stages of banana genotypes which most grown cultivars in Turkey by using classical solid culture, PlantForm, and SETIS temporary immersion bioreactor systems, and a mass production model was created by following the plants throughout eight subcultures.

All data were recorded and discussed at multiplication (number of leaves, multiplication rate, plant height, fresh and dry weight), rooting (root number, root length, plant height, fresh and dry weight), and acclimatization stages, and also some physiological parameters were evaluated in all stages of banana micropropagation.

One of the most critical issues in banana clonal propagation is genetic variations occurring during the subcultures. To check the genetic stability of the plant's leaf samples were taken from each system separately at the initial stage, micropropagation stages, rooting and greenhouse stages throughout the eight subcultures, and the variation in plants was investigated using SSR markers.

In conclusion, TIS bioreactor systems present a strong alternative to traditional *in vitro* micropropagation and rooting systems, resulting in a reduction in cost, labor, and time for mass propagation.

Keywords: Banana, bioreactor, SETIS, Plantform, micropropagation

Micropropagation of Tunisian *Thymus capitatus*: a step towards production of polyphenol-rich extracts showing *in vitro* anthelmintic activities

Essia Sebai[2], Amel Abidi[2], Akkari Hafidh[1]

[1]Laboratory of Parasitology, National Veterinary School of Sidithabet, Tunisia
[2]Laboratory of Bioactive Substances, Centre of Biotechnology of Borj Cedria, Box 901, Hammam-Lif, 2050, Tunisia

akkari_hafidh@yahoo.fr

The objective of this study was to develop a rapid system for regeneration of an important endemic medicinal plant of Tunisia, *Thymus capitatus* (Labiatae). Initially *in vitro* grown seedling were exposed to full strength Murashige and Skoog, 1962 (MS) medium or reduced to ½ MS or ¼ MS hormone-free. Then, for axillary shoots proliferation, BAP, KIN, NAA and IAA were tested for their ability to multiply *T. capitatus*. Shoots obtained on proliferation medium were exposed to elongation medium containing gibberellic acid (GA3) (0.5, 1.0, 2.5, or 5.0 µM). The effect of the auxins IAA, IBA and NAA, on the *in vitro* rooting of the shoots was studied. Maximum number of shoots (5.25±0.52) was observed on the ½ MS medium containing 2.22 µM of BAP. Incorporation of 1 µM of GA3 in ½ MS medium significantly improved the shoot elongation within 3 weeks of culture. For rooting, rhizogenezis was promoted on half strength MS medium hormone-free. Regenerated plants were transferred to dimpled plates filled by peat and vermiculite (2/3:1/3 v/v) mixture. Micropropagated *T. capitatus* plants had a 95% survival rate, and showed vigorous and uniform growth. The qualitative HPLC analyses confirmed the presence of phenolic acids and flavonoids in the extracts. The extracts from both shoot cultures and the leaves from field-grown plants revealed in vitro anthelmintic activity. The conducted research confirmed the regeneration potential of genetically-stable plants of *T. capitatus* under in vitro conditions, the ability of the plantlets to produce polyphenols as those present in field-grown plants, as well as their anthelmintic potential.

Establishment of an embryogenic culture of *Hylocereus undatus*: cultivation in bioreactors and analyses via *-omics*

Roberto Berni[1], Sylvain Legay[1], Samuel Jourdan[1], Diego Rios-Salgado[1], Sébastien Planchon[1], Kjell Sergeant[1], Jenny Renaut[1], Jean-Francois Hausman[1], Gea Guerriero[1]

[1] Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and Technology (LIST), L-4940 Hautcharage, Luxembourg

roberto.berni@list.lu

Hylocereus undatus is a tropical/sub-tropical climbing vine cactus belonging the family Cactaceae. Its fruits, commonly referred to as dragonfruit or pitahaya, are source of several phytochemicals, such as flavonoids, tannins and betalains, which are powerful reactive oxygen species scavengers. Because of their bioactivities, these molecules are largely used in cosmetics, nutraceutics and pharmaceutics to manufacture plant-based products with antioxidants and antiaging properties. Therefore, their demand is huge, and it is necessary to satisfy it while limiting the sourcing of the plants from the wild and the impact on the environment. The technology based on the dedifferentiation of plant tissues to calli and their subsequent cultivation as cell suspensions allows to meet such a demand, because cell suspensions can be scaled up in volume to pilot- and industrial scales.
In this work, an embryogenic cell line of *H. undatus* was established, its metabolome analysed and the transcriptomic changes followed during 7 days of growth. The cultivation in stirred-tank bioreactors was optimised at the laboratory scale and the culture was scaled up to 30 l. The data obtained with metabolomics and transcriptomics show that the embryogenic cell suspension produces flavonoids and that the culture can be grown in stirred-tank reactors with an average batch duration of 12 days at 26 degrees and a final yield of 9 g dry biomass per liter of culture.

Physiological and biochemical responses of clonally propagated *Prunus* spp. GF677 rootstocks to water-deficit and stress recovery

Mariana Correia[1], Tatiana Soares[2], Tércia Lopes[1], Elsa Baltazar[1], Maria Celeste Dias[1], Jorge Canhoto[1], Mónica Zuzarte [2,3], Sandra Correia [1,4]

[1] Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2] Quality Plant - Investigation and Production in Plant Biotechnology, Lda., Coimbra, Portugal
[3] University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal
[4] InnovPlantProtect CoLab, Estrada de Gil Vaz, Elvas, Portugal

mjcorreia324@gmail.com

Prunus spp. interest reside in their edible fruits. Most stone-fruit trees are grafted on clonally propagated rootstocks. One of the most used rootstocks, due to drought tolerance, is the outcome of the natural hybridization between *Prunus dulcis* and *Prunus persica*- GF677.
This work' aim was GF677's physiological and biochemical evaluation during recovery after drought stress. Clonally propagated rootstock plants were exposed to 18-days period of water deficit conditions (no watering), followed by a 30-days post-stress recovery period (regular watering). Relative water content (RWC), photosynthetic parameters and pigments content were determined after each period.
Water deficit reduced RWC, photosynthesis and stomatal aperture. After recovery, the increase of the net CO2 assimilation rate, stomatal conductance and transpiration rate, suggest the re-establishment of the plants physiological performance. This recovery was accompanied by an increase of pigments and RWC to control levels.
Lower gene expression values for *RBCS1* (coding for RuBisCO small subunit) revealed that a degradation of the protein may have occurred during drought stress, but the slight increase in the post-drought period suggest some recuperation. This was also found for the gene expression of *PIP1* (coding for a plasma membrane intrinsic protein). Drought stress induced damages in the plants, but recovery was noticeable, validating the association of *Prunus* spp. rootstocks to water-deficit tolerance.

Optimizing late stages of *Pinus radiata* somatic embryogenesis: a previous stage to scaling up

Itziar A. Montalbán[1], Ander Castander-Olarieta[1], Paloma Moncaleán[1]

[1]Neiker-BRTA, Centro de Arkaute, Apdo. 46, 01080 Vitoria-Gasteiz, Spain.

imontalban@neiker.eus

Somatic embryogenesis is a technique with multiple applications, being one of the most important the implementation of multi-varietal forestry. However, to be cost-effcicient some steps must be optimized and when possible, hand labor reduced. For these purposes, we tried to improve maturation and germination stages of somatic embryogenesis in radiata pine. We tested different preculture conditions before maturation, the effect of several modifications at maturation and germination stages and the implications of these changes for plant conversion; months later, acclimatization was also monitored.

After considering all the modifications performed in this work, a preculture of 14 days in a medium devoiding plant growth regulators seemed to be beneficial for plant conversion. After the somatic embryos were formed, they could be stored at 4ºC with no detrimental effects and even an increased plant conversion in some embryogenic cell lines. At germination stage, adding glutamine to the culture medium and reducing sucrose content to a half (15 g/L) had a significant effect on germination and doubled the percentage of plants suitable for *ex vitro* acclimatization. In this way, germinated explants were influenced by the light source; although fluorescent light enhanced root formation, blue LED light increased the shoot height of somatic plants.

In vitro tissue culture and genotyping innovations to accelerate mutation-assisted breeding of *Coffea arabica*

Radisras Nkurunziza[1,2], Joanna Jankowicz-Cieslak[1], Ivan L. Ingelbrecht[1], Stefaan Werbrouck[2]

[1]Plant Breeding and Genetics Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA Laboratories Seibersdorf, International Atomic Energy Agency, Vienna International Centre, PO Box 100, A-1400 Vienna, Austria
[2]Laboratory for Applied In Vitro Plant Biotechnology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin vaerwyckweg 1, Schoonmeersen – C 9000 Ghent, Belgium

radisras.nkurunziza@ugent.be

Coffee (*Coffea arabica*) is an important cash crop for many households in Asia, Africa and South America. It accounts for about 75% of world coffee trade due to its superior cup quality and aromatic characteristics. *C. arabica* has a unique biology as it is self-fertile and allotetraploid (2n=4x=44). Other coffee species are diploid (2n=2x=22) and self-incompatible. Nevertheless, *C. arabica* varieties are low yielding and highly susceptible to various biotic and abiotic stresses. Genetic improvement of *C. arabica* to resist these stresses through classical breeding can take 25-30 years. Mutation breeding offers a powerful tool to increase genetic variation in the *C. arabica* gene pool. The integration of chemical and/or physical mutagenesis with advanced in vitro tissue culture techniques, such as somatic embryogenesis (SE) can reduce breeding time and induce novel agronomic traits including enhanced climate change adaptation and resilience to transboundary pests and diseases. Unfortunately, with current protocols, few explants respond to embryogenesis and the number of regenerated plants is extremely low. This is the main bottleneck for the use of SE in mutation breeding which requires large populations of mutants. The aim of this research is to increase the efficiency of SE. To this end, new compounds such as cytokinin oxidation inhibitors are being exploited. Finally, these new SE methods need to be integrated into a mutation breeding programme. The timing of the mutagenesis treatment and the number of regenerated plants are crucial.

Embryo rescue as a tool in Pitaya (*Hylocereus,* Cactaceae) breeding programs

Noemi Tel-Zur

French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Sede Boqer Campus, Ben-Gurion University of the Negev, Israel

telzur@bgu.ac.il

Interest in the night-blooming pitaya species (*Hylocereus,* Cactaceae) has greatly increased due to their high economic potential as fruit crops. Three decades ago, at Ben-Gurion University of the Negev, Israel, started a long-term R&D project aiming to introduce and domesticate these group of species for the Israeli Negev desert, where conditions are not suitable for conventional crops. The present study was undertaken to establish an embryo rescue system for *Hylocereus* species. Fertilized ovules of *H. monacanthus* and *H. undatus* were excised from ovaries and surrounding tissue including funiculus and placenta. They were transferred to half-strength MS medium containing 680 µM glutamine, 0.54 µM α-naphthaleneacetic acid, 0.45 µM thidiazuron and sucrose at different concentrations. The funiculi were removed after 30 days in culture and the immature embryos were transferred to fresh medium. *In-vitro* embryo development was influenced by the basal media and the sucrose concentration. The half-strength MS medium with 0.17 M of sucrose resulted in the best growth response. Embryo conversion and seedling development had a high success rate five days after pollination. This study provides substantial evidence that seedling production in *Hylocereus* species from embryos at globular stages is possible using a two-step culture procedure: firstly a fertilized ovule with placental tissue is cultured and then the placental tissue is removed to isolate the embryo.

Cultigar, a project for the conservation, production, and improvement of plant species

Marga Fraga, Beatriz Vidal, Cristina Vigo, Adriana Romero, Aránzazu Rodriguez, Minia Bralo, Gloria Saccatoma

Cultigar (Plant Biotechnology Laboratory), Fundación Paideia Galiza. Liñares Nº 53, 15845-Brión, A Coruña, Spain.

contacto@cultigar.es

Cultigar is a plant biotechnology laboratory owned by the Paideia Foundation whose purpose is to use of Biotechnology for the development of projects responding to the needs and problems of the agricultural and forestry sector.

Our goal is to identify elite genetic resources, making selections and improvement of some species or multiplying others that are not easily propagated by traditional methods because they are recalcitrant. The aim is to contribute to the conservation of biodiversity while facilitating development of a more competitive agroforestry sector. We believe this approach can lead to new green and more sustainable business initiatives actively contributing to economic and social development. The species under study are normally selected by phenotypic attributes, arising from their disease resistance or some other character that has economic value and is worthy of large-scale production.

Cultigar has been working for more than 20 years on improvement and development of micropropagation protocols for forest species such as chestnut, oak and wild cherry, fruit species and rootstocks such as pistachio, kiwi, blueberry, apple and pear trees plus ornamental species such as magnolia and liquidambar (2 new varieties registered with UPOV).

We are currently developing a project for the selection and genetic improvement of forest species including *Quercus robur* and *Betula celtiberica* and development of protocols for *in vitro* production of olive tree varieties of Galician origin.

Potential of metabolic engineering for resveratrol production in peanut cell cultures

Hajer Ben Ghozlen[1,2 *], Stefaan Werbrouck[1], Sven Mangelinckx[2]

[1] Laboratory of Applied *In Vitro* Plant Biotechnology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent university, Valentin Vaerwyckweg 1, B-9000 Belgium
[2] SynBioC, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

hajer.benghozlen@ugent.be

Plants are known to produce a wide range of secondary metabolites with potent biological activities, making them particularly attractive for in vitro production. Stilbenes are well-known phytoalexins from the group of polyphenolic compounds. Their biosynthesis often needs to be stimulated by elicitors as they are defense molecules. Due to their potent bioactivities, including antioxidant, anti-tyrosinase, photoprotective and antibacterial activities, they are used as important ingredients in pharmaceuticals, dietary supplements and cosmetics. Resveratrol (3,5,4'-trihydroxystilbene) is the most studied stilbene and is produced by a limited number of woody plants such as grapes, pine, pistachios, berries and legumes such as peanuts. However, the low production of resveratrol does not meet the increasing industrial demand. Recently, metabolic engineering of plant cell cultures has become a biotechnological approach to increase resveratrol production. Here, we used peanut as a model species and focused on two strategies. Firstly, engineering the biosynthetic pathway for fatty acids by adding an inhibitor. The second was to short-circuit the pathways to increase malonyl-CoA, which is condensed by stilbene synthase to produce the phenolic ring A of resveratrol.

New approaches for *in vitro* micrografting of chestnut

Juan Luis Fernandez-Lorenzo, Ana Couso

Escola Politecnica Superior de Enxenaria (EPSE-USC) Benigno Ledo St., 27002 Lugo (Spain)

juanluis.fernandez@usc.es; ana.couso.viana@usc.es

The high demand for grafted chestnut (*Castanea sativa* L.) trees in Galicia makes it necessary to develop new production strategies and to deepen the knowledge of compatibility of selected varieties with Eurasian hybrids resistant to "ink disease", used as rootstocks. First results of in vitro micrografting of five chestnut varieties on four artificial hybrid rootstocks were obtained in our laboratory, which permitted to assess short-term affinity and evaluate in vitro micrografting to produce grafted plants. For the micrografting procedure, two general approaches have been compared: 1) use of unrooted rootstocks followed by rooting induction after graft-take, and 2) use of rooted rootstocks. In the latter, we propose a simple method to sidestep the extraction of the root system from the medium during the micrografting procedure, which facilitates the procedure and reduces contamination risks. The initial results show that grafting success in most scion/rootstock combinations is high (usually over 70%).

On one side, micrografting on unrooted rootstocks permitted to accelerate graft-take and scion growth (which makes it suitable for compatibility tests), but obliges to implement an additional rooting step, and causes apical necrosis of the scion due to the auxin treatment. The second approach (using rooted rootstocks) proves to be more useful if the objective is the acclimation and production of grafted plants.

(Poster nº 60 not presented)

Direct somatic embryogenesis from leaves of coffee plants in different phenological stages

Claudia Ruta, Giuseppe Basile, Patrick Guarini, Giuseppe De Mastro

Department of Soil, Plant and Food Science, University of Bari Aldo Moro Via Amendola, 165/A, 70126 Bari, Italy.

claudia.ruta@uniba.it

Coffee is an important crop cultivated in about 80 countries around the world and its demand is always high. In recent years, the improvement of alternative technologies for its propagation has been evaluated trying to increase its production. Somatic embryogenesis is one of in vitro techniques studied on different species of coffee. However, the induction of somatic embryos and their subsequent development depend on the species and genotype, the explant source and the culture conditions. Somatic embryogenesis can occur both indirectly or directly. *Coffea arabica* genotypes usually respond more readily to the indirect than to direct pathway. The aim of this study was the definition of an efficient protocol to induce direct somatic embryogenesis of *C. arabica* L. Nakombu, starting from leaf explants. The results showed that the best phenological stage of mother plants to take the leaves is the time of vegetative restart and the young leaves are the more reactive explants. Furthermore, healthy and functional somatic embryos were obtained in growth chamber at a temperature of 22 ± 1°C under light conditions (8 h light, 30 μE m^{-2} s^{-1}), by adding thidiazuron (2.0 mg l^{-1}) to induce direct somatic embryogenesis to the nutritive medium and reducing its concentration at 0.2 mg l^{-1} for the conversion of the embryos.

Somatic embryo induction in the Indonesian elite *Theobroma cacao* clone

Mirni Ulfa Bustami[1,2], Stefaan Werbrouck[1]

[1]Department of Plant and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium
[2]Faculty of Agriculture, Tadulako University, Central Sulawesi, Indonesia

meetmot@yahoo.com

In this study, staminode and petal explants from cacao flower buds were used to test the ability of the Indonesian elite cacao 'Sulawesi 2' clone to produce somatic embryos. Callus was initiated in gelled medium containing various concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 0.25^{-1} L kinetin. It was then transferred to free hormone gelled medium. Embryogenic callus expression was also carried out in hormone-free medium, either on gelled medium or in shaking flasks.
The somatic embryo was only obtained on staminode and petal explants in the medium supplemented with 2 mg L^{-1} 2,4-D and 0.25 L^{-1} kinetin, either gelled or liquid medium. However, the production of embryos was greater on the liquid medium than on the gelled medium. Direct somatic embryo formation was only observed from the petal explant. The somatic embryos were converted into plantlets after transfer to the germinating medium. Our study confirms the embryogenic competence of this Indonesian elite clone.

Keywords: somatic embryo, cacao, 2,4-D

Micropropagation of woody plants: common oak (*Quercus robur* L.) and pear (*Pyrus communis* L.) rootstock OHF 333 as examples

Lilyana Nacheva[1], Nataliya Dimitrova[1], Ivaylo N. Tsvetkov[2]

[1]Department of Breeding, Genetic Resources and Biotechnology, Fruit Growing Institute, Plovdiv, Bulgaria
[2]Department of Genetics, Physiology and Plantations, Forest Research Institute –BAS, Sofia 1756, Bulgaria

tsvet_i@yahoo.com

The activities of two research tissue culture labs are presented through plant species being subjects of more intensive studies. The labs are established during the late 80s and are specialized in experimenting with forest and fruit species, respectively. The common oak is a key forest-forming broadleaved species within the lowland forest vegetation zone. A system for direct organogenesis based on using apical/ nodal bud segments from young seedlings has been developed. The basic elements of a system for regeneration via somatic embryogenesis from immature zygotic embryos has been elaborated and emblings has been successfully obtained. As an idea for medium term storage of propagules, successful regeneration of alginate-encapsulated bud segments from *in vitro* grown plants has been achieved. A valued feature of the USA bred pear (*P. communis* L.) rootstock OHF 333 is its moderate resistance to fire blight (*Erwinia amylovora*). A reliable system for micropropagation of pear rootstock OHF 333 has been developed. Experiments were conducted to optimize the production system (application of culture vessels with improved gas exchange, replacement of BAP with meta-topolin in the proliferation stage, application of biostimulator "Charkor" in *in vitro* rooting, ex *vitro* rooting). An efficient system for regeneration from leaf segments of *in vitro* grown plants has been developed as well.

Competences and skills of the team of Research Centre for Vegetable and Ornamental Crops in Sanremo

Barbara Ruffoni[1], Marco Savona[1]

[1] CREA Research Centre for Vegetable and Ornamental Crops (CREA OF), Corso degli Inglesi 508, 18038 Sanremo (IM), Italy

marco.savona@crea.gov.it; barbara.ruffoni@crea.gov.it

The Tissue Culture team in Sanremo has a 30-year experience in many aspects concerning *in vitro* culture and participated to several COST actions concerning the in vitro culture The research involved virus eradication through meristem tips in order to produce virus-free lines for floriculture market and the study of new or adapted protocols for many ornamental herbaceous, bulbous species and woody and semiwoody Mediterranean plants, such as *Lentiscus* sp., Carob tree, *Myrtus* sp., *Rosa x hybrida* spp. to overcome recalcitrance to *in vitro* multiplication. The morphogenic developmental pathway has been studied for many specie as prerequisite for NGTs (New Genomic Techniques) for improvement of aesthetic and physiological characters.
The clonal shoot and/or cell multiplication was also carried out with automatized techniques named Temporary Immersion Systems with several containers (Plantform®, RITA®) or simple bioreactors.
Furthermore, the attention has been focused on somatic embryogenesis of *Genista monosperma*, *Eustoma grandiflorum* and *Cyclamen persicum*, in order to study and set up precommercial protocols also for the development of artificial seeds.
Nowadays, the laboratories of CREA OF is spread over about 600 mq for: *in vitro* culture, molecular biology, microscopy, biochemistry, equipped with advanced instruments, useful for many research lines involving ornamental, aromatic plants and recently, edible flowers.

Maintaining leaf variegation during *Polemonium caeruleum* micropropagation

Irum Saadia Khan[1], Stefaan P.O. Werbrouck[2], Stephen Page[3]

[1] Walter Blom Plants, Veenenburgerlaan 108A, 2182 DC Hillegom, The Netherlands
[2] Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

[1] Walter Blom Plants, Veenenburgerlaan 108A, 2182 DC Hillegom, The Netherlands
[2] Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

irum@walterblom.nl

Maintaining variegation during in vitro propagation is essential for maintaining the aesthetic and commercial value of chimeras. However, variegation is often not stable. *Polemonium caeruleum*, valued for its decorative and medicinal properties, is no exception. In this study, we aimed to develop an effective in vitro protocol for different *Polemonium* cultivars while preserving their leaf variegation. We compared the use of traditional benzyladenine with meta-topolin cytokinins. We found that some of them were very efficient in reducing the loss of variegation, resulting in a higher market value. Our results demonstrate the importance of careful selection of growth regulators for successful micropropagation of ornamental crops. They also highlight the potential of meta-topolins for stabilizing meristem integrity during in vitro propagation.

Effect of LED light on the in vitro micropropagation of fruit trees

Lilyana Nacheva, Nataliya Dimitrova

Fruit Growing Institute, Agricultural Academy, 12 Ostromila Str., 4004, Plovdiv, Bulgaria

lilyn@abv.bg

In recent years light emitting diodes (LED) have become an alternative to fluorescent lamp (FL) source of light for plant tissue culture because of their low energy consumption, low heat emission, specific wavelength irradiation etc. The aim of this study was to investigate the effects of different LED light regimes on the *in vitro* micropropagation of fruit trees. Two rootstocks were studied – pear rootstock OHF 333 (*Pyrus communis* L.) and plum rootstock Saint Julien (*Prunus domestica* spp. Insititia). The plantlets were cultivated *in vitro* under an illumination system based on Philips GreenPower LED research module. Four groups of LEDs emitting in white (W), red (R), blue (B), mixed (W:R:B:far-red=1:1:1:1) spectral regions and fluorescent lamps (FL, control) were used in our studies. Growth parameters, some physiological and biochemical characteristics of the plantlets were measured. Multiplication of plantlets under mixed light (WBR) favoured biomass accumulation. Red light stimulated elongation of shoots of both studied genotypes. In addition, the red LED light improved the rooting of plum plantlets (over 98% rooting).

Keywords: micropropagation; shoot culture; photosynthetic pigments; chlorophyll fluorescence

Improving hazelnut micropropagation: a comparison of *in vitro* and *ex vitro* rooting

Fabiano Gattabria[1], Massimiliano Meneghini[1], Alice Patella[1], Giuliano Dradi[1], Gianluca Magnani[1], Romano Roncasaglia[1]

[1] Battistini Vivai, via Ravennate 1500, 47522 Cesena (FC), Italia

fgattabria@battistinivivai.com

Hazelnut (*Corylus avellana* L.) is one of the most important species in the Mediterranean area. Traditionally, hazelnut propagation has been carried out by suckers, often by growers themselves. The use of micropropagation to produce healthy plant material provides plants loyal to the selected cultivars and meets the certification system developed in Italy (QVI, Qualità Vivaistica Italia) for the production of Virus-free plants. The main problems for hazelnut micropropagation are maintaining a sterile process for *in vitro* establishment, low multiplication rate, long time between subcultures, a lack of axillary branching and low rooting rate. An efficient protocol for mass micropropagation of the main Italian varieties (Tonda Romana, Tonda Gentile delle Langhe, Tonda di Giffoni, Nocchione) has been developed at Battistini, to put on the market millions of plants of high-quality nursery stock. The production concern plants in different sizes ranging from young plants in multi tray containers to potted plants suitable for planting in an orchard. The aim of this research was to set up an efficient micropropagation protocol focused on improving the hazelnut rooting in order to overcome the *in vitro* rooting induction (in lab on agar medium with auxins) and obtain an efficient protocol for *ex vitro* rooting (in greenhouse, directly into preforma containers). Both *ex vitro* rooting and the quality of plants give the best way to obtain over 85% plants ready for commercial purpose.

Photoautotrophic micropropagation of trees cultured in bioreactors with forced ventilation

Saleta Rico[1], Diego Gago[1,2], Conchi Sánchez[1], Anxela Aldrey[1], Mª Ángeles Bernal[2], Beatriz Cuenca[3], Bruce Christie[4], Nieves Vidal[1]

[1] Dept. of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
[2] Dpto de Biología, Facultad de Ciencias, Universidade da Coruña, Campus da Zapateira s/n, 15071 A Coruña, Spain
[3] Maceda Nursery, Tragsa-SEPI Group, Carretera de Maceda a Baldrei km 2, 32700 Maceda, Ourense, Spain
[4] The Greenplant Company, Palmerston North 4410, New Zealand

nieves@mbg.csic.es

In vitro plants normally need exogenous carbohydrates as a carbon and energy source. We are using liquid media and forced ventilation to evaluate the effect of decreasing sugar supplementation during micropropagation of three tree species: chestnut, plum and willow. Our results suggest that the feasibility of achieving photoautotrophic growth is species-dependent. Willow shoots can be easily cultured without sucrose in RITA or Plantform bioreactors under high light intensity and CO_2 supplementation, and proliferate more than when sucrose is added to the medium. Plum and chestnut shoots can be grown photoautotrophically in temporary or continuous immersion, but proliferation decreases sharply with less than 1 and 0.5% sucrose, respectively. Although sucrose positively influences root formation in the three species, willow shoots form functional roots and acclimate without loss when cultured without carbohydrate. In chestnut, the use of low sucrose during the multiplication and rooting stages can reduce the percentage of rooted shoots, whereas plum shoots grown without sucrose form roots and acclimate successfully even if they performed poorly during the multiplication stage. In conclusion, our experience shows bioreactors can be used for photoautotropic propagation, but it is necessary to adapt the physical environment and the protocols to physiological requirements of each species to achieve useful results that may be applied more universally in a commercial environment.

Funding: This work was funded by Xunta de Galicia (Spain) through the projects IN607A/2021 and "Contrato Programa" 2021 (AGI/CSIC I + D + I 2021, Ref-ACAM 20210200033), by the CSIC program I-COOP + 2020 (RefCOOPB20584).

Development of an in-house bioreactor system to micropropagate ornamental plants

Santiago Silva[1], Juan Carlos Codesido[2]

[1] INEX Plants, Vivero de Empresas. CIFP Politécnico de Santiago, Avda Rosalía de Castro 133, 15706 Santiago de Compostela, A Coruña, Spain
[2]Dpto de Química, CIFP Politécnico de Santiago, Avda Rosalía de Castro 133, 15706 Santiago de Compostela, A Coruña, Spain

santiagosilva7676@hotmail.com

Bioreactors were developed to facilitate automation and improve the economics of large-scale propagation. Although the use of bioreactors reduces the costs of consumables and personnel once the protocols are optimized, the initial outlay (for vessels, pumps, solenoid valves, filters, etc.) is high. Commercial bioreactors are expensive and some parts have to be replaced after a few autoclaving cycles. We present INEX-Plants, a collaborative project for developing a low-cost micropropagation laboratory. This project has been carried out in-house with the support of a technical school, we combined materials or devices designed items for different purposes with 3-D printing. We also built our own laminar flow cabinet, and culture room with a temporary immersion bioreactor system equipped with LED lights. We used our in-house bioreactors to culture ornamental plants as *Drosera venusta*, *Cephalotus follicularis*, *Nepenthes veitchii*, *Paphiopedilum villosum*, as well as starting experiments with woody plants as *Camellia sinensis*. More than 6000 plants were produced, and 4800 of them were recently used for educational purposes in the IFEMA Education Week (Madrid), an event that every year brings together more than 115,000 students, families, teachers, guidance counsellors, heads of educational centres and education professionals. We would welcome opportunities to discuss partnering with people on economically and environmentally important plant tissue culture projects.

Keynote Conference WG4&5

Addressing stakeholder concerns together with EU and national legislation – somatic embryogenesis of Norway spruce in Finland

Tuija Aronen[1], Terhi Latvala[2], and Mikko Tikkinen[1]

[1] Natural Resources Institute Finland (Luke), Production systems, Vipusenkuja 5, FI-57200 Savonlinna, Finland
[2] Natural Resources Institute Finland (Luke), Bioeconomy and environment, Latokartanonkaari 9, FI-00790 Helsinki, Finland

tuija.aronen@luke.fi

Forests and forestry will encounter changes of unknown magnitude within the coming decades, due to changing climate and increased concern on biodiversity loss with the simultaneously growing demand of bio-based raw materials. In the Nordic, long rotations challenge anticipation to the future changes. Tree breeding can help in coping with these changes. The time span of implementing genetic gains may be shortened by using of somatic embryogenesis (SE). Introducing SE to forest regeneration in practise depends on perceptions about new technology, and on addressing legislation for forest reproductive material (FRM). Luke made a survey about perceptions on applying SE, among forest owners and professionals. The majority of the over 3000 respondents accepted the use of SE material to some extent, the average being 30 % of FRM. They considered depletion in genetic diversity as the biggest risk. The most valued traits in FRM were improved resilience and pest and pathogen resistance together with securing species' gene pool. Willingness to pay more for improved traits was indicated. In 2017, Luke registered SE basic material (type "*Parents of families*", category "*Qualified*") of Norway spruce *(Picea abies*) as FRM, enabling marketing and piloting mass-propagation. Currently Luke is composing novel SE lots for registration with higher genetic gain and/or differing deployment area. The trade of FRM within EU is regulated by Council Directive 1999/105/EC – and addressing this together with stakeholder expectations and national regulations is discussed.

Oral WG4&5-1

Harmonized communication by active members – the foundation for CopyTree's collaborative innovation

Valbona Sota[1,2]

[1]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania
[2]Science Communication Coordinator of CopyTree

valbona.sota@fshn.edu.al

One of the most important things in our society is doing the extraordinary through hard work and dedication. But if these findings are not communicated and disseminated in an understandable way to a large audience, all that is done has no value. Effective communication is a product not only of devotion but also teamwork. This is why WG5 (with 49 members so far), responsible for communication and dissemination, is a crucial point of CopyTree.
The two major goals of CopyTree are Research Coordination and Capacity Building, with the latter seeking to advance innovation and large-scale *in vitro* plant production by sharing and disseminating the existing experience in all facets of the micropropagation of woody plants. But how are we going to accomplish it? COPYTREE's WG5 aims to increase the visibility of the project objectives and results through communication channels, tools, and activities. The website, containing the online community platform and various social media channels are established to raise awareness among potential users and encourage them to participate in project events. This will attract potential beneficiaries/users of the project results and engage with key stakeholders and the public.
What's next? Creating useful and pertinent content, identifying the best ways to communicate with a particular audience, and, most importantly, collectively promoting. And this will work if there is teamwork and active members. The interrelations between WG5 and other WGs of the project must work harmonized and synergic to achieve higher visibility. If we stay connected, actively collaborate, and work hard for success, it undoubtedly will come back positively.

Oral WG4&5-2

CopyTree's Community Platform- A communication tool supporting strategic collaborative innovation

Rober Ahmad Kreimech [1,2]

[1] Strategic Advisor, Ghent University, Belgium
[2] Founder of BE4 Management, Belgium

info@be4management.be

In today's digital era, online platforms are essential for building thriving communities, as they have proven being effective tools to enhance efficiency through productive communication and remote collaboration.
Considering CopyTree's dual mission, "*Research Coordination*" and "*Capacity Building*", the need for an online platform has emerged soon and culminated in the construction of the CopyTree Community Platform (CCP).
Through its members-only area, the CCP aims to stimulate *intra-community* interactions and collaboration among community members. Whether members are in their office or on the go, by using CopyTree's mobile application, they should always be able to interact and publish posts and polls or upload and download documents using the file-share page.
Along with optimizing *internal* communication and collaboration, CopyTree's parallel challenge is to connect with and *inform external stakeholders* about relevant activities and innovative scientific results.
The latter challenges can only be overcome if the CopyTree community heads unidirectionally and cohesively along with a well-defined strategy, mission and *vision*.
In conclusion, efficient communication is fundamental for any community that tries to reach the intended outcomes. With this in mind, the CCP will be a self-evident tool to be used by the CopyTree community that aims to evolve towards its vision.

Oral WG4&5-3

Increasing the participation of young talents in biotechnological studies through effective communication language and channels

Şule Yalçin

Schulee Consulting, Istanbul, Turkey

sule.yalcin@schulee.com

The problems created by climate change in our world have turned all the attention to ingestion habits and making life sustainable. At the same time, the field of biotechnology is rapidly growing, and there is a need for young talent to contribute to the industry's development. However, the participation of young people in biotechnological studies is still relatively low. Effective communication language and channels are crucial in encouraging young talent to participate in biotechnological studies.

Social media is a powerful tool that can be used to reach out to young people and encourage them to pursue biotechnological studies. It can also be used to connect young people with industry professionals who can provide guidance and mentorship.

An effective communication effort is required to ensure public awareness of the results of laboratory research to be conducted within the extent of the European Network for Innovative Woody Plan Cloning Project and innovative strategies. Within the extent of this communication study, in addition to the public, academicians conducting research on plant science, biotechnology, and texture culture, as well as doctoral and master's students undergoing education should be targeted as a priority. Creating discussion groups in global scientific networks in the selected communication channels, sharing up-to-date project outputs on social media, and ensuring that young people are proactive on these platforms will increase awareness. In addition, offering internship opportunities to young people in the research laboratories where the project takes place and ensuring their active participation will contribute more to the awareness of the project.

In conclusion, effective communication language and channels are crucial in encouraging young talent to participate in biotechnological studies. By using social media, engaging with schools and communities, and providing mentorship and educational resources, we can increase their participation and help young people develop an interest in biotechnology. This can contribute to the growth and development of the industry while providing young people with exciting and rewarding career opportunities.

Micropropagation of forest trees: a tool for breeding programmes and production of new Forest Reproductive Material (FRM)

Francisco J. Lario, Beatriz Cuenca

TRAGSA, Vivero de Maceda, Carretera Maceda-Baldrei Km.2, 32700 Maceda, Ourense, Spain

bcuenca@tragsa.es

In breeding programmes for forest genetic resources, vegetative propagation techniques are essential because they exploit non-additive genetic variation and thus transmit the genetic potential of the selected genotypes. TRAGSA laboratory has been using micropropagation for this purpose since 2000, when we started a chestnut breeding programme to produce new rootstocks tolerant to *Phytophthora cinnamomi* (Pc) and better adapted to the climate and soil characteristics of Galician orchards. The new clones are currently being micropropagated in large quantities to supply the market. TRAGSA also carried out a selection programme of cork oak for high cork production and, later, for tolerance to the combined stress of Pc and drought, recognised as the main causes of "La Seca" disease. Clones were produced by somatic embryogenesis to deploy field trials, so they could soon be registered. Somatic embryogenesis of *Pinus pinaster* has become a very valuable tool in our laboratory also, to reproduce the progeny of genotypes tolerant to the pine wood nematode (*Bursaphelenchus xylophagus*). The germinated somatic embryos are evaluated while the collection is cryopreserved. Superior genotypes would be de-cryopreserved and amplified by somatic embryogenesis and rooted cuttings in series. *Alnus ssp.* is the other species for which TRAGSA is currently conducting a breeding programme for tolerance to *P. xalni* complex. Once surviving seedlings are available, micropropagation will be used to k copies for field trials and commercial purposes.

Applications of *in vitro* tissue culture technologies in Kosovo: Past and future perspective

Bekim Gashi[1], Valbona Sota[2], Mirsade Osmani[1], Efigjeni Kongjika[3]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina, 10000 Prishtina, Kosovo
[2] Deparment of Biotechnology, Faculty of Natural Sciences, University of Tirana, Tirana, Albania
[3]Academy of Sciences of Albania, Tirana, Albania

bekim.gashi@uni-pr.edu

Kosovo, as a developing country, with late research development and transition due to the last war, has made progress recently due to opening up to the scientific world. However, until now, no laboratory or institution has dealt with in vitro plant methodologies. Our primary collaboration is with the University of Tirana, with which, in the past, we have also done some joint studies in the laboratories of the Department of Biotechnology, FNS, UT. The outlook for this field is very good because at the University of Prishtina we are building the new building of the Faculty of Natural Sciences and the research laboratory of plant biotechnology, financed from IPA funds. We will implement our previous experiences in this field's research, particularly for the micropropagation and conservation of rare species from fruit trees. During our collaboration with the University of Tirana, we have worked on micropropagation and *in vitro* conservation *Ramonda serbica* and *Ramonda nathaliae* which are rare and endemic relict plant species from Balkan Peninsula. The highest number of shoots and multiplication rate was observed on JG-B medium supplemented with BAP and IAA (0.5 mg l^{-1} each). This experience constitutes an essential basis for implementing and optimizing *in vitro* methodologies aiming micropropagation and preservation of endangered plant species with high economic values in Kosovo.

"Sound of the forest", using science and music for an interactive experience about trees

Nieves Vidal[1], Conchi Sánchez[1], Tomé Mouriño[2], Marta Soto de Paz[3], Lidia Mouriño[3], Purificación Covelo[1]

[1] Dept. of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
[2] De Ninghures Musical Group, Santiago de Compostela, Spain
[3] Faculty of Educational Sciences-USC, Avda. Xoan XXIII, Santiago de Compostela, Spain

purificacion.covelo.abeleira@csic.es

The world of trees is important in Galician oral tradition. The memory of our often-illiterate ancestors is kept alive in traditional songs in which trees are sung about due to their beauty, symbolism, and importance in people's daily lives.
For years, our research group has been organising scientific events about trees attempting to bring them alive and closer to audiences of different ages and interests. We focused on showing *in vitro* cultures (micro-forests) to children and adult people, so they could touch everything and turn into micropropagators for a day. Recently we tried something different. Together with three young musicians involved in traditional music, we organised an event called "Son do bosque". In Galician this has a double meaning, "The sound of the forest" and "I am from the forest". Five traditional songs focused on trees, and a new instrumental work composed by our musicians were used for the event. We wrote nine stories giving a voice to an oak, a hazelnut, a cedar, an olive, a lemon, a wild pear, a cork oak, and a chestnut, plus our micropropagated trees, interspersed with the songs performed by all of us with audience participation. We presented it in the Galician City of Culture, within the cycle "Month of Science in the Library". Our aim was to highlight the importance of trees, their relations with other organisms and people lives, as well as to share some of the scientific research we carry out on them.

Funding: This work was funded by Xunta de Galicia (Spain) through the projects IN607A/2021 and "Contrato Programa" 2021 (AGI/CSIC I + D + I 2021, Ref-ACAM 20210200033).

Transmission of research results in the field of micropropagation to students of technical schools - case study

Liva Purmale, Rafaels Joffe, Anna Korica, Anta Sparinska

Bulduri Biotechnology center, Bulduri Technical School, Jurmala, LV-2010, Latvia

Liva.purmale@bulduri.lv

CopyTree is a platform that disseminates information to various stake holders - also Technical schools' students. Bulduri Agri-Food Technical school is located in Latvia and has experience over 110 years. It is the only Technical school in Baltic States that has a module on plant tissue culture. In spring 2023, the school hosted an International Symposium. The programme consisted of 5 e-poster, 4 live and 8 online presentations, half of which were devoted to plant tissue cultures and the rest-to the other plant biology areas (including a presentation about CopyTree). During the event students were given questioner (11 students, 17-19 years old, enrolled in 40-hour course about plant tissue culture). The questions were: Who is giving the presentation? What is the presentation about? What new things I have learned? Which one would you single out as your favourite presentation? After the event students had the option of submitting their work immediately or reading the abstract booklet, fill-in the missing information and hand it in later. Several students marked that their favourite presentations were from Ghana and Germany. It was stated that geographical origin of the speaker was important. Thus, we conclude that an invited speaker from foreign country is more likely to convey the desired message. Comparing the ways of giving presentations, we concluded that presentation (both face-to-face or online) is better format than e-poster to reach the Technical school students.

Production of ornamental plants commercially in biotechnological companies in Adana

Yeşim Yalçın Mendi[1] and Yıldız Aka Kaçar [1]

[1] Department of Horticulture, Faculty of Agriculture, University of Çukurova Balcalı, 01330 Adana, Turkey

ymendi@gmail.com; yildizakakacar01@gmail.com

With globalization in the world, the consumption of ornamental plants is increasing day by day. With the increasing consumption demand, production areas and production amount have increased both in the world and in Turkey. The rapid increase in energy prices in Europe, the lack of personnel to work, the new generation's disapproval of the sector, the rapid increase in transportation prices in the world and the opening of greenhouse areas have led European producers to regions that are close to them as a region and where production and energy costs are lower than themselves. In this case, as in many agricultural products in Turkey, in the ornamental plant's sector; stands out due to its geopolitical and geostrategic location. Therefore, as Turkey, we have to take steps to turn this problem of Europe into an advantage as a country.

Two tissue culture laboratories located in Adana Province produce ornamental plants such as *Phalenopsis* orchids, Anthurium, *Spathyphillium*, *Ficus*, *Monstera*, *Aloe vera*, *Philodendron* and Ferns using organogenesis, somatic embryogenesis, meristem and shoot tip culture. In addition to these species, they would like to start ornamental citrus production using shoot tip grafting techniques. However, recently, researchers aiming at higher quality and more efficient production as an alternative to classical tissue culture to increase international competitiveness have started to use bioreactor and semi-solid culture systems. Some of these projects such as micropropagation of *Anthurium*, *Phalenopsis*, *Aloe vera*, *Monstera*, and ornamental *Citrus* using bioreactor systems are supported by Tübitak (The Scientific and Technological Research Council of Turkey).

Biotechnological Studies in *Cyclamen sp*

Yeşim Yalçın Mendi[1], Metin Koçak[2], Aycan Alp[3], Başar Sevindik[4], Mehmet Tütüncü[5], Tolga İzgü[6], Senem Uğur[1]

[1] Department of Horticulture, Faculty of Agriculture, University of Çukurova Balcalı, 01330 Adana, Turkey
[2] Department of Agricultural Biotechnology, Faculty of Agriculture, Van Yuzuncu Yil University, Tuşba 65080, Van, Turkey
[3] Alata Horticultural Research Institute, Mersin, Turkey
[4] Department of Horticultural Plants, Vocational High School of İzmir Demokrasi University, İzmir, Turkey
[5] Department of Horticulture, Faculty of Agriculture, University of Ondokuz Mayıs, Samsun, Turkey
[6] National Research Council of Italy (CNR) IBE/Institute of BioEconomy, Sesto Fiorentino (Firenze), Italy

ymendi@gmail.com

Biotechnological techniques were used on *Cyclamen* breeding and production. A somatic embryogenesis protocol was developed using leaf and petiole explants from ten *C. coum* plants. In a half-strength MS medium containing different concentrations of NAA + Kinetin and NAA + BA, somatic embryogenesis was achieved from leaf and petiole explants. In addition, somatic embryos were encapsulated with sodium alginate (3% w/v) supplemented with MS, WPM, or ROM. In addition, he production possibilities of somatic embryos and synthetic seeds from the tuber tissues of *C. persicum, C. coum,* and *C. graecum* which are naturally grown in Türkiye, were investigated.

Haploidization possibilities via ovule and anther culture were also investigated in *C. persicum* and Melody F1. In *C. persicum* species, haploid embryo and diploid plants derived from these embryos formed from anther explants were cultured on BS medium containing 5µM NAA. As a result of ovule culture experiments of *C. persicum* and Melody F1 commercial variety, callus, embryo formations and shoots occurred from MS medium supplied with 2 mg/l 2,4-D + 0.8 mg/l 2iP and 2 mg/l 2,4-D + 0.5 mg/l 2iP, respectively.

In addition to haploidization, *in situ* parthenogenesis was tested in cyclamen first time and the effects of different irradiation doses on pollen viability, pollen germination and embryo formation after pollination with irradiated pollens were investigated. Flower buds were irradiated with different dosages of gamma light (0, 50, 100, 150, 200, 300, 450 Gy) using Co-60source. The flower buds pollinated with irradiated pollens were collected after pollination and appropriate developmental stages of the embryo for embryo rescue (ovule culture) were determined via histological studies. Moreover these studies, mutation breeding studies were also carried out. Two different cultivars of *Cyclamen persicum* were used. Seeds (0, 2, 5, 10, 15, 20, 35, 45, 65, 85, 110, 130 Gy) and microtubers (0, 2, 5, 10, 15, 20, 35, 45 Gy) Co-60 gamma irradiated with irradiation and suitable conditions were provided *in vivo*.

ISBN 978-84-09-50864-8