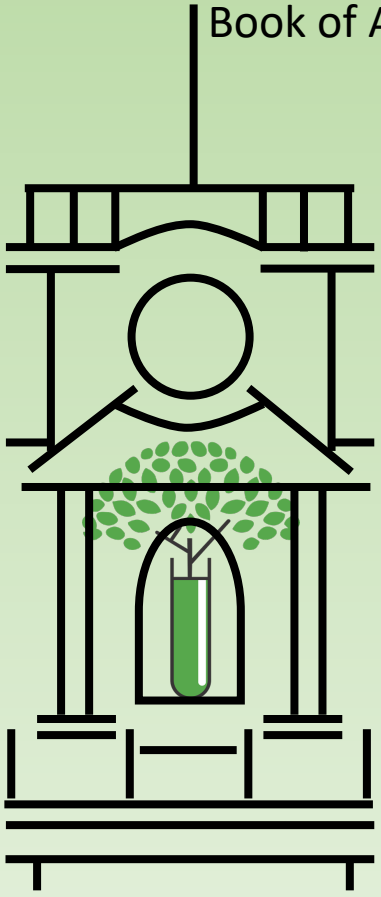
European Network for Innovative Woody Plant Cloning (CopyTree)

3rd Conference of Cost ACTION CA21157

Trees for the future: Cloning and beyond

Book of Abstracts



Centre for Functional Ecology
Department of Life Sciences
University of Coimbra

12 – 14 May, 2025

Funded by the European Union

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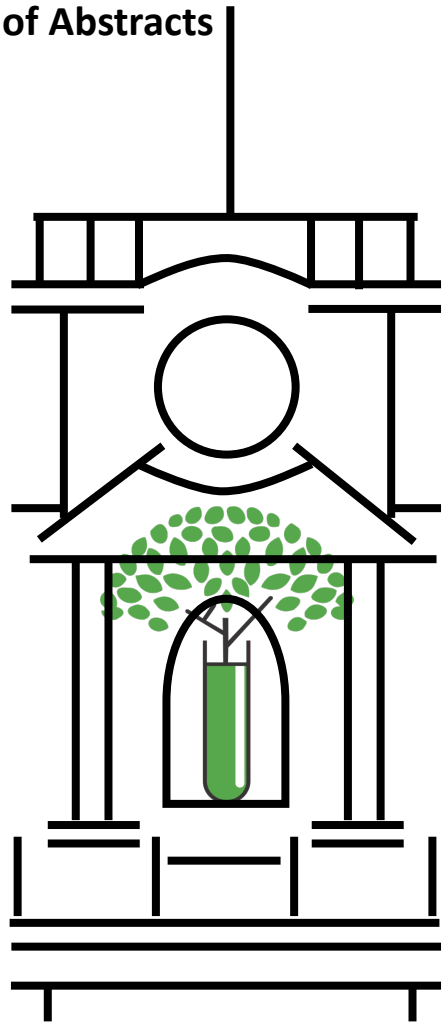
The third conference of Cost action CA2115, titled "**Trees for the future: Cloning and Beyond**", is organized by the Center for Plant Ecology of the Department of Life Sciences of the University of Coimbra.

Book of Abstracts



Venue:

Auditorium of the Rectorate of the University of Coimbra
Rua Larga, Coimbra, Portugal

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Tamarilo (*Solanum betaceum* Cav.), Solanaceae

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The Man Who Planted Trees (1953)

...The shepherd, who did not smoke, took out a bag and poured a pile of acorns out onto the table. He began to examine them one after another with a great deal of attention, separating the good ones from the bad. I smoked my pipe. I offered to help him, but he told me it was his own business. Indeed, seeing the care that he devoted to this job, I did not insist. This was our whole conversation. When he had in the good pile a fair number of acorns, he counted them out into packets of ten. In doing this he eliminated some more of the acorns, discarding the smaller ones and those that that showed even the slightest crack, for he examined them very closely.

...Having arrived at the place he had been heading for, he begin to pound his iron rod into the ground. This made a hole in which he placed an acorn, whereupon he covered over the hole again. He was planting oak trees.

Jean Giono, French, 1895-1970

http://www.pinetum.org/GionoUK.htm

Welcome

Dear colleagues,

A warm welcome to the third conference of our COST Action COPYTREE, entitled "Trees for the future: Cloning and Beyond." This event is a heyday of our scientific community and marks an important milestone in our common quest to share knowledge and stimulate innovation in the vital field of *in vitro* tree and woody plant production, achieved through robust networking and collaboration.

Our European COST Action is dedicated to woody plants that provide essential resources - timber, edible fruits, nuts, berries, fodder and herbal medicines - as well as those that have significant ecological, ornamental or cultural value. With the growing importance of micropropagation of trees and shrubs, driven by changing food demands and climate change, this conference directly addresses the critical challenges identified by our working groups: (1) recalcitrance, (2) sanitation and germplasm conservation, (3) scaling up and automation, (4) proactive risk management and public acceptance, and (5) commercialization.

This conference serves as an essential platform for researchers, industry leaders, and stakeholders to share key insights, present groundbreaking findings, and engage in impactful discussions that will undoubtedly shape the future trajectory of our discipline. The chairs and vice-chairs of the working groups have carefully selected the oral presentations, with an interesting line-up of keynote speakers. The poster session will further enhance this exchange, providing ample opportunities to share expertise and make valuable connections.

I would like to express my deep appreciation to Jorge Canhoto, Sandra Correia, and their dedicated colleagues for their extraordinary efforts in bringing this conference to fruition. I also extend my sincere gratitude to our Science Communication Coordinator, Valbona Sota, whose hard work behind the scenes has been instrumental in further developing our communication platform and the success of this meeting, and our Grant Holder, Lucie Fischerova, for her skillful management of the financial complexities over the past years. Our sincere thanks go to our supportive COST Science Officer, Ralph Stübner, and our Administrative Officer, Katchamon Nimprang, for their trust and guidance.

In closing, I extend my sincere gratitude to all our speakers and participants. for their invaluable contributions, which are essential to make this conference a truly collaborative and enriching experience. I wish you a highly productive and enjoyable congress, filled with fruitful learning and networking opportunities.

Yours sincerely,

Stefaan Werbrouck

Program

Monday, May 12

08.00 – 09.00 Registration and poster installation

09.00 – 09.15 Opening session

Working Group 1 – Recalcitrance
Chairs: Itziar Montalbán & Sandra Correia

09.15 – 10.00 **Plenary Lecture 1** - **Regulatory pathways for *de novo* root regeneration in forest tree species**. Carmen Díaz-Sala, University of Alcalá, Spain

10.00 – 10.15 **Somatic embryogenesis of native plants from Brazil: lessons learned for recalcitrant species**. Miguel Pedro Guerra, University of Santa Catarina, Brazil

10.15 – 10.30 **New understanding of meta-topolin riboside dynamics in micropropagated woody plants**. Maroua Grira, Ghent University, Belgium

10.30 – 11.00 **Coffee break**

11.00 – 11.15 **Exploring the role of rRNA methyltransferases in tamarillo (*Solanum betaceum* Cav.) somatic embryogenesis**. Ricardo Ferraz, University of Coimbra, Portugal

11.15 – 11.30 **Establishment of *in vitro* cultivation protocols for the recalcitrant European beech (*Fagus sylvatica* L.)**. Virginia Zahn, Thünen Institute of Forest Genetics, Germany

11.30 – 11.45 **Elucidating the effect of single gene modifications on drought stress tolerance of poplars (*Populus*)**. Alexander Fendel, Thünen Institute of Forest Genetics, Germany

11.45 – 12.00 **How poplar epitypes may serve as an experimental model to identify new drugs acting on *in vitro* tree regeneration (COPYTREE)**. Elena Carneros, CSIC, Spain

12.00 – 12.15 **Promising compounds for improving root formation in tissue culture shoots of *Pistacia vera* L**. Nur Koyuncu, Field Crops Central Research Institute, Turkey

12.15 – 12.30 **Enhanced root regeneration in olive *via* nanoparticle-mediated slow release of auxin**. Saba Taheri, Ghent University, Belgium

12.30 – 14.00 **Lunch**

14.00 – 14.15 **Exploring natural biostimulants for overcoming tissue culture recalcitrance: mechanisms and applications**. Paula Oros, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

14.15 – 14.30 **Innovative survey technique for the optimization of Blackberry micropropagation: The influence of subculture duration and initial explant density**. Luca Regni, University of Perugia, Italy

STSM Session 

14.30 – 14.45 **Isolation and characterization of extracellular vesicles from Pinus nigra embryogenic tissue**. Ján Jurčík, Institute of Plant Genetics and Biotechnology, Slovakia

14.45 – 15.00 **Overcoming rooting recalcitrance in olive micropropagation combining a controlled hormone release system under different light spectra**. Nourhene Jouini, University of Palermo, Italy

Working Group 4 – Risk assessment, commercialization
Chairs: Neslihan Yeşim Yalçin Mendi & Barbara Ruffoni

15.00 – 15.45 **Plenary Lecture 2** – **Propagation of *Eucalyptus* spp.: a key step to tackle biotic and abiotic threats**. Luís Fontes, Altri Florestal, Portugal

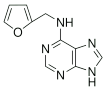
15.45 – 16.15 **Coffee break & poster session** 

16.15 – 16.30 **Deployment of vegetatively propagated forest trees: technical possibilities coupled to regulatory restraints and possibilities - A general outlook with a Swedish example**. Magnus Hertzberg, SweTree Technologies AB, Sweden

16.30 – 16.45 **Challenges and strategies for acclimatizing ornamental plants in the Mediterranean climate**. Soner Yağ, Çukurova University, Turkey

16.45 – 17.00 **From conventional to innovative *in vitro* production of saffron: Temporary Immersion Systems (TIS) and machine learning**. Waed Tarraf, Institute of BioEconomy, Italy

17.00 – 17.15 **How to inform the public about *in vitro* culture of woody plant species?** Paweł Chmielarz, Polish Academy of Sciences, Poland

17.15 – 17.45 **Ring test meeting** 

18.00 – 20.00 **Trees, flowers and drinks – Botanic Garden** 

Tuesday, May 13

08.30 – 09.00 Desk open

Working Group 3 – Scalling up and automation
Chairs: Nieves Vidal & Liva Purmale-Trasune

09.00 – 09.45 **Plenary Lecture 3** – **Are automation and robotization the future of micropropagation?** Maurizio Lambardi, IBE-Institute of BioEconomy, National Research Council, Italy

09.45 – 10.00 **Designing culture media: From cause-effect analysis to optimization using AI tools.** Pedro P. Gallego, University of Vigo, Spain

10.00 – 10.15 **Advancing automated somatic embryo analysis and germination prediction in Pinus radiata using AI and robotics.** Jana Krajnakova, Scion, New Zealand

10.15 – 10.30 **Computing artificial neural network and computational models for the feature optimization of in vitro organogenesis of ornamental woody plant - royal purple (*Cotinus coggygria* Scop).** Muhammad Asim, Sivas University of Science and Technology, Turkey

10.30 – 11.15 **Coffee break & poster session**

11.15 – 11.30 **Maturation temperature´s effect on amino acid, polyamine and ploidy levels in *Pinus radiata* somatic embryos**. Itziar Montalbán, NEIKER. Campus Agroalimentario de Arkaute, Spain

11.30 – 11.45 **Scaling up the Norway spruce SE production – maturation matters**. Saila Varis, Natural Resources Institute, Finland

11.45 – 12.00 **Electrochemical impedance spectroscopy for real-time monitoring of tamarillo (*Solanum betaceum* Cav.) embryogenic callus using a 3D porous electrode**. André Caeiro, University of Coimbra, Portugal

12.00 – 12.15 **RING TEST Investigation: Comparative analysis of CKX inhibitors in semi-solid and Plantform™ bioreactor cultivation of *Prunus domestica* L.** Brunilda Çuko, Research Center of Biotechnology and Genetics, Academy of Sciences, Albania

12.15 – 12.30 **Temporary immersion system for mass propagation of *Coffea arabica* shoots.** Giuseppe Natale Basile, University of Bari Aldo Moro, Italy

12.30 – 14.00 **Lunch**

14.00 – 14.15 **Optimizing sweet cherry rootstock micropropagation: improved performance of SETIS™ bioreactors supported by physiological and biochemical analysis.** Elsa Baltazar, University of Coimbra, Portugal

14.15 – 14.30 **Effect of different silver nanoparticle formulations on apricol micropropagation using Temporary Immersion System.** Nuria Albuquerque, Department of Plant Breeding, CEBAS-CSIC, Spain

14.30 – 14.45 **Tiny messengers from Trees: Exploring extracellular vesicle production in poplar and tamarillo calli.** Miguel Rito, University of Coimbra, Portugal

Working Group 5 – Communication
Chair: Valbona Sota

14.45 – 15.30 **Plenary Lecture 4** – ***Florestas.pt* an information benchmark for Portuguese forests.** Cristina Marques, RAIZ, Instituto de Investigação da Floresta e Papel, Portugal

15.30 – 16.15 **Coffee break & poster session**

16.15 – 16.30 **Perception about the potential use of micropropagated trees in forestry and reforestation in European countries.** Nieves Vidal, Misión Biológica de Galicia (MBG), CSIC, Spain

16.30 – 16.45 **A comparative analysis of tissue culture strategies in commercial propagation: A network analysis approach.** Buhara Yücesan, Bolu Abant Izzet Baysal University, Turkey

16.45 – 17.00 **Biotechnological strategy for *ex situ* plant propagation and reintroduction into the Atlantic islands of the Galicia National Park.** Tomás A Arteta, University of Vigo, Spain

17.00 – 19.00 **Working group meetings**

19.30 **Dinner and Fado**

Wednesday, May 14

08.30 – 09.00 Desk open

Working Group 2 – Diagnosis, sanitation & conservation
Chair: Claudia Ruta

09.00 – 09.45 **Plenary Lecture 5** – **Elimination of systemic pathogens from plants: advances, results and limits.** Ivana Gribaudo, Consiglio Nazionale delle Ricerche, Torino, Italy

09.45 – 10.00 **Preliminary attempts for implementing electrotherapy to eliminate pathogens in apricot plants.** Francisco J. Alfosea-Simón, Department of Plant Breeding, CEBAS-CSIC, Spain

10.00 – 10.15 **Recovery from viral infections through *in vitro* techniques in the autochthonous grapevine cultivar "Shesh I Zi".** Elektra Papakosta, Center of Agricultural Technology Transfer, Albania

10.15 – 10.30 **Using meristematic clumps as explants for long term conservation.** Hannes Wilms, INBO, Team Genetische Diversiteit, Belgium

10.30 – 11.15 **Coffee break & poster session** 

11.15 – 11.30 **Cryopreservation of embryogenic cell suspensions of *Aesculus flava*.** Slađana Jevremović, University of Belgrade, Serbia

11.30 – 11.45 **Effect of different plant vitrification solutions on cryotherapy efficiency of apricot shoot tips.** Marina Martín-Valmaseda, Department of Plant Breeding, CEBAS-CSIC, Spain

11.45 – 12.00 **Transcriptional reprogramming of defense responses in chestnut genotypes against *Phytophthora cinnamomi* infection.** Saleta Rico, Misión Biológica de Galicia-CSIC, Spain

12.00 – 12.15 **FTIR detects changes in macromolecules induced by iron and zinc in *in vitro* banana shoots under cadmium toxicity.** Doaa Elazab, Assiut University, Egypt

12.15 – 12.30 **Closing**

12.30 – 14.00 **Lunch** 

14.00 – 16.00 **MC meeting** 

16.00 – 18.00 **Walking guided tour**

Drinks by the river or in any other place

Thursday & Friday, May 15-16

Post congress tour 

Plenary lecture WG1

Regulatory pathways for *de novo* root regeneration in forest tree species

Daniela Cordeiro, Alberto Pizarro, Carmen Díaz-Sala
Department of Life Sciences, University of Alcalá, 28805 Alcalá de Henares, Madrid, Spain
carmen.diazsala@uah.es

Vegetative propagation has been used in commercial forests to propagate elite genotypes expressing yield traits of economic or ecological interest, such as wood quality or resistance to biotic or abiotic factors. The formation of adventitious roots by stem cuttings is an important step for successful vegetative propagation. Loss of competence to form adventitious roots impairs the propagation of high-quality genotypes for commercial production and forest plantations. In trees, the decline in the ability to form adventitious roots is a dramatic effect of age and maturation. Although the basic nature of the maturation-related decline in adventitious root formation has been the subject of many investigations, the mechanisms of cell-fate switching of fully differentiated progenitor cells to induce a root meristem in ectopic locations are still unknown. A role for the interaction between cell wall-plasma membrane and cytoskeleton has been proposed as being involved in the maturation-related decline of adventitious root formation in pine. Specific and dynamic changes in cell wall-cytoskeleton interactions affecting polarity in rooting progenitor cells prior to and after the irreversible decline of adventitious root formation may represent possible targets for the hormonal, developmental, environmental, and epigenetic regulation of the maturation-related decline in adventitious root formation.

Keywords: adult cell reprogramming, conifers, polarity

Plenary lecture WG2

Elimination of systemic pathogens from plants: advances, results and limits

Ivana Gribaudo

Consiglio Nazionale delle Ricerche, Torino, Italy

ivana.gribaudo@gmail.com

Diseases caused by plant pathogens such as viruses, viroids and phytoplasmas can cause significant economic losses in crop yield and quality, also limiting the safe movement of plant material across borders. The impact is particularly important in vegetatively propagated plants, as those pathogens are more likely to be passed on to the offspring with the propagating material. Suitable plant protection techniques are not available against systemic pathogens, therefore various methods – mainly based on tissue culture – have been developed for sanitation of candidate materials. Many factors can influence the efficacy of the applied strategy, including complex pathogen-host interactions, and pathogen eradication can be quite challenging. Most used are thermotherapy and meristem culture, but advanced methods such as chemotherapy, cryotherapy and somatic embryogenesis have been proposed and appear to be promising approaches.

Recently the availability of high-sensitivity molecular diagnostic tools (such as those based on High Throughput Sequencing) allows reliable and early diagnosis of pathogen infection as well as better evaluation of the efficiency and the accuracy of the adopted sanitation method.

Plenary lecture WG3

Are automation and robotization the future of micropropagation?

Maurizio Lambardi

IBE-Institute of BioEconomy, National Research Council (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino (Firenze), Italy

maurizio.lambardi@ibe.cnr.it

In Italy, micropropagation is an important sector of the national nursery industry. It is presently characterized by 26 commercial laboratories operating in the territory, almost 52 million high-quality plants produced annually, and a growth trend in the last decade of 2 million more plants each year. In particular, Italian laboratories hold a recognized leadership at European level in the *in vitro* production of a wide range of rootstocks and cultivars required in fruit growing. On the other hand, the commercial micropropagation cycle is still affected by a high level of hand labour in the various stages of the production cycle. As a result, some European countries, such as Italy, face the competition of micropropagated plants from Mediterranean areas characterized by lower labour costs. It is therefore increasingly necessary to invest in innovation in the sector. Numerous advances have been made in the automation and robotization of the activities that precede and follow the subculture phase, such as the preparation of the culture jars, the organization of the growth chamber, the transplant operations from *in vitro* to *in vivo* for acclimatization. The situation is different with regard to the subculturing activity, *i.e.*, the central heart of the micropropagation cycle: in this area, the prototypes of robotization of the procedure are always developed on species characterized by a certain "simplicity" of cutting and subculturing, while the development of subculturing prototypes suitable for "complex" species, that multiply *in vitro* through the division of crowded clusters of shoots, remains problematic.

Keywords: automation, fruit trees, micropropagation, robotization, rootstocks

Plenary lecture WG4

Propagation of *Eucalyptus* spp.: a key step to tackle biotic and abiotic threats

Luís Fontes, Ana Reis, Mário Louro, Luís Ferreira, Luís Leal

Altri Florestal SA, Quinta do Furadouro, 2510-582 olho-Marinho, Portugal

luis.fontes@altri.pt

Effective propagation of *Eucalyptus* spp. is crucial to address environmental and phytosanitary challenges particularly relevant in the context of climate change. Biotic threats, such as pests and diseases, alongside abiotic factors like drought and rising temperatures significantly negatively impact the productivity of eucalyptus plantations. Operational propagation strategies will be addressed, including both seed and clonal approaches, aimed at enhancing the production of *E. globulus* genotypes and eucalypt hybrids, with improved traits for resilience and adaptability. Key topics include the selection of resistant plant material, optimized operation and propagation techniques, and the integration of genetic and environmental knowledge to promote forest health and mitigate adverse impacts. Ongoing work aims at producing more resilient eucalyptus forests in the context of climate change and increasing pests and diseases.

Plenary lecture WG5

Florestas.pt an information benchmark for Portuguese forests

Cristina M. P. Marques

RAIZ, Instituto de Investigação da Floresta e Papel, Qta S Francisco Apt 15, 3801-501 Eixo, Portugal

Cristina.marques@thenavigatorcompany.com

From early time, the diversity and composition of forests have been profoundly influenced by humans. Overall, the global forest area has been reducing, currently occupying around 30% of the earth's surface. In Europe we have 38% forest coverage and in Portugal the forest represents 36% of the territory.

Our country hosts a diversity of trees, adapted to the landscape's topography, soil and climate: from 84 native (in mainland Portugal), to around 40 exotic species, and (alas) around 13 species that have become invasive (including 8 acacias).

Besides their economic and recreational use, forest areas perform other significant functions, contributing to conserve habitats, genetic resources and geological monuments. No less important it's their role in microclimatic protection, regulation of the hydrographic network and mitigation of erosion. However, public information about this invaluable ecosystems is scarce and often negatively biased.

The *Florestas.pt* platform, created in 2020, is an initiative of The Navigator Company under the technical scientific coordination of RAIZ, and the support of the national scientific community and various institutions related to the forest. Review contents result from the identification of reference sources, contextualized with historical and geographical information. Often times, they are revised by experts.

After almost 5 years online, we became an information benchmark. We invite you to browse state-of-the-art knowledge about Portuguese forestry and agroforestry spaces. Enjoy their relevance, challenges and opportunities in a clear, inspiring and accessible way!

Oral communications – WG1

Somatic embryogenesis of native plants from Brazil: lessons learned for recalcitrant species

Miguel Pedro Guerra[1], Ricardo Bittencourt[2]

[1]Full Professor, Graduate Programs in Plant Genetic Resources, Federal University of Santa Catarina, Admar Gonzaga Road, 1346, Florianópolis, Santa Catarina, 88034-000, Brazil, and visiting scientist FAPESC-FURB, Regional University of Blumenau, Forestry Engineering Graduate Program, São Paulo Street, 3250. Blumenau, SC, 89030-000, Brazil.

[2]Full Professor, FURB, Regional University of Blumenau, Forestry Engineering Graduate Program, São Paulo Street, 3250. Blumenau, SC, 89030-000, Brazil, ricbittencourt@furb.br.

miguel.guerra@ufsc.br

Brazil is home to the greatest biodiversity on the planet, with 8,715 tree species, representing 14% of the 60,065 woody species worldwide. An expressive portion of them are endemics and endangered, which reinforces the need to proper characterization, conservation and sustainable use. Somatic embryogenesis (SE) and associated techniques comprise effective tools for large-scale propagation of endangered species and/or elite plants. When combined with cryopreservation, they enable the establishment of cryogenic banks of endangered germplasm and elite embryogenic lines. Since the first SE protocol developed with the native palm *Euterpe edulis* in 1988, our research group expanded the research focus with other perennial and non-domesticated species from different Brazilian biomes. This is a challenging task since wild species are well known by their recalcitrancy to SE. Accordingly, we decided to establish at least one SE model system for each of the main plant taxonomic groups: monocots, dicots, and gymnosperms. Since then, we focused efforts to unraveling the bottlenecks in the development of SE protocols for (i) *Bactris gasipaes*, an amazon multipurpose palm; and native bamboo species; (ii) *Acca sellowiana*, a Myrtaceae fruit species native to south Brazil, under domestication in its diversity center, and the cocoa (*Theobroma cacao*); (iii) *Araucaria angustifolia* and *Podocarpus lambertii*, both native conifers threatened with extinction. The main bottlenecks of these model systems are presented and discussed.

New understanding of meta-topolin riboside dynamics in micropropagated woody plants

Maroua Grira[1], Els Prinsen[2], Stefaan Werbrouck[1]

[1]Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium.

[2]Integrated Molecular Plant Physiology Research, Department of Biology, University of Antwerp, Groenenborgerlaan 170, 2020 Antwerp, Belgium.

Maroua.grira@gmail.com

Topolin cytokinins have emerged as valuable tools in micropropagation. This study investigates the metabolism of meta-topolin riboside (mTR) in three distinct woody species: *Handroanthus guayacan*, *Tabebuia rosea* (*Bignoniaceae*), and *Tectona grandis* (*Lamiaceae*). Using labeled N^{15} mTR, we elucidated the complex mechanisms underlying cytokinin homeostasis, identifying N9-glucosylation as the dominant deactivation pathway. Remarkably, *T. rosea* and *H. guayacan* demonstrated the ability to reposition the hydroxyl group on the cytokinin molecule, a previously unexplored metabolic pathway. This study also highlights significant interfamilial and interspecies variations in mTR metabolism, challenging conventional views on callus tissue as a primary cytokinin storage site. These findings deepen our understanding of mTR metabolism and its implications for plant tissue culture, offering illuminating perspectives on cytokinin dynamics and their role in plant physiology.

Keywords: Cytokinin homeostasis, labeled meta-topolin riboside

Exploring the role of rRNA methyltransferases in tamarillo (*Solanum betaceum* Cav.) somatic embryogenesis

Ricardo Ferraz[1,2], Carlos Cação[3], Joana Ferreira[3], Paulo Gameiro[4], Sílvia Coimbra[2,5], Sandra Correia[1,6], Jorge Canhoto[1]

[1]Universidade de Coimbra, Centro de Ecologia Funcional, Laboratório Associado Terra, Departamento de Ciências da Vida, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[2]LAQV Requimte, Sustainable Chemistry, Universidade do Porto, 4169-007 Porto, Portugal
[3]Multidisciplinary Institute of Ageing (MIA-PORTUGAL), University of Coimbra
[4]NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Campo Mártires da Pátria 130, 1169-056 Lisboa, Portugal
[5]Faculdade de Ciências da Universidade do Porto, Departamento de Biologia, Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal
[6]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. Tamarillo is a solanaceous tree with well-established *in vitro* culture protocols that make it an appropriate experimental system to 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. Although the understanding of plant RNA-MTases is still emerging, recent studies have shown their role in development. In this study, firstly, a protocol for the genetic transformation of tamarillo NEC cells was developed, opening the way to reverse genetic analysis using this callus. Then, to determine the targets of the family of this rRNA-MTase, the CLIP (CrossLink and ImmunoPrecipitation) method will be performed. In this *in vivo* technique, tamarillo NEC cells are submitted to UV-C light to induce the crosslink between rRNA-MTases and their targets, which are subsequently immunoprecipitated. After isolating and reverse transcribing the RNA targets, the respective cDNA is amplified and sequenced. An antibody previously raised against a conserved exposed region of NEP-TC and validated through Western Blot and immunoprecipitation is being used. With this study, we expect to characterise rRNA-MTases targets to better understand their mode of action during SE.

Keywords: *Agrobacterium*-mediated transformation, non-embryogenic callus, RNA-protein crosslinking, Solanaceae, somatic embryogenesis

Establishment of *in vitro* cultivation protocols for the recalcitrant European beech (*Fagus sylvatica* L.)

Virginia Zahn[1], Franka Thiesen[2], Paweł Chmielarz[3], Alexander Fendel[1], Alice-Jeannine Sievers[1], Matthias Fladung[1], Ben Bubner[2], Tobias Bruegmann[1]

[1]Thünen Institute of Forest Genetics, Grosshansdorf, Germany

[2]Thünen Institute of Forest Genetics, Waldsieversdorf, Germany

[3]Institute of Dendrology, Polish Academy of Sciences, Kórnik, Poland

virginia.zahn@thuenen.de

European beech (*Fagus sylvatica*), the most common deciduous tree in Central Europe, is crucial for forest ecosystems and economically significant. Challenged by increasing drought and diseases ("beech dieback"), a sustainable *in vitro* cultivation of beech can contribute to its conservation and further biotechnological research using transgenic and CRISPR/Cas-based approaches. Despite its importance, beech remains highly recalcitrant to *in vitro* propagation, with only a few research groups addressing this challenge since the 1980s.

We established a sustainable *in vitro* culture of beech, covering initiation, propagation, and rooting. Various plant materials from provenance trial trees and seedlings were tested. The material source and the provenance were key factors for successful establishment. Explants from seedlings led to shoot formation rates of up to 94% with optimized media and antibiotics, with 26% of lines remaining sub-cultivable. Autotrophic growth conditions mitigated growth arrestment or bacteria overgrowth. Mass propagation was conducted with beech explants in a temporary immersion system and with refined media compositions. Stable mass propagation for over two years was successful for 109 clones. Rooting experiments and acclimatization are ongoing, with rooting rates of 70% in preliminary studies. Initial experiments for biotechnological approaches, including transient *Agrobacterium*-mediated transformation and protoplast-based techniques, have been successfully tested.

Keywords: recalcitrance, provenance trial, antibiotics, temporary immersion systems

Elucidating the effect of single gene modifications on drought stress tolerance of poplars (*Populus*)

Alexander Fendel[1], Matthias Fladung[1], Tobias Bruegmann[1]

[1]Thünen Institute of Forest Genetics, Genetic Technologies, Großhansdorf, Germany

alexander.fendel@thuenen.de

Climate change and fast-changing fluctuations of local climate conditions strongly affect forest tree viability. Especially drought stress appears as a harmful factor; however, the underlying genes related to drought stress tolerance are still not well understood. The late initiation of reproductive organs in forest trees decelerates tree breeding and hinders the research for genetic characterizations. However, for many tree species, the *in vitro* culture and its subsequent biotechnological modification represent an excellent system for functional gene studies. We apply genetic engineering and genome editing mechanisms of single genes in *in vitro*-grown plants of the European poplar hybrid *Populus* × *canescens*. Single genes, especially those potentially involved in stomatal aperture/density or proline accumulation, were either modified by CRISPR/Cas-generated loss-of-function mutations or constitutively overexpressed.

The rapid *in vitro* propagation of genetically engineered trees provided optimal conditions for comparative functional genetic analyses. Modified trees were then analyzed on their altered drought stress tolerance under controlled stress conditions in the greenhouse. Amongst others, the improvement of survivability rates of more than 40 % could be observed in different modified trees compared to wild types.

The *in vitro* culture approach enables a fast and reliable understanding of single gene contributions to the complex network of drought stress tolerance in trees. The identified key genes can be considered in targeted forest plant breeding approaches to obtain stress-tolerant trees, either by molecular selection from natural forests or via biotechnological approaches.

Keywords: Drought stress, *in vitro*, genetic modifications, *Populus* × *canescens*, gene networks

How poplar epitypes may serve as an experimental model to identify new drugs acting on *in vitro* tree regeneration (COPYTREE)

Elena Carneros[1], Alain Delaunay[2,§], Isabelle Le Jan[2], Alexandre Mary[2], Pilar S. Testillano[1], Stéphane Maury[2]

[1]Pollen Biotechnology of Crop Plants group, Margarita Salas Center of Biological Research (CIB) CSIC, 28040 Madrid, Spain

[2] P2e, Université d'Orléans, INRAE, EA 1207 USC 1328, Orléans 45067, France

[§]Deceased 7 August 2024.

ecarneros@cib.csic.es, testillano@cib.csic.es, stephane.maury@univ-orleans.fr

Epigenetics refers to heritable changes that occur during cell division (mitosis or meiosis) and affect the activity of genes and/or transposable elements without altering the DNA sequence. Plant *in vitro* culture involves various cellular processes such as cell dedifferentiation, acquisition of totipotency, and organogenesis or embryogenesis that involve epigenetic modifications. There is strong supportive and experimental evidence that recalcitrance is linked to epigenetic modifications and other omics layers, but the causality remains to be elucidated (Maury, 2024, https://doi.org/10.3390/f15010141; COPYTREE white paper 2025). Recent studies have shown a crucial role of genome-wide changes of DNA methylation in somatic embryogenesis of a tree, cork oak (Carneros et al. 2024, https://doi.org/10.1111/ppl.14143). Independent epigenetically modified poplar lines or epitypes (individuals with the same genome but with different epigenomes leading to different phenotypes) have been generated in recent years. They provide a relevant biological model to test the causality of DNA methylation variations on tree regeneration *in vitro*, but also to study the potential effects of different drugs and hormones on this process. Here, we will present our collaboration in the frame of COPYTREE with poplar epitypes, *in vitro* regeneration systems, preliminary results and next steps including new COPYTREE collaborators.

Keywords: Regeneration, Somatic embryogenesis, memory, epigenetics.

Acknowledgements: Work partially supported by the ANR EPIMYC Project (ANR-24-CE20-5751-01), and Projects PID2023-146824OB-I00, PID2020-113018RB-I00 (funded by MCIN/AEI /10.13039/501100011033), TED2021-129633B-I00 and CPP2021-008750 (funded by MCIN/AEI /10.13039/501100011033 and NextGenerationEU/ PRTR).

Promising compounds for improving root formation in tissue culture shoots of *Pistacia vera* L.

Nur Koyuncu[1], Yaprak Bölük[2], İ.Halil Yiğit[2]

[1]Department of Biotechnology, Field Crops Central Research Institute, Şehit Cem Ersever cd. 06170, Ankara, Türkiye

[2]Department of Plant Growing Techniques, Pistachio Research Institute, 27070, Gaziantep, Türkiye

nur.koyuncu@tarimorman.gov.tr

Advances in micropropagation techniques have helped produce true-to-type clones of many horticulturally important plants. A successful *in vitro* propagation system for commercial plants depends on a fast multiplication rate to produce shoots and a high rooting rate of these shoots. In recent years, the demand for clonal rootstocks well adapted to environmental conditions and high-yielding elite pistachio varieties has increased rapidly due to the request to establish new plantations. However, pistacia species have challenges at each stage of micropropagation that need to be carefully addressed. In this study, we explored the challenges faced during the rooting stage and investigated the impact of IBA (4,92, 9,84, 14,76 µM), phloroglucinol (PG, an auxin synergist), and paclobutrazol (PBZ, an anti-gibberellin), in combination or alone, on shoot rooting. During propagation of shoots on DKW basal medium, the growth regulator meta-topolin was used in combination with IBA to enhance shoot proliferation. In the first rooting experiments high doses of PG (392, 792, 1188 µM) and PBZ (1.7, 3.4, 6.8 µM) caused defoliation in agar-solidified medium. Then, adjusted PG and PBZ doses in combination with IBA on liquid culture medium increased both rooting rate and root number per shoot. These findings suggest that the use of these compounds may help to increase our understanding of the rooting pathways and alleviate rooting barriers in micropropagated pistachio shoots.

Keywords: Pistachio, *in vitro*, phloroglucinol, paclobutrazol

Enhanced root regeneration in Olive via nanoparticle-mediated slow release of auxin

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

[1]Department Applied Plant Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

[2]Nano-BioTechnology Group, Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

SeyehehSaba.Taheri@ugent.be

Slow release of plant growth regulators may be of great importance for regulating the induction of adventitious shoot or root meristems *in vitro*, as this is known to be influenced by gradients. We will present a novel delivery system employing auxin-loaded calcium carbonate microparticles (4 ± 0.5 µm) encapsulated within calcium alginate milliparticles (1 ± 0.2 mm), containing more than 10 million microparticles. The efficacy of this system was assessed in *Arabidopsis thaliana*, *Nicotiana tabacum*, and *Olea europaea* (olive), a species known for its recalcitrance to rooting. Results demonstrated a significant enhancement of root induction across all species. This research highlights the potential of nanotechnology-driven slow-release systems as a precise and effective tool for improving root regeneration efficiency, with additional applications for adventitious shoot induction and protoplast regeneration of recalcitrant woody plants.

Keywords: Recalcitrant, new tool, slow-release, auxin, rooting, microparticles

Exploring natural biostimulants for overcoming tissue culture recalcitrance: Mechanisms and applications

Paula Oros, Corina Cătană*

Center for Biodiversity and Conservation, Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5, Manastur Street, 400372 Cluj-Napoca, Romania

paula.oros@usamvcluj.ro, *corina_catana@yahoo.com

Tissue culture recalcitrance in woody plant species remains a significant challenge in plant biotechnology, limiting the efficiency of *in vitro* propagation for economically and ecologically valuable species. Biostimulants derived from organic sources such as algal extracts, yeast extract, casein hydrolysate, coconut water, chitosan, humic acids, amino acids, polyamines and plant growth-promoting microbes, have shown considerable promise in enhancing micropropagation success. This study categorizes their applications into four key functional roles. Yeast extract and casein hydrolysate provide essential amino acids and vitamins that stimulate cell division and differentiation, leading to improved organogenesis and somatic embryogenesis. Coconut water and algae extracts can reduce oxidative stress and phenol accumulation, acting as natural antioxidants. Chitosan and polyamines promote plantlets rooting and survival during *ex vitro* transfer. Additionally stimulating beneficial microbial interactions can enhance nutrient uptake and stress tolerance. By integrating the knowledge of the mechanisms through which these biostimulants improve micropropagation performance, along with case studies demonstrating their efficacy and optimal application methods for recalcitrant species, this paper demonstrates the adoption of biostimulant-based micropropagation, an approach that aligns with current global goals of sustainability and cost-effectiveness.

Keywords: biostimulants, natural growth promoters, tissue culture, sustainable micropropagation

Innovative survey technique for the optimization of Blackberry micropropagation: The influence of subculture duration and initial explant density

Luca Regni[1], Arianna Cesarini[1], Silvia Calisti[1], Raffaella Brigante[2], Laura Marconi[2], Sara Zollini[3]

[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italia

[2]Department of Engineering, University of Perugia, Via G. Duranti 93, 06125 Perugia, Italy

[3]Department. of Civil, Construction-Architectural & Environmental Engineering, University of L'Aquila, Piazzale Ernesto Pontieri 1, Monteluco di Roio - 67100 L'Aquila, Italy

luca.regni@unipg.it

Blackberry consumption and cultivation is increasing and plants are mainly propagated *in vitro*. This study examined the effects of explant density and subculture duration on the micropropagation of the 'Thornfree' cultivar. Four initial densities (10, 15, 20, and 30) and two subculture durations (30 and 45 days) were tested. Explant density did not affect the viability, number and length of shoots, presence and weight of callus, rooting percentage, number and length of roots, weight of fresh roots, weight of fresh shoots, and total dry weight of explants. On the contrary, the subculture duration had a significant impact. A 45-day subculture promoted more shoots, though shorter, and increased callus incidence and weight and rooting percentage. The area covered by proliferated shoots was evaluated through an Apple iPhone 15 Pro Max equipped with the viDoc RTK Rover. Through the integration with the Pix4Dcatch app, high-accuracy 3D models were generated. The obtained RGB point clouds were filtered by color to extract points related to the shoots. The highest area covered by each explant was at 45 days, especially with 30 explants. In the vessel with 30 initial explants, the available area was occupied faster than in other densities. This study provides insights into optimising micropropagation techniques for blackberries, considering for the first time the influence of subculture duration and initial explant density, also using innovative tools to monitor the shoot growth.

Keywords: *Rubus* spp, *in vitro* culture, berry crops, viDoc RTK rover

Oral communications – STM

Isolation and characterization of extracellular vesicles from *Pinus nigra* embryogenic tissue

Ján Jurčík[1], Miguel Rito[2], Miroslav Perniš[1], Katarína Klubicová[1], Sandra Correia[2,3]

[1]Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Akademická 2, P.O. Box 39A, 95007 Nitra, Slovakia

[2]Centre for Functional Ecology, Laboratory Associate TERRA, Departamento de Ciências da Vida, Universidade de Coimbra, Portugal

[3]InnovPlantProtect CoLAb, Estrada de Gil Vaz, 7350-478 Elvas, Portugal

jan.jurcik@savba.sk

Extracellular proteins are critical in regulating somatic embryogenesis (SE) by modulating cell division, cell-to-cell adhesion, communication, proliferation, and signal transduction. These proteins are released via the conventional protein secretion pathway, which involves an N-terminal signal peptide. However, many proteins present in the extracellular space lack this signal sequence. We hypothesize that extracellular vesicles (EVs) mediate the transport of proteins and other essential molecules to the extracellular space, analogous to mechanisms observed in different systems. In this study, we focused on isolating EVs from the embryogenic callus tissue of *Pinus nigra*. The callus tissue was infiltrated with a buffer solution, followed by sequential centrifugation and ultracentrifugation. The isolated samples were analyzed using nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) to confirm the vesicle-sized particles presence and assess their abundance profiles. Protein quantification demonstrated that the isolated EVs contained measurable levels of proteins. Our results indicate that EVs can be successfully isolated with adequate protein concentrations in sufficient quantities for downstream analysis, and LC-MS analysis is being conducted for further functional characterization.

Keywords: somatic embryogenesis, extracellular proteins, extracellular vesicles, calli

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Overcoming rooting recalcitrance in olive micropropagation combining a controlled hormone release system under different light spectra

Nourhene Jouini[1], Saba Taheri[3], Elsa Baltazar[2], Stefaan P.O. Werbrouck[3], Jorge Canhoto[2], Tiziano Caruso[1]

[1]Department of Agricultural, Food and Forestry Sciences (SAAF), University of Palermo, Viale delle Scienze - Ed. 4, 90128 Palermo, Italy

[2]Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

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

nourhene.jouini@unipa.it

Olive propagation is hindered by the species' recalcitrance to rooting, thus limiting large-scale production. To address this challenge, we explored in vitro olive micropropagation to identify rootstocks with high rooting potential for in vitro micrografting (IVM). Rooting experiments were conducted on diverse cultivars, including ‘Galega Vulgar’, ‘Arbequina’, ‘Chemlali’, ‘G28’, and ‘G35’ (Koroneiki-derived F2 genotypes developed by the University of Palermo, SAAF Department). In collaboration with the university of Ghent, a novel slow-release system utilizing mesoporous $CaCO_3$ microparticles was employed for sustained auxin delivery, achieving 100% rooting success in ‘Arbequina’ and ‘Chemlali’. Additionally, optimized light spectra significantly improved rooting in the recalcitrant cultivars ‘Galega Vulgar’. These findings demonstrate the effectiveness of combining controlled hormone release and targeted light treatments to enhance olive micropropagation, facilitating the selection of superior rootstocks for future IVM applications.

Keywords: Micrografting, microparticles, rootstocks, slow-release system

Oral communications – WG2

Preliminary attempts for implementing electrotherapy to eliminate pathogens in apricot plants

Francisco J. Alfosea-Simón[1], Liva Purmale-Trasune[2], Lorenzo Burgos[1], Nuria Alburquerque[1]

[1]Group of Fruit Tree Biotechnology, Department of Plant Breeding, CEBAS-CSIC, University Campus at Espinardo, Bldg. 25, Murcia, 30100, Spain

[2]Bulduri Biotechnology Centre, 6 Viestura Street, LV- 2010 Jurmala, Latvia

fjalfosea@cebas.csic.es

Electrotherapy is a simple, cost-effective plant sanitation technique and a promising alternative to other methods. During a Short-Term Scientific Mission (STSM) in Latvia, this technique was tested on apricot (*Prunus armeniaca* L.) shoots infected with *Plum Pox Virus* (PPV) and *Hop stunt viroid* (HSVd). A total of 60 shoots (30 per pathogen, 10 per treatment) were subjected to electrotherapy inside an electrophoresis tank. Three intensity treatments (10 mA, 20 mA and 30 mA) were applied for 10 minutes. After electrotherapy, meristems were rescued under sterile conditions. Twelve weeks later, pathogen elimination was evaluated by RT-PCR on surviving meristems. Survival ranged from 50% to 90% and the best PPV elimination rate was found in the treatment with the highest intensity. However, it was not possible to eliminate HSVd.

To improve the methodology, the technique was adapted in Spain performing all steps under sterile conditions in a laminar flow hood. The electrophoresis tank was sterilized with 50% sodium hypochlorite for 30 minutes, followed by UV exposure. This adjustment simplifies the workflow for routine protocols. Given the high survival rates, further research aims to apply more intensive treatments or combine electrotherapy with other methods (chemotherapy, silver nanoparticles, etc.) to enhance pathogen elimination.

Keywords: electrotherapy, pathogen elimination, *in vitro*, *Prunus armeniaca*, methodological approach

Recovery from viral infections through *in vitro* techniques in the autochthonous grapevine cultivar "Shesh i Zi"

Elektra Papakosta[1], Orges Cara[2]

[1]Center of Agricultural Technology Transfer, Vlora, Albania
[2]University "Aldo Moro", Bari, Italy

elektrapapakosta@gmail.com

Local grapevine cultivars in Albania hold significant importance as they represent rich genetic diversity of the country, its cultural heritage, and potential for producing unique, high-quality wines that can compete in international markets. The preparation and implementation of scientific protocols for analysing the genetic identity and phytosanitary status of propagating material are essential for the production of healthy grapevine seedlings. This study, conducted during the years 2021–2023, analysed the autochthonous grapevine cultivar " Shesh i Zi " to evaluate its phytosanitary status. The analyses were performed using the DAS-ELISA serological method to detect the presence of GFLV, GFkV, GLRaV-1, GLRaV-3, and GVA viruses, employing kits from Agritest, Italy. The results showed that 6 out of 18 samples tested were infected with the GLRaV-3 virus (*Grapevine Leafroll-associated Virus 3*). To eliminate this virus, the meristem culture technique was applied using MS medium. A total of 35 meristems were isolated from apical shoots. After 40 days in the vegetative growth chamber, 27 explants were obtained and their micropropagation was carried out. The grapevine plantlets were also subjected to *in vitro* thermotherapy, treated for 2 months: initially at 30 °C, followed by therapeutic treatment at 38 °C for one week. After the acclimatization and growing in "screen house", 83% of the analysed plants were confirmed virus-free from GLRaV-3, demonstrating the success of the procedure. The recovery of virus-free plants is an important step to start a rigorous certification program for the selection of the most interesting autochthonous cultivars before they are eventually lost and to provide greater market opportunities for local nurserymen, aiming toward the future accession of Albania to the EU.

Keyword: local grapevine cultivar, meristem culture, thermotherapy, GLRaV-3, DAS-ELISA

Using meristematic clumps as explants for long term conservation

Hannes Wilms[1,2] ,Natalia Fanega Sleziak[3], Bart Panis[2,3]

[1]INBO, Team Genetische Diversiteit, Gaverstraat 4, 9500 Geraardsbergen, Belgium

[2]KU Leuven, Department of Biosystems, Willem de Croylaan 42, 3001 Heverlee, Belgium

[3]Bioversity International, Belgian office, Willem de Croylaan 42, 3001 Heverlee, Belgium

Hannes.wilms@inbo.be

Breeding programmes but also agriculture in general depend on the availability of the widest possible genetic diversity within of a species. The conservation of genetic resources is thus extremely important Whilst for many plant species multiple conservation methods have been developed, for some species with recalcitrant seeds such as the coconut palm (*Cocos nucifera*) the tools are limited. However, with the recent development of a new type of explant, coconut meristematic clumps, resulting from a novel shoot micropropagation method, this type of explant could be used for the long term conservation via cryopreservation. Meristematic clumps contain multiple meristems per explants which increases the chance for survival after cryopreservation since each meristems on the explant has the potential to grow. We opted for droplet vitrification and optimized the protocol by varying preculture time and composition, PVS2 treatment time, and explant size. After cryopreservation the explants were checked if they were still able to proliferate. In the current protocol around 50 % of explants survived liquid nitrogen exposure. However, subsequent browning during growth caused a 30% drop, resulting in 22% or less of explants that formed new meristems after cryopreservation. The material that survived was able to proliferate and regenerate into rooted plants, showing the potential of this explant for long term conservation purposes.

Keywords: Cryopreservation, Cocos, Meristematic tissues, Droplet vitrification

Cryopreservation of embryogenic cell suspensions of *Aesculus flava*

Slađana Jevremović, Dušica Ćalić, Jelena Milojević, Maja Belić, Snežana Zdravković-Korać

Department of Plant Physiology, Institute for Biological Research Sinisa Stankovic – National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11108, Belgrade, Serbia

sladja@ibiss.bg.ac.rs

In woody plants, obtaining highly regenerative embryogenic cell lines is a difficult and time-consuming process and the lines can be lost through constant subcultivation. Cryopreservation is the recommended method for long–term storage until they are needed for use. In this work, different methods for cryopreservation of *Aesculus flava* embryogenic cell suspensions of were investigated. The efficacy of three cryopreservation methods was evaluated, which included slow-freezing, encapsulation slow-freezing and encapsulation-vitrification method. Cell suspensions were pretreated with 7.5% DMSO prior to slow-freezing with the MrFrosty system prior to immersion in liquid nitrogen. Immobilised cell suspensions were pre-cultured in liquid medium for three days and in 0.9 M sorbitol for two days before pretreating with 5% DMSO and slow freezing and immersion in liquid nitrogen. In the encapsulation-vitrification method, the immobilised cell suspension beads were pretreated with PVS2 solution for 30, 60 and 90 minutes before being directly immersed in liquid nitrogen. The best results were obtained with the slow-freezing method and not with the direct immersion method after vitrification. According to our results, slow cooling is the most efficient method for cryopreservation of embryogenic cell cultures of *A. flava*. Treatment with DMSO prior to immersion in liquid nitrogen enabled 75-100% regrowth of suspension and subsequent reconstitution of *A. flava* embryogenic suspensions.

Keywords: yellow buckeye, slow freezing, DMSO, vitrification, somatic embryos

Effect of different plant vitrification solutions on cryotherapy efficiency of apricot shoot tips

Marina Martín-Valmaseda, Cristian Pérez-Caselles, Elena Yelo, Lorenzo Burgos, Nuria Alburquerque

Group of Fruit Tree Biotechnology, Department of Plant Breeding, CEBAS-CSIC, Campus de Espinardo, 25, 30100 Murcia, Spain

mmartin@cebas.csic.es

Virus infection has negative effects on stone fruit trees production, being Plum pox virus (PPV) one of the most important viruses infecting *Prunus* species worldwide. Therefore, there is an increasing interest in the development of protocols to produce virus-free *Prunus* plants. Cryopreservation protocols, such as droplet-vitrification, can be applied as cryotherapy for virus eradication. These usually consist of the excision of the shoot tips, a preculture phase for dehydration, application of a loading solution, followed by a Plant Vitrification Solution (PVS), freezing on liquid nitrogen and rewarming in Unloading Solution.

In droplet-vitrification protocols PVS are essential for achieving proper vitrification of meristematic cells, ensuring their survival. The choice and concentration of cryoprotectants can significantly impact on shoot tip survival. In this work, PVS2 and two PVS3 with different sucrose and glycerol concentrations (40%-40% and 50%-50%), have been applied to apricot shoot tips infected with PPV. Regeneration after each step of the protocol has been analysed. The best regrowth rate was obtained when applying PVS3 40%-40%. Virus infection of surviving shoots from cryotherapy was evaluated by RT-PCR 5 and 7 months after cryotherapy. Good efficiency of virus eradication after cryotherapy has been obtained with all three PVS used.

Keywords: Droplet-vitrification, cryoprotectants, *Plum pox virus*, *Prunus armeniaca*, virus eradication

Transcriptional reprogramming of defense responses in chestnut genotypes against *Phytophthora cinnamomi* infection

Saleta Rico[1], Jesús Vielba[1], Patricia Fernández-Calvo[1], Beatriz Cuenca[2], Nieves Vidal[1], Conchi Sánchez[1]

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

[2]Maceda Nursery, TRAGSA-SEPI Group, Carretera de Maceda a Baldrei km 2, 32700 Maceda, Spain.

saleta@mbg.csic.es

Ink disease, caused by *Phytophthora cinnamomi*, threatens European chestnut (*Castanea sativa*) populations. Resistance varies among genotypes, suggesting a genetic basis for tolerance and highlighting the need to understand molecular defense mechanisms.

This study investigates the transcriptional response of two genotypes with different resistance levels to *P. cinnamomi*: CS12 (*C. sativa, susceptible) and* PO11 (*C. sativa* × *C. crenata*, resistant). *In vitro* plantlets were inoculated and sampled at 24, 48, and 72 hpi for RNA-seq analysis. Our results reveal a dynamic activation of defense responses with progressive engagement of diverse mechanisms. DEGs involved in signal transduction, transcriptional regulation (including MYB, WRKY, and NAC factors), and defense-related processes (e.g., kinases, lectin-domain receptors, and pathogenesis-related proteins) were identified. Pathways such as plant-pathogen interactions, MAPK signaling, and hormone-mediated defense are also highlighted, with differences between genotypes. PO11 exhibits a stronger, more coordinated response over time, contributing to its resistance. In contrast, CS12 shows an impaired early activation followed by a sharp increase at later times, suggesting a failure in prompt pathogen detection and immune response.

These findings provide insights into the temporal dynamics of chestnut immunity to *P. cinnamomi* and identify key molecular players for breeding and biotechnological strategies against ink disease.

Keywords: Chestnut, *Phytophthora cinnamomi*, ink disease, transcriptomics, defense

FTIR detects changes in macromolecules induced by iron and zinc in *in vitro* banana shoots under cadmium toxicity

Doaa Elazab[1,2], Aylin Ozudogru[3], Claudia Ruta[4], Maurizio Lambardi[2]

[1]Department of Pomology, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt.
[2]IBE—Institute of BioEconomy, National Research Council (CNR), 50019 Sesto Fiorentino (Florence), Italy.
[3]Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics,Istinye University Istanbul, Zeytinburnu, Istanbul 34010, Turkey.
[4]Department of Agricultural and Environmental Science, University "Aldo Moro" of Bari, 70125 Bari, Italy

doaa.elkassas@agr.au.edu.eg

Cadmium (Cd) is a widespread and strongly toxic environmental pollutant. This study investigated the interaction between Cd and essential nutritional metals such as iron and zinc in banana plants (Musa spp., cultivar Grand Nain), cultured *in vitro*, using Fourier transform infrared (FT-IR) and physiological analysis. Plantlets were treated *in vitro* with Fe and Zn (200 and 500 mg/L) under 500 mg/L Cd exposure. The results showed that Cd toxicity increased Cd uptake and raised % of damage. However, Fe and Zn addition ameliorated the negative impact of Cd by enhancing Fe, Zn, phosphorus, and potassium contents. The FT-IR analysis showed alterations within the bands correlated to the foremost macromolecules in plants under Cd stress and its interactions with Fe or Zn. The peak of some functional groups for carbohydrates, proteins, alcohols, phenolic compounds, lipids, amide I, amide II, cellulose and hemicellulose, and aromatic compounds were negatively shifted by Cd stress. However, Fe and Zn regulated the transmittance and intensity of these bands, showing improved tolerance to Cd. Moreover, Fe and Zn modulated the total antioxidants and enzymatic antioxidant activities for catalase and ascorbate peroxidase. The study showed that the nutrition with Fe and Zn enhanced in vitro banana tolerance against Cd toxicity. It also highlighted the powerful role of FT-IR in understanding the mechanisms involved in minimizing Cd toxicity in banana shoots under Fe and Zn.

Keywords: Heavy metals; Cd-damage; FT-IR; antioxidants, banana

Oral communications – WG3

Designing culture media: From cause-effect analysis to optimization using AI tools

Pedro P. Gallego, Tomás A. Arteta

Agrobiotech for Health, Department of Plant Biology and Soil Science, Faculty of Biology, University of Vigo, Vigo, Spain.

pgallego@uvigo.es

Artificial intelligence (AI) tools revolutionize plant biotechnology, offering intelligent solutions for novel culture media development. AI surpasses traditional statistics with its flexibility and ability to incorporate and reanalyze data for enhanced insights. Neurofuzzy logic, combining fuzzy logic with artificial neural networks (ANNs), exemplifies this, modeling complex data and expressing results as interpretable linguistic rules. This technology identifies key factors influencing plant growth in vitro. Once these factors are understood, genetic algorithms (GAs), optimization algorithms inspired by natural evolution, are employed to design optimized culture media. AI-driven experimental design and modeling, like neurofuzzy logic and GAs, provide advantages over traditional methods, especially with numerous variables common in biological processes. This approach facilitates efficient and informed culture medium design.

Keywords: neurofuzzy logic, genetic algorithms, experimental design, data modeling, plant tissue culture

Advancing automated somatic embryo analysis and germination prediction in *Pinus radiata* using AI and robotics

Sam Davidson[1], Cathie Reeves[1], Sakari Valimaki[2], Taryn Saggese[1], Celine Mercier[1], Guillermo Salvatierra[1], Elmar Bernhardt[3], Mikko Tikkinen[2], Tuija Aronen[2], Jana Krajnakova[1]

[1]Scion, Rotorua, New Zealand

[2]Natural Resources Institute (Luke), Finland

[3] South-Eastern Finland University of Applied Sciences (Xamk), Finland

jana.krajnakova@scionresearch.com

The advancement of somatic embryogenesis automation relies on the integration of machine vision, machine learning, and deep learning techniques. We have analyzed sets of individual microscopic images of *Pinus radiata* mature somatic embryos, representing different embryogenic cell lines, and have achieved notable progress using machine learning and deep learning. Our machine learning model accurately predicts germination success, achieving performance comparable to expert biologist assessments.

Additionally, our deep learning pipeline precisely segments cotyledon and hypocotyl regions in images of mature somatic embryos, with instance segmentation automatically detecting and counting individual cotyledons.

Furthermore, we report the first trials of using the robot (developed by Luke & Xamk, Finland) for automated embryo sorting in radiata pine, demonstrating its potential to enhance selection efficiency in large-scale propagation systems.

Key words: *Pinus radiata*, deep learning, machine vision, robotics, germination algorithm

Automation in tissue culture – Transforming the transplanting process of tissue-cultured plants

Marten Elsinga, Michiel van Bennekom

Iribov R&D Tissue Culture, The Netherlands

marten.elsinga@iribov.com

The large-scale production of tissue-cultured plants remains a labour-intensive process. While much of the propagation is carried out in low-cost regions, hardening often takes place closer to the market, in areas where labour is increasingly expensive or unavailable. This presents a bottleneck in upscaling tissue culture production and highlights the need for innovative, labour-efficient solutions.

At Iribov, an industrial plant tissue culture company, we have developed and implemented an automated transplanting system based on our proprietary TC-tray. This tray is designed to enable automated transplanting of tissue-cultured plantlets from agar-plugs into soil-plugs, allowing for a large reduction in manual handling while upholding high quality standards. In this poster, we present an overview of our TC-tray system and its application in an automated transplanting workflow.

With our automated process as example, we will also provide an overview of the considerations that preceded this development, the challenges we have solved to come to the current process, the effectiveness of the process compared to conventional manual transplanting and the next steps to further improve the product.

While this project originates from an industrial need to increase labour efficiency, it also opens opportunities for collaboration with academia. For example, insights from our automation-related challenges can inspire new research on future culturing systems and automation focussed propagation. By sharing our experience, we hope to contribute to a broader understanding of how automation can support sustainable growth in plant tissue culture, and how industry-developed tools like our TC-tray can be of interest to both industry and academia.

Keywords: Agar-plug, automation, Labour-efficiency, T TC-tray, Tissue Culture

Computing artificial neural network and computational models for the feature optimization of in vitro organogenesis of ornamental woody plant - royal purple (*Cotinus coggygria* Scop)

Muhammad Asim

Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, Sivas, Turkiye

mshazim@gmail.com

This study presents the in vitro regneration protocol for Royal purple [(*Cotinus coggygria* Scop. (syn.: *Rhus cotinus* L.)] from nodal segment explants followed by optimization. Optimization of input factors were done by response surface optimization with factorial regression optimization of design of experiment. Results were compared with artificial intelligence based Genetic Algorithm (GA), aided with PyTorch ANN. Experimental setup was comprised of two different nutrient medium (MS & WPM), two BAP concentrations (1.0 & 2.0 mg/L), with 0.1 mg/L or without IBA. Application of different machine learning models revealed the highest R^2 values for both output variables, acquired by using MLP model in PyTorch platform. Response optimization was perfromed was based on type of basal medium, while GA provided optimized protocol by considering all combinations. Combination of 1.25 mg/L BAP, 0.02 mg/L NAA, and 0.03 mg/L IBA in WPM medium was found optimum for 100 % regeneration with the help of NSGA-II algorithm. Whereas, model yielded multiple combinations for shoot counts parameter. Results revealed the successful use of computation models and AI models for optimizing multiple input variables for in vitro micropropagation.

Keywords: Artificial intelligence, Genetic algorithm, Optimization, Woody ornamental, Design of experiment

Maturation temperature´s effect on amino acid, polyamine and ploidy levels in *Pinus radiata* somatic embryos

Ander Castander-Olarieta[1], Antonia Maiara Marques Do Nascimento[1], Luiza Giacomolli Polesi[2], Franklin Back[2], Neusa Steiner[3], Miguel Pedro Guerra[2], Paloma Moncaleán[1], Itziar Aurora Montalbán[1]

[1]NEIKER. Campus Agroalimentario de Arkaute, s/n, N 104 – Km 355, 01192. Arcaute, Álava. Spain.
[2]Laboratório de Fisiología do Desenvolvimento e Genética Vegetal, Universidade Federal de Santa Catarina, Florianópolis, Brazil
[3]Departamento de Botânica, Universidade Federal de Santa Catarina, Florianópolis, Brazil

imontalban@neiker.eus

One of the hypotheses behind our work during the last years is based on the application of high temperatures at different stages of somatic embryogenesis to obtain plants better adapted to climate change. However, it is important to assess the effect of these temperatures on plant development. In this context, we investigated the impact of high temperature pulses applied during the maturation phase (control 23 ºC for 16 weeks, 40 ºC for 90 min, 50 ºC for 30 min or 60 ºC for 5 min) on the genetic stability and on the profile of different metabolites of somatic embryos. Further, at the end of the germination phase, different morphological parameters of the plantlets (number of needles and secondary roots, total length, length of the aerial part, width of needles, stem diameter and length of the primary root) were measured.
The results indicated that maturation temperature had no significant effect on the ploidy and genome size of phenotypically normal somatic embryos. Significant differences were observed between somatic embryos from different maturation temperatures for the levels of several amino acids involved in the synthesis of polyamines, such as glycine, arginine, lysine, and ornithine. In this sense, putrescine levels were higher in somatic embryos from embryogenic tissue subjected to the highest maturation temperature. Finally, the maturation temperature did not significantly impact on the morphology of the generated plantlets.

Keywords: climate change, genetic stability, germination, metabolites

Scaling up the Norway spruce SE production - maturation matters

Saila Varis, Sakari Välimäki, Mikko Tikkinen, Tuija Aronen

Natural Resources Institute Finland (Luke), Vipusenkuja 5, FI-57200 Savonlinna, Finland

saila.varis@luke.fi

When scaling up the somatic embryogenesis for commercial production well performing cell lines should be initiated, i.e., lines that have vigorous proliferation, survive cryopreservation, and produce a large number of somatic embryos after cryopreservation. The embryo yield is one of the essential traits when the profitability of individual cell line is evaluated. Many steps during the process affects the outcome of embryo maturation.

We analyzed the data from the Norway spruce SE lines initiated in six different years, including a total of 119 families and over 13,000 cell lines. Over 8,700 maturation results are included from continuously subcultured lines and selected group are also cryopreserved and thawed. Also, we have a selected group of lines subcultured and maturated regularly over two years' time. We used several genetic, environmental, and operational variables to explain the survival from cryopreservation and maturation success. For example, the choice of gelling agent in the maturation media had a clear impact on maturation yield.

Overall, the mean embryo production capacity was 87 E/g FW among the cell lines which produced embryos. The best cell lines within families produced between 6 to 687 E/gFW (119 families). Good embryo producer (cell line yielding least 100 E/gFW) was found from family when at least 16 cell lines could be matured (65 families).

Further improving the maturation protocols is important to produce more cotyledonary embryos and reduce the occurrence of premature embryos, facilitating automated germination. Additionally, more robust maturation protocols are vital for utilizing a wider range of genotypes have shown strong performance in field tests.

Keywords: cryopreservation**,** embryo productivity, subculturing, tissue aging

Electrochemical impedance spectroscopy for real-time monitoring of tamarillo (*Solanum betaceum* Cav.) embryogenic *callus* using a 3D porous electrode

André Caeiro, Francisco C. Cotta, Jorge Canhoto, Paulo R.F. Rocha

Centre for Functional Ecology, Laboratory Associate TERRA, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

andrecaeiro91@gmail.com

Tamarillo (*Solanum betaceum* Cav.) is a solanaceous tree cultivated globally for its edible fruits. In tamarillo, somatic embryogenesis (SE) has been extensively described, with several protocols starting from different explants. For instance, when leaf segments are cultured in Murashige & Skoog medium (1962) supplemented with picloram, an indirect pathway occurs, generating embryogenic (EC) and non-embryogenic (NEC) *callus*. Somatic embryo development is then triggered by auxin removal.

Electrochemical impedance spectroscopy (EIS) is an analytical technique that can monitor electrochemical systems across a wide frequency range. It has various biological applications, namely crop monitoring. Previously, we have demonstrated that an EIS apparatus with planar gold electrodes can track NEC development and its response to environmental stimuli (auxin withdrawal), correlating well with cell growth and mass increase. However, likely due to its more complex morphology, EC was not completely compatible with this setup.

Here, we present a 3D porous electrode coated with PEDOT:PSS that overcomes these limitations. Our results show that the porous electrode also supports both *calli,* with the variation of impedance in low frequency regime, specifically at 0.1 mHz, correlating with mass increase. The better performance of 3D electrode is probable due to the much higher surface area conductive surface, allowing a higher sensitivity of the biological interaction in the electrodes.

Keywords: Embryogenic *callus,* impedance modulus, non-embryogenic *callus,* somatic embryogenesis

RING TEST Investigation: Comparative analysis of CKX inhibitors in semi-solid and Plantform™ bioreactor cultivation of *Prunus domestica* L.

Brunilda Çuko[1], Maurizio Lambardi[2], Stefaan Werbrouck[3], Efigjeni Kongjika[4], Valbona Sota[1, 4]

[1]Department of Biotechnology, Faculty of Natural Sciences, Albania
[2]IBE-Institute of BioEconomy, National Research Council (CNR), Sesto Fiorentino (Florence), Italy
[3]Ghent University, Belgium
[4]Research Center of Biotechnology and Genetics, Academy of Sciences of Albania

cuko.brunilda@gmail.com

This study examines the effects of two CKX compounds (cytokinin oxidase/dehydrogenase inhibitors: No. 52 and No. 77) on the *in vitro* propagation of *Prunus domestica* L., cv Tropojane, in a semi-solid medium, as well as in the Plantform™ bioreactor for liquid culture in Temporary Immersion System (TIS). The CKX compounds, which protect isoprenoid cytokinins from oxidation, were applied at a concentration of 1 µM and tested in combination with two cytokinins: 6-benzylaminopurine (BAP, 4.44 µM) and zeatin (ZEA, 4.56 µM). For each treatment, Quoirin & Lepoivre (LP) basal medium was used, supplemented with 0.54 µM of 1-naphthaleneacetic acid (NAA). The morphometric parameters (shoot length and shoot number) and relative growth rates (RGR) of shoots were evaluated, and the data were analyzed and compared against PGR_{Free} and CKX_{Free} controls in both cultivation systems. The highest proliferation rate was attained with the addition of CKX 77 in Plantform™ bioreactor supplemented with BAP, which caused a RGR much higher than the rest of treatments (9.34), as well as shoot length (4.95 cm) and shoot number (20). However, this effect of CKX 77 is not observed during micropropagation on semi-solid nutrient medium, where the addition of CKX 52 improved micropropagation, yielding an RGR of 6.88 and an average shoot number of 7.3. ZEA-containing media gave lower results in terms morphometric parameters observed, compared with BAP-containing media. In all cases, the micropropagation of plum shoots in Plantform™ bioreactor resulted more effective in terms of RGR , length and average number of shoots. These findings highlight the potential of CKX compounds, particularly CKX 77, in optimizing *in vitro* propagation protocols for plum and possibly other woody plant species.

Keywords: plum, cytokinin oxidase/dehydrogenase inhibitors, TIS bioreactors, micropropagation

Temporary immersion system for mass propagation of *Coffea arabica* shoots

Giuseppe Natale Basile[1], Anna Tagarelli[1], Doaa Elazab[2,3], Giuseppe De Mastro[1], Maurizio Lambardi[2], Claudia Ruta[1]

[1]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/a, 70125 Bari, Italy

[2]BE-Institute of BioEconomy, National Research Council (CNR), 50019 Sesto Fiorentino (Florence), Italy

[3]Department of Pomology, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt

giuseppe.basile@uniba.it

The aim of the study is to evaluate and validate an innovative and efficient micropropagation protocol, related to the massive propagation of selected mother plants of *Coffea arabica*, to overcome the issues related to global warming, which is expected to halve the native cultivation extensions by 2050. The trial was performed on the the use of temporary immersion liquid culture (TIS) in comparison with semi-solid agarised culture. Buds of 0.5 cm were excised from seedlings, sterilised and transferred in jars containing a basic culture medium (MB), solidified with agar and enriched with 3 mg l^{-1} 6-Benzylaminopurine (BAP) for the adaptation to the asptic culture. After 60 days, binodal microcuttings, approximately 0.5 cm in length, were transferred to semi-solid media and Plantform™ bioreactors, both containing nutrient medium enriched with 0.05 mg l^{-1}, 1 mg l^{-1}, or 2 mg l^{-1} BAP. The immersion frequency in the bioreactors was 4 minutes every 6 hours (16 minutes/day) and 8 minutes every 6 hours (32 minutes/day). Rooting induction was obtained in vitro adding Indol butyrric acid (IBA, 3 mg l^{-1}) to the agarised substrate or directly in vivo using IBA commercial product (Clonex® 3300 mg l^{-1}). TIS culture with BAP at 0.05 mg l^{-1} proved to be the most effective in terms of efficiency of use, achieving quantitatively and qualitatively superior results in half the time, compared to agarised substrate. In vivo rooting also halved the time of successful transplant, offering economic and practical advantages to optimise large-scale production.

Keywords: Micropropagation, liquid culture, TIS, Plantform, ex vitro rooting.

Optimizing sweet cherry rootstock micropropagation: improved performance of SETIS™ bioreactors supported by physiological and biochemical analysis

Elsa Baltazar[1*], Mariana Correia[1], Tércia Lopes[1], João Martins[1], Jorge Canhoto[1], Sandra Correia[1,2]

[1]University of Coimbra - Center for Functional Ecology Science for People & the Planet, TERRA Associated Laboratory, Department of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, Portugal.

[2]InnovPlantProtect - Estrada de Gil Vaz Apartado 72, 7350-478 Elvas

e.c.s.baltazar@gmail.com

Conventional *in vitro* micropropagation with semi-solid media is precise for large-scale propagation but raises costs. Temporary immersion bioreactors (TIBs) offer a cost-effective alternative for fruit tree crops, including rootstocks and scions, by enhancing propagation rates and increasing production. This study aimed to improve micropropagation of sweet cherry rootstocks, *Prunus avium* and Gisela 6, comparing their multiplication in semi-solid media and TIB using SETIS™. Micropropagation success was evaluated by *in vitro* establishment, shoot length, number of shoots, and Relative Growth Rate (RGR), along with physiological and biochemical parameters. Establishment success was 56% for *P. avium* and 62% for Gisela 6. No differences in shoot number were found between propagation methods or genotypes. However, shoot length differed between media types, with Gisela 6 having longer shoots in TIB. The RGR confirmed TIB's superiority in promoting shoot proliferation. Photosynthetic pigments showed significant differences in *P. avium* in TIB. Total flavonoids were unaffected by method or genotype. MDA content was higher in *P. avium* in TIB, indicating oxidative stress due to hyperhydricity. Proline levels were higher in *P. avium*, indicating genotype-dependent stress responses. These results highlight the importance of selecting suitable micropropagation systems and understanding their effects on plant physiology.

Keywords: Micropropagation, plant physiology and biochemistry, sweet cherry rootstocks, temporary immersion bioreactors (TIB)

Effect of different silver nanoparticle formulations on apricot micropropagation using Temporary Immersion System

Alfosea-Simón F.J., Pérez-Caselles C., Martín-Valmaseda M., Yelo E., Burgos L., Alburquerque, N.

Group of Fruit Tree Biotechnology, Department of Plant Breeding, CEBAS-CSIC, Campus de Espinardo, 25, 30100 Murcia, Spain

nalbur@cebas.csic.es

The application of silver nanoparticles (AgNPs) has emerged as a promising approach for the *in vitro* cultivation of plant species. In the case of apricot (*Prunus armeniaca L.*), the commercial formulation Argovit™-7 has demonstrated effectiveness in promoting shoot growth and enhancing micropropagation. However, the efficiency of AgNPs depends on multiple factors, including synthesis method, particle size or type of coating. Identifying the most suitable AgNP formulation for a specific context is essential to optimize its use under *in vitro* conditions.

This study compares three different formulations: (I) Argovit™-7, stabilized with polyvinylpyrrolidone (PVP); (II) BioArgovit™, coated with protein hydrolysate; and (III) Argovit™-C, which combines both stabilizers. Apricot shoots were cultivated under controlled conditions for a four-week cycle in a Temporary Immersion System (TIS) using liquid medium supplemented with 0, 25, 50, 75, and 100 mg L^{-1} of each Argovit™ formulation. At the end of the cycle, silver accumulation in plant tissues, shoot biomass, and growth parameters were assessed.

Results showed significant differences of silver accumulation, observing an optimum at different concentrations depending on the AgNP formulation. Shoot proliferation improved with increasing concentration of Argovit™-7 and with Argovit™-C an optimum was observed at 75 mg/L. However, the use of BioArgovit™ did not improve the micropropagation of apricot shoots in TIS.

Kewords: Temporary Immersion System, *in vitro*, AgNPs coating, micropropagation, *Prunus armeniaca*

Tiny messengers from trees: Exploring extracellular vesicle production in Poplar and Tamarillo *calli*

Miguel Rito[1]; Beatriz César[1]; Jorge Canhoto[1]; Sandra Correia[1,2]

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

[2]InnovPlantProtect CoLAb, Estrada de Gil Vaz, 7350-478 Elvas, Portugal

miguel9_rito@hotmail.com

Extracellular vesicles (EVs) play a crucial role in intercellular communication and have garnered significant interest for their potential applications in biotechnology, including their role as nanocarriers. Research on plant EVs remains limited, particularly for woody species. In this work, we investigated the production of EVs from *calli* induced in two trees, tamarillo (*Solanum betaceum*) and a hybrid poplar (*Populus tremula x P. alba*).

Starting from well-established protocols for *calli* induction and scalable growth conditions, EVs from both *calli* were isolated. *Calli* was harvested and the buffer was carefully infiltered. Low-speed centrifugation was performed to collect the buffer with EVs, and differential centrifugation was employed to pellet them before fractioning using density gradient centrifugation. Size, abundance and morphology were observed through transmission electron microscopy and nanoparticle track analysis.

We observed that tamarillo *calli* produced a heterogeneous population of EVs with peaks at 120, 300 and 400 nm while poplar *calli* had a more homogenous population with peaks at 120 and 200 nm. EVs observed were round-shaped with their known bilipid layer. Both *calli* produced abundant EVs when compared to other tissues, confirming their potential as valuable sources for the scalable production of bio-nanocarriers. To further characterize EV production in these species, and their roles in signalling, protein analysis is being carried out through LC-MS.

Keywords: *calli*, extracellular vesicles, nanocarriers, plant stress

Oral communications – WG4

Deployment of vegetatively propagated forest trees: technical possibilities coupled to regulatory restraints and possibilities - A general outlook with a Swedish example.

Magnus Hertzberg, Malin Abrahamsson,

SweTree Technologies AB, Skogsmarksgränd 7, SE-903 36 Umeå, Sweden

Magnus.Hertzberg@SweTree.com

SweTree Technologies is a research and technology development company within the area of forest biotechnology. We have for many years been developing an automation solution for large scale (tens of millions) production of conifer plants based on somatic embryogenesis. The last years we have built a pilot facility to test and further develop our automation solutions in preparation for a first large-scale production facility for Norway spruce.

Large scale somatic embryogenesis-based plant production for conifers and other forest tree species could have great value for commercial forestry and conservational forestry. To efficiently catch this value, it is important that the regulatory system is balanced. We will present how clones and clone mixtures based on large scale somatic embryogenic amplification could be used in forestry within Europe and Sweden, in relation to laws and regulation, particularly the European FRM (Forest reproductive Material) regulations and Swedish national law.

Keywords: FRM, Variety mixes, legislation, genetic variation

Challenges and strategies for acclimatizing ornamental plants in the mediterranean climate

Soner Yağ[1], Bora Onur Hallaç[2], Yeşim Yalçin Mendi[1]

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

[2]Bolu Abant İzzet Baysal University, Postgraduate Education Seed Science and Technology, Bolu

soneryag@gmail.com

Woody plants play a significant role in the production activities of the ornamental plant sector. The condition and quality of the starting material in the production of woody plants significantly affect all production processes. In ornamental plant production, where plant quality is targeted and determined as the primary parameter, the method used to produce the starting material becomes critically important across many stages, from production to sales. While the propagation method commonly used in woody plant groups within the ornamental plant sector is cutting production, tissue culture production has become widespread for certain varieties in recent times. Especially in woody plant groups, production through cuttings allows for rapid plant growth, with the plant form transforming into a tree-like shape. Supermarkets and florists tend to purchase plants that can be placed on shelves, have abundant leaves, a thick stem, and a more compact growth habit. Such growth is possible in a production system where roots, stems, and leaves grow together in a homogeneous manner, and this method can be achieved through tissue culture production to reach commercial quantities. This study evaluates the methods and strategies used during the acclimatization phase, an important stage following tissue culture production of *Ficus benjamina, Ficus lyrata, Ficus elastica,* and *Schefflera* plants. It also examines the activities carried out by a commercial producer in the Adana region of Turkey regarding the acclimatization processes following tissue culture production. Acclimatization practices and protocols have been developed on a variety-specific basis, enabling commercial-scale production with very low loss rates. These practices include protective fungicide applications, gradual adjustments of humidity conditions, correct selection of light levels, and optimization of temperature conditions.

From conventional to innovative in vitro production of saffron: Temporary Immersion System (TIS) and Machine Learning

Waed Tarraf[1], Tolga İzgü[1], Özhan Şimşek[2], Nunzia Cicco[3], Carla Benelli[1]

[1]Institute of BioEconomy (IBE), National Research Council (CNR) of Italy, 50019 Sesto Fiorentino, Florence, Italy

[2]Horticulture Department, Agriculture Faculty, Erciyes University, 38030 Kayseri, Türkiye;

[3]Institute of Methodologies for Environmental Analysis, National Research Council, C.da S. Loja, 85050 Tito Scalo, Potenza Italy

waed.tarraf@ibe.cnr.it

Saffron is propagated solely through the corm multiplication, which makes it vulnerable to diseases and environmental stresses. Traditional propagation methods often lead to material loss, while in vitro culture techniques enable the production and growth of pathogen-free corms. This study applied the Temporary Immersion System (TIS) through the Plantform™ bioreactor to propagate saffron corms from the Abruzzo region in Italy. After surface disinfection, the corms were cultured on semisolid MS with BA, NAA, ascorbic acid, and sucrose. Well-developed in vitro corms were used in TIS and semisolid cultures for a comparison between the two in vitro culture systems. Results showed that TIS, with the immersion frequency of 5 min/4 h and aeration of 15 min/4 h, produced significantly more microcorms, shoots, and roots than semisolid medium, after 30, 60, and 90 days. These findings were supported by machine learning analysis to model and predict the micropropagation efficiency of saffron using TIS and the semisolid medium. Notably, the Random Forest model exhibited the best predictive performance for microcorm number, demonstrating the potential capacity of machine learning in forecasting micropropagation outcomes. Overall, the TIS method showed superior efficiency, therefore, this innovative system could be valuable for the large-scale propagation, of healthy saffron corms in commercial laboratories, regardless of the season, reducing hand-labour and costs.

Keywords: *Crocus sativus*, Plantform™, microcroms, machine learning, commercialization

How to inform the public about *in vitro* culture of woody plant species?

Paweł Chmielarz, João Paulo Rodrigues Martins, Juan Manuel Ley-López, Mikołaj Krzysztof Wawrzyniak,

Institute of Dendrology, Polish Academy of Sciences, 62-035 Kórnik, Poland

pach@man.poznan.pl

In contemporary society, there is an increasing focus on trees, yet there is a lack of knowledge regarding the *in vitro* micropropagation of trees under sterile conditions. This has given rise to concerns and questions about the safety of this method. In response, we have used plants derived from *in vitro* culture to educate the public about tree micropropagation methods, using pedunculate oak (*Quercus robur* L.). Oaks do not reproduce vegetativelly in the traditional method, therefore *in vitro* culture has been successfully employed to propagate oak trees. We proved that this approach can be successfully applied also for 800-year-old oaks. However, these studies have demonstrated that only about 50% from 20 oaks examined, could be successfully multiplied *in vitro*. This suggests that the ability to propagate *in vitro* may be genotype-dependent. Promising individuals were selected and successfully acclimated to *in vivo* conditions. The oak trees derived from *in vitro* culture have a root and a shoot genetically identical to the mother tree. Since 2019, oaks derived *in vitro*, measuring 1–2 meters in height, have been planted. Planting a tree with such an old genotype aroused considerable media interest (TV, radio, social media, and press) about the event. It was also the opportunity to engage with the local communities to present the plants, *in vitro* methods, and the historical significance of the original trees. For example, *in vitro* clones of the Rus oak, growing in Rogalin (approximately 800 years old), the Chrobry oak from Piotrowice (of the same age, now dead), as well as the Wybicki oak (c. 500 years old) from Będomin and the Florian oak (now dead) from Zwierzyniec, all originate from well-known locations in Poland. These clones were first planted near their respective mother trees and later in locations such as schools and other sites of community importance. The oak planting procedure begins with establishing personal contact with individuals or institutions interested in acquiring the tree. Then, we have to obtain a permission from the landowner for planting. The tree is then planted on a selected date (often to mark special anniversaries) and accompanied by the placement of an informational plaque next to it. This plaque describes the *in vitro* method, the approximate age of the mother oak, and the institution that funded the research. A brief lecture is given, and a certificate of authentication is presented. The media are informed about the event in advance. In cases where planting does not take place near the original tree, a large photo of the mother tree is displayed on a 2-meter-high roll-up poster next to the planted oak. To document the planted oak trees, a map has been continuously updated since 2019. To date, more than 50 cloned oak trees have been planted in this way.

Oral communications – WG5

Perception about the potential use of micropropagated trees in forestry and reforestation in European countries

Bruce Christie[1], Purificación Covelo[2], Jesús M Vielba[2], Nieves Vidal[2]

[1]The Greenplant Company, Palmerston North, New Zealand

[2]Misión Biológica de Galicia (MBG), CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain

nieves@mbg.csic.es

A primary goal of COPYTREE is to improve the communication with stakeholders and general public in order to increase the acceptance of in vitro technologies. In this study we present the results of a survey carried out by 34 scientists working in Forestry and Ecology belonging to 12 European countries.

The survey was conducted in a session at the final conference of the COST Action "Pan-European Network for Climate Adaptive Forest Restoration and Reforestation" after the presentation of a COPYTREE communication entitled "From the lab to the forest: Can in vitro technologies contribute to environmental restoration?". In this talk we presented eleven examples in which COPYTREE members successfully planted micropropagated trees (poplars, willows, radiata pine, Norway spruce, chestnut, cork oak, wild apple, cherry and linden) in forest land for different purposes such as land stabilization, tree conservation, timber, cork and nut production.

The questionnaire aimed to collect researcher's perception of the risks, disadvantages and opportunities for the application of micropropagation to forestry. Questions were divided into 3 groups according to the type of response: i) simple answers (Yes/No/I don't know) ii) open questions and iii) personal data (area of knowledge, country, age and gender).

The feedback can help us to design dissemination activities directed specifically to this particular audience and to reflect on how we can adapt our work strategies to the needs of this potential market.

Keywords: in vitro, forest trees, public acceptance, stakeholders, survey

A comparative analysis of tissue culture strategies in commercial propagation: A network analysis approach

Buhara Yücesan

Bolu Abant Izzet Baysal University, Dep. of Seed Science and Technology, 14020, Bolu, Türkiye

buhara@ibu.edu.tr

Plant tissue culture (PTC) techniques, including micropropagation, somatic embryogenesis, and temporary immersion systems, play a crucial role in plant biotechnology with expanding applications in commercial-scale plant production and industrial in vitro culture. This study applies network analysis using Gephi to evaluate research trends via citation-weighted keyword co-occurrence in Web of Science (WOS) research articles, excluding reviews. The dataset was selected based on keywords relevant to commercialization, industrial applications, and large-scale propagation, ensuring a comprehensive comparison of different PTC strategies.

The network comprises two connected components, with a dominant, highly interconnected structure centered around *Micropropagation*. Network statistics indicate an average degree of 3.4, with a weighted degree of 49.2, highlighting citation influence. A network diameter of 6 and an average path length of 3.1 indicate a compact structure where key topics remain well linked. A modularity score of 0.5 reveals distinct but interrelated research clusters, including somatic embryogenesis, commercial plant propagation, and regional applications. This citation-weighted co-occurrence approach enhances our understanding of micropropagation's impact on plant biotechnology. From a knowledge share perspective, keyword selection addressing commercial and industrial propagation ensures that the dataset reflects global PTC efforts aimed at economic viability. Research efforts have evolved from fundamental in vitro studies to large-scale propagation, with a growing focus on woody crops. Eucalyptus and Cacao emerge as leading woody crops in PTC, with countries like India, the United States, Brazil, and China playing central roles in shaping industry practices and innovation. In conclusion, this study highlights the role of strategic keyword selection as a tool for business model generation, moving beyond traditional academic analyses. The alignment of research trends with industry needs extends beyond conventional applications, advocating for a broader analytical framework. Network analysis presents a novel tool for uncovering non-linear relationships within PTC commercialization, offering insights into how research trajectories interact with industry demands. Future research should explore how these insights influence market strategies and the large-scale adoption of PTC technologies, fostering innovation in commercial propagation models.

Keywords: Network analysis, Microprogation, Tissue Culture Commercialization, Knowledge Dissemination

Biotechnological strategy for *ex situ* plant propagation and reintroduction into the Atlantic islands of the Galicia National Park

Ángeles Meira, Erea Garcés, Pedro P. Gallego, Tomás A. Arteta

Agrobiotech for Health, Department of Plant Biology and Soil Science, Faculty of Biology, University of Vigo, Vigo, Spain.

pgallego@uvigo.es

Invasive alien species (IAS) significantly impact the conservation of Natura 2000 habitats in the Maritime-Terrestrial Galician Atlantic Islands National Park (NW Iberian Peninsula). The LIFE Insular project aims to restore native flora and develop a conservation strategy for these habitats following IAS removal. Efficient propagation methods, particularly macro and micropropagation, are crucial for large-scale reintroduction efforts. The AgroBioTech for Health research group at the University of Vigo has designed micropropagation protocols for five key native species: *Daphne gnidium*, *Corema album*, *Cistus salviifolius*, *Prunus spinosa*, and *Ulex europaeus*. Four were successfully established *in vitro*, with contamination being a major challenge. Medium optimisation was crucial for growth. *Ulex europaeus* mineral nutrition and growth regulator studies, using an AI tool, neurofuzzy logic, will be presented as an example of the identification of key factors in the culture medium composition. These protocols enhance multiplication rates and facilitate the propagation of wild native species, particularly those with limited prior propagation research. This research contributes valuable knowledge for the conservation and restoration of native flora in threatened island ecosystems.

Keywords: ex situ conservation, micropropagation, conservation, plant tissue culture, plant nutrition

Posters WG1

P1 - Micropropagation of medicinal plants: A bibliometric analysis based on recent publications in Europe

Kassiani Papadopoulou, Antonia Sofianou, Eleni Abraham

Laboratory of Range Science, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

eabraham@for.auth.gr

Micropropagation techniques can be used to conserve and propagate medicinal and aromatic plants that are endangered or difficult to propagate using conventional methods. It is an efficient tool to produce homogeneous, high-quality, disease-free plants suitable for both commercial production and conservation. Scopus has been implemented to analyze 93 documents published between 1961 and 2024 from European countries, Israel and Turkey that include the terms “micropropagation” and “medicinal plants”. According to the results, a rapid increase in the number of publications after 2000 has been reported. Poland followed by Turkey and Bulgaria are the countries with the largest scientific production. Agriculture and biological sciences (44.9%) followed by Biochemistry, Genetics and Molecular Biology (22.2%) are the most prominent fields in the study of micropropagation of medicinal plants. The main purpose of micropropagation is the restoration and/or conservation of natural population of rare, endemic and endangered medicinal plant species. The research predominantly focuses on the composition of nutrient media and conditions for a successful development of micro plants.

Keywords: in vitro propagation, publications, herbaceous species, medicinal

P2 - Micropropagation of juvenile *Argania spinosa* (L.) skeels via axillary budding

Pablo Piñeiro[1], María Teresa Martínez[1], Javier Sampedro[2], Elena Corredoira[1]

[1]Misión Biológica de Galicia (MBG-CSIC), Sede de Santiago de Compostela, Avd. Vigo s/n 15705 Santiago de Compostela, La Coruña, Spain

[2]Departamento de Biología Funcional, Facultad de Biología, Rúa Lope Gómez de Marzoa, s/n. Campus sur, 15782 Santiago de Compostela, A Coruña, Spain

pablomanuel.pineiro@mbg.csic.es

Argan is a horticultural forest species endemic to Morocco and Algeria, well adapted to arid and semi-arid Mediterranean climates and with high social, ecological, economic and agronomic interest. However, populations of this species are threatened by overgrazing, lack of natural regeneration and overexploitation, as argan oil demand is increasing. As conventional propagation is very problematic, the development of micropropagation methods is imperative. Here, an efficient procedure for micropropagation of juvenile material based on axillary budding is described. Forced shoots obtained from potted plants of two different 2-year-old genotypes were employed as initial explants to establish in vitro shoot proliferation cultures. The establishment medium consisted of Murashige and Skoog (MS) medium, supplemented with 1 mg L^{-1} benzyladenine (BA). Optimal shoot proliferation was achieved in MS medium supplemented with 2 mg L^{-1} meta-topolin, 1 mg L^{-1} indole-3-acetic acid, 0.6% Sigma agar and 3% sucrose for 6 weeks at 28°C. Rooting was induced when 1.5–2 cm shoots were cultured in MS medium with half-strength macronutrients, supplemented with 25 mg L^{-1} indole-3-butyric acid (IBA) for 24 or 48 hours, before being transferred to ½MS medium devoid of IBA and supplemented with 20 µM silver thiosulfate. Shoot proliferation and rooting were found to be genotype-dependent.

Keywords: Argan, cytokinins, meta-topolin, mineral medium, silver thiosulfate.

Funding: This work was partly supported by Ministerio de Ciencia, Innovación y Universidades (MICIN, Spain) through the project PID2020-112627RB-C33 (AEI/10.13039/501100011033).

P3 - An initial micropropagation protocol for the endemic Mediterranean seagrass *Posidonia oceanica*

Ricardo Castro-Camba, David Fernández, Laura Guerrero-Meseguer, Inés Castejón, María Rubio, Diego Fansa[1], Jorge Terrados

Department of Marine Ecology, IMEDEA-UIB-CSIC, C/ Miquel Marquès, 21, 07190, Esporles Illes Balears, Spain

ricardo.castro@csic.es

Posidonia oceanica is a Mediterranean endemic seagrass that forms extensive meadows, providing significant ecological benefits. However, the distribution of these meadows has been declining. Restoration efforts could greatly benefit from micropropagation techniques, which enable the production of a large number of individuals for transplantation.

Despite its potential, there is a significant lack of knowledge regarding micropropagation techniques applied to marine seagrasses, with few studies demonstrating limited efficiency. Establishing a micropropagation protocol for *P. oceanica* presents considerable challenges, including the difficulty of obtaining plant material and its extremely slow growth rate (ranging from 0.1 to 6 cm/year, depending on whether the rhizome is vertical or horizontal). In addition, conventional culture media are designed for terrestrial conditions, making them unsuitable for marine environments (pH ≈ 8 and salinity 38 ‰).

Initial trials were conducted to assess the effects of pH and salinity modifications on explant health. Subsequent experiments focused on determining the optimal sucrose concentration, as well as testing different concentrations of meta-topolin (0, 0.25, 1, 2, and 4 mg/L) and naphthalene-acetic acid (0, 0.5, and 1 mg/L) in a PES liquid medium dissolved in artificial seawater. These experiments aim to establish a basic micropropagation protocol (with a multiplication rate > 1) as a starting point for further optimization.

Keywords: *Posidonia oceanica*, ecological restoration, micropropagation, protocols, marine plants

P4 - Selection effective disinfected method for in vitro propagation of cherry rootstock "Gisela 5"

Maia Kukhaleishvili, Iveta Megrelishvili

Georgian Technical University, Biotechnology Center, Kostava st.77, 0160, Tbilisi, Georgia
maia.kukh@gmail.com

This study was conducted to investigate the effects of different disinfectants methods on Chery rootstock "Gisela 5" contamination to determine the best possible way for disinfecting the explant under in vitro conditions.

The 20 cm long and having active vegetative leaf buds from 2–4 years old mature tree are collected in Jigaura, Shida Kartli, Georgia, during dormant months of December-January. The dormant wood was first treated with Captain (@ 0.2%), followed by cold storage at 4±3°C in poly-bags till further use.

The samples were covered with polyethylene bags, and placed in growth chambers with controlled conditions (temperature 25 ± 1°C, humidity 80-82%, 5000 lux, 16/8h light/dark photoperiod) for initiation during 20 days. Initiated explants was sterilized in various disinfection methods:

1) 0.8% (v/v) sodium hypochlorite for 10 minutes; 2) 0.1% mercuric chloride for 5-6 minutes; 3) the combination of 0.5 % benzalkonium chloride (BAC) and 5 % chlorhexidine digluconate (CHX), 1-2 minutes, followed by dipped in 70% (v/v) ethanol solution and washed four times in sterile deionized water in all cases. 40% modified MS medium (Murashige & Skoog) was used for initiation in vitro culture of Cherry rootstocks "Gisela 5" under a 16-hour photoperiod in phytotrone.

Overall, the 0.1% mercuric chloride provided the highest survival rate (34.09%) among the treatments, following the combination of 0.5% benzalkonium chloride (BAC) and 5.0% chlorhexidine digluconate (CHX (25. 67%) and minor survival rate was shown under 0.8% (v/v) sodium hypochlorite treatment.

This study was he first successful in vitro culture establishment of Chery rootstocks "Gisela 5" from open field sources in Georgia.

P5 - *In vitro* culture of tree species of agricultural and environmental interest. Advances and limitations

Juan J. Silva Pupo[1], Karel Chala Arias[1], Jorge L. Perez Pérez[1] and Stefaan Werbrouck[2]

[1]Centro de Estudios de Biotecnología Vegetal, Universidad de Granma, Cuba

[2]Laboratory In vitro Plant Biotechnology, Gent University, Belgium

jsilvapupo@gmail.com

Woody plant species, whether fruit trees, forest trees, or others, present serious difficulties in responding satisfactorily to in vitro culture conditions, which is why they are called recalcitrant species. In our laboratory, we have worked with different species that respond in varied ways. The mulberry (*Morus alba* L.), used for animal feed and medicinal purposes, has developed each stage of in vitro propagation with the use of specific growth regulators. The resulting seedlings were established under field conditions as a propagule bank. In vitro germination of seeds was achieved for another species known as ylang-ylang (*Cananga odorata*), and research is underway on the development of the other stages of the process. However, in the case of the Jamaican walnut (*Juglans jamaicensis*), an endangered species, in vitro establishment has not been achieved, presenting problems of necrosis and microbial contamination of buds obtained from cuttings under semi-controlled conditions. Research is also being conducted on the endemic fruit species *Annona cubensis*, for which only seed disinfection has been achieved. For these last two species, the available material is scarce, which limits the implementation of experiments.

P6 - Effect of pH and carbon source on *in vitro* propagation of plum, sour cherry and raspberry

Tatjana Anđelić, Mirjana Mićević, Tatjana Vujović

Fruit Research Institute (FRI), Department of Fruit Physiology, Kralja Petra I 9, 32000 Čačak, Serbia

tvujovic@institut-cacak.org

The aim of this study was to investigate the effects of medium pH and different types of carbon sources (CSs) on the *in vitro* multiplication of plum (*Prunus domestica* L.), sour cherry (*Prunus cerasus* L.), and raspberry (*Rubus idaeus* L.). In the first experimental setup, Murashige and Skoog (MS) medium (1.0 mg l^{-1} BAP, 0.1 mg l^{-1} IBA, and 0.1 mg l^{-1} GA_3) was set at an initial pH 5.7. Five types of CSs (each at final concentration of 30 g l^{-1}) were tested: sucrose, fructose, glucose, sorbitol, and a combination of all four sugars (mix). The change in media pH was monitored after autoclaving and after four weeks of subculturing. The results indicated that the pH of all media containing different CSs decreased after autoclaving. However, after four weeks of multiplication, an increase in pH was observed only in the medium with fructose. Fructose significantly enhanced multiplication in plum and raspberry, while the sugar mix yielded the highest multiplication index in sour cherry. In the second experimental setup, fructose and the sugar mix were further evaluated at different pH levels (5.2 and 4.7), and compared with sucrose as the standard CS. In sour cherry, a sugar mix at pH 4.7 increased shoot lenght and leaf number. In raspberry, fructose yielded the highest multiplication index at pH 5.2, whereas the lowest index was observed at pH 4.7 with the same sugar. The study found that pH and CS significantly affect multiplication, with fructose and the sugar mix having the most favorable effects, depending on pH conditions.

Key words: medium pH, fructose, sucrose, glucose, sorbitol, *in vitro* multiplication

P7 - Woody plant propagation at Deroose Plants: overcoming recalcitrance at various stages

Filip Vandenbussche[1], Cynthia Marbun[1], Sofie Van Gijseghem[1], Reinout Impens[2], Lucas Vanhaelewyn[1,3]

[1]R&D Department, Deroose Plants NV, Weststraat 129A, 9940 Evergem, Belgium

[2]Presco Plc Obaretin Estate, Km 22 Benin / Sapele Road, Ikpoba-Okha Local Government Area. P.O. Box 7061, Benin City, Edo State, Nigeria

[3]Department of Agricultural Economics, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

Filip.Vandenbussche@DeroosePlants.com

Since the 1980's Deroose Plants is using plant tissue culture to propagate ornamental, fruit and industrial commodity crops. To provide young plants at a commercial scale, a thorough research and development trajectory is needed to overcome bottle necks in the micropropagation cycle. Many of these bottle necks depend on the genus, the species, the variety or broadly speaking, the genotype. Overcoming the recalcitrance of rooting of Eucalyptus *gunnii*, Argania *spinosa*, Elaeis *guineensis* and Rubus sp. are clear examples of this. Along the same lines, overcoming the variety dependent recalcitrance to somatic embryogenesis of Hevea *brasiliensis* is necessary to successfully propagate these trees in vitro. By refining this methodology, Deroose Plants has developed a protocol to multiply more than 10 different varieties. Moreover, recently, protocols for clonal root stock production have been developed, thus providing the basis for future grafting procedures with certified clean material of clonal varieties that can be used as scions and clonal rootstocks of Hevea. These developments pave the way to commercial propagation of quality woody plant varieties, offering a safe source of high-quality, pathogen-free material for growers worldwide.

Keywords: micropropagation, somatic embryogenesis, in vitro culture, woody plants, improved rooting

P8 - Callogenesis induction attempts of *Welwitschia mirabilis* Hook f. - gymnosperm species recalcitrant to micropropagation

Teresa Hazubska-Przybył[1], Marzenna Guzicka[1], Ewa Kazimierczak-Grygiel[2], Sara Zwolenkiewicz [3], Justyna Wiland-Szymańska[2,3]

[1]Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

[2]The Botanical Garden of the Adam Mickiewicz University in Poznań, Dąbrowskiego 165, 60-594 Poznań, Poland

[3]Department of Systematic and Environmental Botany, Faculty of Biology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland

hazubska@man.poznan.pl

Welwitschia (*Welwitschia mirabilis* Hook f.) is a species endemic to the Namib Desert, growing in Angola and Namibia and reproducing exclusively by seed. Monitoring of natural sites has confirmed a rare occurrence of young *W. mirabilis* plants. The reason is most likely an infection of seeds by the fungus *Aspergillus niger*, which limits their germination. Due to the uniqueness of this plant, as well as the natural limitations associated with its propagation from seeds, the UAM Botanical Garden and the Institute of Dendrology PAS began researching the possibility of its micropropagation. Generative explants were sterilized in 30% hydrogen peroxide (H_2O_2) and placed on MS medium supplemented with dichlorophylloxyacetic acid (2.4-D; 9 µM) and benzyladenine (BA; 4.4 µM), then incubated in the dark for 8 weeks. The induced callus was transferred to two proliferation media: MS and WPM with 2,4-D (9 µM), BA (4.4 µM), kinetin (KIN, 4.6 µM) and &-naphthaleneacetic acid (NAA, 5.4 µM). The disinfection efficiency was of 100%, using H_2O_2. Preliminary tests confirmed the possibility of obtaining callus induction from the tested explants. The appearance of a soft, light cream-colored callus was observed, which was capable of multiplying in several subsequent passages. Microscopic examination revealed callus tissue composed of cells of various shapes and sizes. At this stage of callus formation, morphogenetic centres and superficial meristematic zones were not observed yet.

Keywords: Gymnospermae, Gnetopsida, *Welwitschiales*, callus *in vitro*, generative explants

P9 - Topophysis-driven uniformity in woody plant cultures: for automated micropropgation.

Maroua Grira[1], Els Prinsen[2], Stefaan Werbrouck[1]

[1]Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium.

[2]Integrated Molecular Plant Physiology Research, Department of Biology, University of Antwerp, Groenenborgerlaan 170, 2020 Antwerp, Belgium.

Maroua.grira@gmail.com

Topophysis, the influence of the explant's original position, significantly impacts the uniformity of in vitro cultures, which is a key factor in enabling the robotization of plant tissue culture processes. This study investigates topophysis in three tropical woody species: *Handroanthus guayacan*, *Tabebuia rosea* and *Tectona grandis*. Shoots from stock cultures were divided into upper, middle, and basal sections and cultured on a modified MS medium. After 8 weeks, distinct topophysical responses were observed across species. In *H. guayacan*, the middle section produced the most shoots, the longest shoots, and the highest number of nodes. Conversely, in its related species *T. rosea*, basal explants exhibited superior performance, generating the highest shoot numbers, longest shoots, and most nodes per plant. For *T. grandis*, middle-section explants yielded the highest shoot and node counts, while apical explants produced the longest shoots. To elucidate the underlying mechanisms, aromatic cytokinin were analyzed in all three species, providing valuable insights into the topophysical effects. These findings emphasize the importance of considering explant topophysis when designing multiplication protocols to reduce heterogeneity in woody plant cultures. Furthermore, understanding these topophysical responses is crucial for the efficiency of an automated robotic systems capable of processing uniform microshoots.

Keywords: explant position, microshoot uniformity, robotization

P10 - Novel approaches for shoot regeneration in *Lobelia* and *Petunia* using flower explants

Elnara Sadigova, Stefaan Werbrouck

Ghent University, Belgium, Faculty of Bioengineering Sciences, Dept. Plant & Crops, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium

elnara.sadigova@ugent.be

This study investigated the feasibility of inducing adventitious shoot regeneration from floral explants of *Lobelia erinus* and *Petunia hybrida*. The establishment of such a protocol would significantly enhance mutagenesis programs, enabling the recovery of novel flower color mutants from sectorial chimeras and facilitating the propagation of plants with inaccessible leaf or shoot material. Both *L. erinus* and *P. hybrida* are widely cultivated ornamental species valued for their prolific flowering and diverse floral morphologies. Furthermore, their ability to flower *in vitro* makes them ideal model systems for studying petal-based shoot regeneration.

Given the sensitivity of petal tissue to conventional sterilization techniques, an alternative protocol utilizing 70% ethanol and 0.5% Umonium 38 was successfully developed. Following sterilization, *Lobelia* explants were cultured on Murashige and Skoog (MS) medium supplemented with various combinations of novel compounds, including 2-isopentenyladenosine (2iPR), cytokinin oxidation inhibitors, and an anti-senescence agent (ASES). *Petunia* explants were cultured on MS medium supplemented with those compounds and a range of cytokinins. The inclusion of ASES proved particularly beneficial in mitigating petal senescence and giving the cells enough time for callus formation.

This study demonstrates the efficacy of novel compounds in promoting adventitious shoot regeneration from floral explants, representing a promising new strategy for the biotechnological improvement of ornamental flowering species.

P11 - In vitro rooting of holm oak shoots derived from plants resistant to *Phytophthora cinnamomi*

María Teresa Martínez[1], Eduardo Piñas[1], Emma López[1], Cristina Buceta[1], Beatriz Cuenca[2], Felipe Pérez[3], Elena Corredoira[1]

[1]Misión Biológica de Galicia (MBG-CSIC), Sede de Santiago de Compostela, Avd. Vigo s/n, 15705 Santiago de Compostela, La Coruña, Spain

[2]Grupo TRAGSA, Vivero de Maceda, Carretera Maceda-Baldrei, - Km 2 - 32700 Maceda, Orense, Spain

[3]Dirección General de Biodiversidad, Bosques y Desertificación, Ministerio para la Transición Ecológica y el Reto Demográfico (MITECO), Avd. Gran Vía de San Francisco 4 – 6, 28005 Madrid, Spain

temar@mbg.csic.es

Quercus ilex L. is the most representative tree species in Mediterranean forests. In recent years, this species has been plagued by oak decline, mainly caused by the oomycete *Phytophthora cinnamomi* (Pc). The main strategy currently used for combating the disease involves cloning tolerant genotypes selected following infection with Pc. Holm oak is considered highly recalcitrant to micropropagation and is particularly difficult to root. As successful rooting and acclimatization of shoots is essential for the mass production of plants, the research reported here aimed to improve the rooting ability of axillary shoots of holm oak. Shoot cultures established from plants selected for their tolerance to Pc were subjected to rooting experiments. Successful root induction depended on the concentration of indole-3-butyric acid (IBA) used and the time of exposure to this auxin. The best rooting rate was obtained by culture in medium containing 25 mg l^{-1} IBA for 48 h and subsequent transfer to an auxin-free medium with 20 µM silver thiosulfate. The composition of the mineral medium used as the basal rooting medium was also an important factor. In more than 90% of the genotypes evaluated, the highest rooting rates were achieved using Murashige and Skoog (MS) medium with half-strength macronutrients. The application of an initial dark period or the addition of activated charcoal, 5-Azacytidine or phloroglucinol to the expression medium did not have any positive effects on rooting rates.

Keywords: Axillary budding, mineral medium, indole-3-butyric acid, *Quercus ilex*, recalcitrant species.

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P12 - Effect of dichlorprop auxin ester prodrugs on the rooting response of chestnut

Hajer Darouez[2], Stefaan P.O. Werbrouck[2], Nieves Vidal[1], Jesús Vielba[1], Saleta Rico[1], María José Cernadas[1], Conchi Sánchez[1]

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

[2]Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, 9000 Ghent, Belgium

conchi@mbg.csic.es

Adventitious rooting is a critical step for successful vegetative propagation, especially in woody species. Chestnut is known for its recalcitrance to rooting, making the micropropagation of selected and tested genotypes highly dependent on the development of efficient protocols to produce high-quality rooted shoots and acclimatized plantlets. The auxin Indole-butyric acid has been widely used to induce adventitious roots in chestnut microshoots.In this study, we investigated the effect of the auxin prodrug Dichlorprop Ester (DCPE), commercialized as Corasil® on the *in vitro* adventitious rooting of chestnut. Root induction was performed by treating the shoots with DCPE at varying concentrations (0, 0.1, 1, 2.5, and 5 µM) and for different exposure periods, ranging from 1 to 3 days. Following treatment, the microshoots were transferred to a DCPE-free medium.

Preliminary results showed that, among the tested concentrations, microshoots have to be DCPE-treated for at least 24h to form roots. Additionaly, DCPE was found to promote adventitious rooting in a dose- and exposure time-dependent manner. Rooted microshoots look healthy with low levels of apical necrosis, and acceptable acclimation rates.

Keywords: adventitious roots, chestnut, DCPE, micropropagation

P13 - *In vitro* rooting of poplar: Effects and metabolism of dichlorprop auxin ester prodrugs

Hajer Darouez, Stefaan P. O. Werbrouck

Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium

hajerdarouez@yahoo.fr, stefaan.werbrouck@ugent.be

Optimizing adventitious root formation is a key challenge in micropropagation. Auxin prodrugs, which act as inactive precursors that convert into active auxins within plants, offer a promising strategy for improving rooting control while reducing phytotoxicity. In this study, we assessed the potential of dichlorprop ester (DCPE), a compound originally developed to enhance grapefruit size, as an in vitro root inducer in *Populus* × *canadensis*. Compared to its hydrolyzed form (DCP) and a related compound (C77), DCPE demonstrated superior root-inducing effects, particularly at low concentrations (0.01 and 0.1 µM). At higher concentrations (1 µM), both DCPE and DCP triggered the unexpected formation of aerial roots, a phenomenon not previously observed in poplar with conventional auxin treatments. Metabolite analysis revealed that DCPE undergoes rapid hydrolysis into DCP, enabling a faster and more systemic distribution of the active auxin throughout the plant. In contrast, C77 exhibited slower uptake and gradual conversion into DCP, limiting its efficiency. These findings highlight the potential of auxin prodrugs, particularly DCPE, as innovative tools for refining in vitro rooting protocols in woody plant species.

Keywords: adventitious root induction; auxin prodrugs; aerial roots; dichlorprop; *Populus* × *canadensis*

P14 - Pulsed dichlorprop and light spectrum: A combined approach to enhance rooting in UCB-1 Pistachio.

Chaima Ben Othmen[1,2], Taoufik Bettaieb[2], Stefaan P.O. Werbrouck[1]

[1]Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium.

[2]Laboratory of Horticultural Sciences, National Agronomic Institute of Tunisia, University of Carthage, 43 Av. Charles Nicolle, Tunis 1082, Tunisia

chaimabenothmen11@yahoo.fr

Pistacia vera L. is a high-value nut crop, and the increasing demand for uniform pistachio cultivars has led to the widespread use of clonal rootstocks, particularly UCB-1, which enhances growth and disease tolerance. However, in vitro propagation is often hindered by rooting difficulties. This study aimed to improve rooting efficiency by investigating the effects of dichlorprop (DCP) and its ester DCPE, at varying concentrations (0.01-10 µM) using a one-hour pulse treatment. The influence of fluorescent light (FL), red light (R), and red + far-red (RFR) LED light was also evaluated. Results indicated that DCP consistently outperformed DCPE. Maximum plant length was observed under RFR light with a 1 µM DCP pulse, while the highest number of adventitious roots and the longest lateral roots were achieved with 10 µM DCP under the same light condition. The highest number of secondary roots and minimal callus weight, indicative of a good root/stem vessel connection, were obtained under FL with 0.1 µM and 0.01 µM DCP pulses, respectively. These findings suggest that DCP, particularly under specific light conditions, can significantly enhance rooting efficiency in UCB-1 pistachio rootstocks.

Keywords: rootstock, LED light, micropropagation, DCPE (Dichlorprop ethyl ester), callus

P15 - Effect of LEDs on the *in vitro* rooting and *ex vitro* acclimatization of peach rootstock GF 677

Lilyana Nacheva[1], Nataliya Dimitrova[1], Diyana Aleksandrova[1], Ivaylo Tsvetkov[2]

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

[2]Forest Research Institute – BAS, 132, Kliment Ohridski Blvd., Sofia 1756, Bulgaria

lilyn@abv.bg

The peach rootstock GF 677 (*Prunus amygdalus* × *Prunus persica*) is the most commonly used rootstock in Europe, propagated almost exclusively by tissue culture. Due to its drought resistance and tolerance to Fe deficiency, it is very suitable for low-fertility and carbonate soils, as well as those with low water availability. In recent years light emitting diodes (LEDs) have become an alternative to fluorescent lamps (FLs) as a 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 is to investigate the effects of different LED light regimes on the *in vitro* rooting and *ex vitro* acclimatization of peach rootstock GF 677. The plantlets were cultivated *in vitro* under an illumination system based on the Philips GreenPower LED research module. Four groups of LEDs emitting in white (W), red (R) and blue (B) spectral regions plus a mixed source of illumination (W:R:B:far-red=1:1:1:1, WBR) were used d. Fluorescent lamps (FLs) were used as a control. The rooted plantlets were acclimatized to *ex vitro* conditions in a greenhouse with controlled temperature (22 ± 2 °C) while some plant growth parameters were monitored simultaneously. Plants grown and rooted under blue LED light showed the weakest growth rate and lowest rooting percentage (16%) among all the tested variants. Subsequently, during the process of *ex vitro* acclimatization they died. The red light stimulated the rooting (rooting percentage 92%), the mean number of roots (4.4) and root length (14.87 mm). In the acclimatization stage, the best biometric indicators (stem length and diameter, number of leaves and biomass) were recorded in plants cultivated *in vitro* under mixed light (WBR), followed by plants grown under red (R) light. The presented results would be useful in improving the micropropagation of this valuable peach rootstock.

P16 - Unraveling drought tolerance mechanisms in Persian walnut (*Juglans regia* L.)

Hojjat Ataee[1,2,3], Conchi Sánchez [2], Saleta Rico[2], Jesús Vielba[2], Mahdi Alizadeh[1], Kourosh Vahdati[3]

[1]Department of Horticulture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

[2]Misión Biológica de Galicia, Consejo Superior de Investigaciones Científicas, 15780 Santiago de Compostela, Spain

[3]Department of Horticulture, College of Aburaihan, University of Tehran, Tehran, Iran

conchi@mbg.csic.es

Climate change has intensified drought stress worldwide, posing a major challenge to plant cultivation, particularly for trees. Persian walnut (*Juglans regia* L.) a valuable species in temperate regions is highly sensitive to drought stress. In Iran, these trees are widely distributed, exhibiting diverse germplasm that has led to different drought adaptation mechanisms depending on local geographic conditions.

To investigate drought tolerance, RNA sequencing was used to analyze transcriptomic responses in drought-sensitive and drought-resistant walnut families. Additionally, key physiological and biochemical parameters (chlorophyll content, leaf greenness index, relative water content, proline levels, and malondialdehyde) were measured. Results revealed significant differences between sensitive and tolerant walnut plants. Under drought stress, tolerant plants upregulated genes involved in stress adaptation, including heat shock proteins, redox homeostasis enzymes, metabolic regulators, and hormonal signalling components while sensitive plants overexpressed genes linked to generic stress responses and oxidative damage. This suggests that drought tolerance is driven by targeted protective mechanisms, whereas sensitivity results from disrupted metabolic activity and insufficient stress mitigation. Biochemical analyses showed reductions in photosynthetic traits, proline, and relative water content under severe drought stress, although resistant families were less affected.

Keywords: climate change, drought stress, population dispersion, Persian walnut, resistant families

P17 - Use of TIS and agar-solidified medium for *Mertensia maritima* and *Tripolium pannonicum* salinity tolerance studies

L. Purmale-Trasune[1,2], G. Ievinsh[2]

[1]Bulduri Biotechnology Centre, 6 Viestura Street, LV- 2010 Jurmala, Latvia

[2]Department of Ecology, Faculty of Medicine and Life Sciences, University of Latvia, 1 Jelgavas Street, LV-1004 Riga, Latvia

liva.purmale@bulduri.lv

Salinity is one of the main threats in agriculture, and coastal plant species are potential model organisms to understand salinity tolerance mechanisms. Halophyte species used in this study, *Mertensia maritima* subsp. *maritima* (L.) Gray, *Mertensia maritima* subsp. *asiatica* (Takeda) S.L. Welsh, and *Tripolium pannonicum* (Jacq.) Dobrocz., are edible, making them interesting for potential agricultural use.

In vitro cultures were initiated on agar-solidified medium using seeds as explants. From established cultures, shoots were transferred in Plantform temporary immersion system (TIS) reactors and jars with agar-solidified medium for 4 weeks, then the experiment was terminated. K^+, Na^+, electrical conductivity (EC), fresh and dry biomass were determined. MS medium was used as a control; for treatments, Na^+ in the form of NaCl in concentrations 1 – 5 g/L was added.

Increased Na concentration in the medium correlated with increased Na^+ accumulation in tissues and increased values of EC. Increased Na^+ concentration in the medium had no effect on K^+ concentration in *T. pannonicum* tissues, but in the case of *Mertensia*, K^+ concentration had a negative correlation with Na^+ added to the medium. Plants grown in TIS tended to be bigger and with higher fresh and dry weight, than these on agar-solidified medium. In the case of *Mertensia*, Na^+ 1 g/L had a positive effect on plant growth on agar-solidified medium, but the effect was ambiguous in TIS. TIS-grown plants showed hyperhidricity symptoms that require further optimization.

Keywords: halophytes, temporary immersion system

P18 - Preliminary results on the effect of CKX inhibitors on shoot development in wild cherry micropropagation

Sanja Bogunović

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

sanjam@sumins.hr

Wild cherry (*Prunus avium* L.) is an economically and ecologically significant forest tree species, valued for its high-quality hardwood and as a food source for various bird and mammal species. Conventional propagation methods are often slow and limited by the irregular availability of viable seeds. Micropropagation of selected elite genotypes represents the most efficient approach for producing high-quality seedlings for afforestation and clonal seed plantations. Cytokinins play a crucial role in regulating plant development, with their endogenous levels primarily controlled by cytokinin oxidase/dehydrogenase (CKX), an enzyme responsible for cytokinin degradation. Synthetic CKX inhibitors can suppress this degradation, thereby increasing cytokinin levels and potentially enhancing shoot development. This study aimed to evaluate the effects of three novel synthetic CKX inhibitors on shoot development in an elite wild cherry genotype. The results revealed significant differences between treatments. The application of these three compounds, in combination with two concentrations of the cytokinin 6-(γ,γ-Dimethylallylamino) purine (2iP), led to an increase in leaf and shoot biomass in explants. These findings contribute to the understanding of CKX inhibitors applications in wild cherry micropropagation and may facilitate the development of more efficient *in vitro* propagation protocols for this species.

Keywords: biomass, CKX inhibitors, *Prunus avium*, tissue culture

P19 - Effect of cytokinin oxidase/dehydrogenase inhibitors on shoot multiplication in *Buxus* L.

Hanzal Šedivá Jana[1], Pekařová Michaela[1], Zahradník Daniel[1], Nisler Jaroslav[2]

[1]Landscape Research Institute v. v. i. (VÚK), Department of Plant Biotechnology, Květnové nám. 391, 252 43 Průhonice, Czech Republic

[2]Institute of Experimental Botany, Laboratory of Growth Regulators, Czech Academy of Sciences and Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic

sediva@vukoz.cz

Buxus L. is a significant slow-growing evergreen shrub. It is widely used for shaped hedges in gardens. One of the possibilities for improving shoot production in recalcitrant plant species is a new group of chemical compounds (CKX inhbibitors) that protect cytokinins in in vitro culture from degradation. The aim of our study was to evaluate the effect of cytokinin oxidase/dehydrogenase (CKX) inhibitors (diphenylurea derivates) in two distinct boxwood genotypes, *Buxus sempervirens* 'Marginata' and *B. microphylla* 'Winter Gem'. We tested three CKX inhibitors, compounds 20, 52, and 77 alone or in combination with isoprenoid cytokinins (2iP or zeatin). Preliminary experiments showed promising results in the boxwood. A positive effect on shoot regeneration in the presence of CKX inhibitors was demonstrated. The tested genotypes exhibited varying regeneration capacity.

Keywords: *Buxus*, cytokinin oxidase/dehydrogenase inhibitors, shoot production, *in vitro*

P20 - Preliminary results of CKX inhibitors in poplars

Vladislava Galović[1], Tatjana Vujović[2], Florin Huiban Adrian[3], Saša Orlović[1]

[1]Institute of Lowland Forestry and Environment, Antona Čehova 13d, 21000 Novi Sad, Serbia

[2]Fruit Research Institute, Kralja Petra I br. 9, 32000 Čačak; Serbia

[3]University of Life Sciences ''King Mihai I'' Timișoara, Faculty of Engineeing and Applied Technology, Timisoara, Calea Aradului Street, number 119, Romania

vladislava.galovic@gmail.com

Endogenous cytokinin inhibitors reduce growth and affect premature senescence in poplars. Inhibitors of cytokinin oxidase/dehydrogenase (CKX) can reduce the degradation of cytokinins in plants. Recently invented two compounds, 52 and 77, of series of CKX inhibitors based on diphenylurea were used in this study. We examine their influence on the processes of clonal propagation by somatic embryogenesis and callus derived somaclonal variation in poplar *in vitro* culture. For clonal propagation, shoot apex of 2 months cultured poplar clones, L-12 and L-111 and 36 days old callus regenerants were used in the experiments. The treatments included two inhibitor compounds, 52 and 77, each applied at two concentrations 1µM and 0.1µM in solid MS medium, in combination with two cytokinins, 2iP (1µM) and BAP (1µM). After 48 days in the culture the following analyses were performed: multiplication index, dry mass, pigment content and biochemistry. The best results for multiplication index regarding L-12 were the combinations: 1 µM 77 with 1 µM 2iP and 0.1 µM 77 with 1 µM BAP. For L-111 that was 1µM 77 with 1 µM 2iP and 0.1 µM 77 with 1 µM BAP. Both compounds were also tested on solid WPM shoot induction medium (SIM) at 1µM and 0.1µM, respectively, for callus-based somaclonal variation in the L-12 poplar clone, with no further exogenous cytokinins introduced. After 36 days of subculture on SIM, the best results in both cases were achieved with 1 µM 77. All explants reach height of average 5 cm with fully developed leaves and roots without any signs of senescence. Positive effect of CKX inhibitors on poplar growth can be exploited in agriculture and in plant tissue culture.

Keywords: cytokinin oxidase/dehydrogenase, compound 52, compound 77, *Populus alba* L.

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P21 - Molecular basis of the role of ethylene in adventitious rooting of mature chestnut microshoots

Ricardo Castro-Camba[1,2], Conchi Sánchez[1], Saleta Rico[1], Nieves Vidal[1], Puri Covelo[1], María José Cernadas[1], Anxela Aldrey[1], Jesús Vielba[1]

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

[2]Dept. of Marine Ecology, IMEDEA-UIB-CSIC, C/ Miquel Marquès, 21, 07190, Esporles, Illes Balears, Spain

jmvielba@mbg.csic.es

Sweet chestnut (*Castanea sativa* Mill.) is a forest species with significant ecological, cultural and economic relevance in Europe. One of the major bottlenecks in the micropropagation of this species is the formation of adventitious roots. This challenge is particularly accentuated in microshoots established from trees with mature characteristics. While auxin is the primary plant growth regulator involved in adventitious rooting, other plant hormones, such as ethylene, can modulate the response to auxin and negatively influence the rooting process.

Rooting experiments were conducted to evaluate the effects of the ethylene perception inhibitor silver thiosulfate (STS) on the adventitious rooting performance of mature chestnut microshoots. STS pre-treated plants showed an improvement in their rooting ability after auxin treatment, with a higher rooting rate and a better developed root system.

A transcriptomics analysis was performed to explore the genetic basis underlying these physiological responses. Our results suggest that the STS pre-treatment significantly modified auxin signaling and transport processes during root induction, highlighting the influence of ethylene in the recalcitrant behavior of mature chestnut microshoots. Specific molecular processes identified here help explain the complex interactions between auxin and ethylene at the molecular level, and their influence in developmental responses.

Keywords: adventitious roots, auxin, chestnut, ethylene, recalcitrance

P22 - Ethylene regulates somatic embryogenesis in tamarillo by altering cytokinin and global DNA methylation levels

Mariana Neves[1], Sandra Correia[2], Michal Karady[3], Jorge Canhoto[1]

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Dep. of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[2]InnovPlantProtect CoLab, 7350-478 Elvas, Portugal
[3]Laboratory of Growth Regulators, Institute of Experimental Botany, The Czech Academy of Sciences & Palacký University, Šlechtitelů 27, CZ-779 00 Olomouc, Czech Republic

Tamarillo (*Solanum betaceum* Cav.) has been studied to understand the pathways of in vitro organogenesis and somatic embryogenesis (SE) as well as the recalcitrance associated with those morphogenic pathways. Despite the potential importance of ethylene in micropropagation, its effect on regeneration processes in woody species has not been thoroughly studied. Therefore, the main aim of this work was to evaluate how ethylene affects the SE process, using tamarillo as an experimental model, with potential application to other woody species. The results showed that inhibiting ethylene biosynthesis reduced the totipotency acquisition by decreasing the embryogenic callus (EC) induction. Nevertheless, this inhibition improved further somatic embryo differentiation. Ethylene immediate non-volatile precursor 1-aminocyclopropane-1-carboxylic acid (ACC) and cytokinin (CK) phytohormones endogenous profiling revealed that quick rises in ACC levels and a higher ratio ACC/CK were associated with totipotency acquisition, whereas the opposite profile was associated with embryo development. During EC induction, the inhibition of ethylene biosynthesis led to an upregulated *ARR1* expression, a positive regulator of CK response, previously reported as a disruptor of auxin-induced callus formation, while an ethylene precursor, ethephon, drove significant changes in expression patterns of several DNA methyltransferases genes and global DNA methylation levels. The combination of a DNA methyltransferase activity inhibitor, 5-AzaC, with ethephon improved EC induction, underscoring the synergy between hormonal regulation and epigenetic modifications in SE induction. Overall, our findings highlight new possible molecular mechanisms governing plant regeneration. The stage-specific role of ethylene points out its potential as a tool for improving micropropagation strategies in tamarillo and other woody plants.

Keywords: DNA methylation, ethylene modulation, in vitro culture, plant regeneration, woody species

P23 - Application of topolin-type cytokinins during the proliferation of Norway spruce embryogenic cultures

Lucie Fischerová[1], Zuzana Vondráková[1], Karel Doležal[2], Jana Pavlíčková[1], Daria Penzina[1], Marzieh Mohri[1], Kateřina Eliášová[1]

[1]Laboratory of Biologically Active Compounds

[2]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, Prague, Czech Republic

fischerova@ueb.cas.cz

The process of conifer somatic embryogenesis (SE) is controlled by phytohormones exogenously supplied into the culture medium. The early phases of SE (induction and proliferation), which significantly influence the later development of somatic embryos and the germination, are controlled by auxins and cytokinins (CKs). In traditional protocols, N^6-benzyladenine (BA) is the dominant CK. However, BA and its degradation products are stable, causing adverse effects in many in vitro plant propagation systems.

We applied aromatic topolin-type CKs meta-topolin (mT), methoxy meta-topolin (memT), and meta-topoline riboside (mTR) during the proliferation stage of Norway spruce SE and monitored the developmental process until the germination stage. The effect of different CKs on the anatomical structure (specifically the area of meristematic centres) and the overall growth of proliferating cultures were assessed. The yield of high-quality mature embryos was recorded, and the percentage of germinating embryos, along with morphological parameters of emblings (including root and hypocotyl length), were monitored across various types of pre-germination treatments. Despite high genotype-dependent variability, the area of meristematic centres and yield of mature embryos were explicitly affected by the applied CKs. The germination parameters, however, were more dependent on the type of pre-germination treatment than on the CK applied.

Keywords: somatic embryogenesis, topolin-type cytokinins, image analysis, phytohormone analysis, emblings

P24 - New phytohormone derivatives as a tool to optimize micropropagation of recalcitrant plant species

Karel Doležal[1,2], Magdalena Bryksová[2], Vlasta Matušková[2], Kristýna Bieleszová[2], Asta Žukauskaitė[2], Marek Zatloukal[2], Dardan Klos[2], Lucie Plíhalová[1,2], Ondřej Novák[1], Miroslav Strnad[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany AS CR, & Palacký University, Šlechtitelu 27, 78371 Olomouc, Czech Republic

[2]Department of Chemical Biology, Palacký University, Šlechtitelu 27, 78371 Olomouc, Czech Republic

karel.dolezal@upol.cz

Cytokinins (CKs). in concert with auxin, are critical for successful plant regeneration in tissue culture, where the most frequently used CK is 6-benzylaminopurine (BAP). However, due its fast *in-situ* N9-glucosylation, it can also generate diverse negative effects, which complicate micropropagation processes, especially in recalcitrant species. N9-glucosylation could be suppressed by appropriate N9 purine substitution of BAP or hydroxylation of its benzyl ring. To solve this problem, series of CK derivatives substituted at N9-position by various sugars and protective groups have been recently prepared and are already successfully used in many plant micropropagation systems. Moreover, by small change in CK structure, a potent cytokinin antagonists and/or inhibitors of their inactivation have been generated, including their isotopically and fluorescently labelled analogues.

Phytohormones auxins, thanks to their capacity to determine plant architecture, also have been successfully employed to obtain more economically advantageous plants. The ratio between activity of auxin and cytokinin needs to be tightly controlled to achieve proper shoot generation as well as rooting and acclimation. Recently we prepared several groups of new PEO-IAA-inspired anti-auxins, capable of antagonizing auxin responses *in vivo*.

Here, recent results of synthesis, characterization and biological activity testing of several new phytohormone derivatives will be presented.

Keywords: recalcitrance, cytokinin, auxin

P25 - Investigating the impact of compound interactions on plant development *in vitro*

Dicle Dönmez[1], Stefaan P.O. Werbrouck[2]

[1]Biotechnology Research and Application Center, Çukurova University, Adana, Türkiye

[2]Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, 9000 Ghent, Belgium

dicledonmez4@gmail.com

In vitro plant regeneration remains challenging due to various factors influencing successful growth and development. Genetic and physiological differences among plant species can further complicate establishing reliable protocols. Overcoming these obstacles is crucial for improving the success rate of plant regeneration in vitro and advancing biotechnological applications. This study aimed to identify effective plant growth regulators and compound combinations to enhance plant tissue responses to callus formation and shoot development. In this experiment, a range of compounds were used, including cytokinin oxidation inhibitors (21, 77), cytokinin derivatives (Zeatin, mT, 2iP, iPR), a cellular communication enhancers (CPZ), and an anti-auxin (PEO-IAA). Nine different plant species (*Betula utilis*, *Coleus esculentum*, *Lavendula angustifolia*, *Kalanchoe daigremontiana*, *Petunia hybrida*, *Drosera rotundifolia*, *Gynura proscumbens*, *Actiniada arguta* (kiwiberry), *Nicotiana tobacum* (control plant)) were used as plant material. Nine different compound combinations were prepared and applied to leaf disks and root explants. Additionally, the effects of blue and white light were investigated. The results of this screening will be discussed. For instance shoot induction was observed in lavender in media containing 4 µM PEO-IAA, 2 µM PEO-IAA + 1 µM IPR + 0.5 µM 77, and 10 µM CPZ + 1 µM 2iPR + 0.5 µM 21.

Keywords: cytokinin oxidation inhibitors, recalcitrant, plant tissue culture

P26 - Multi-scale characterizations of various desiccation treatments to produce somatic embryos closer to zygotic embryos of hybrid larch.

Savane Parisa[1], Eliášová Kateřina[2], Dobrev Petre I[2], Vondráková Zuzana[2], Delile Armelle[1], Boizot Nathalie[1], Rogier Odile[1], Motyka Václav[2], Ridel Céline[1], Claverol Stéphane[3], Teyssier Caroline[1*], Lelu-Walter Marie-Anne[1*]

[1]INRAE, ONF, BioForA, UMR 0588, F-45075 Orléans, France
[2]Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czechia
[3]University Bordeaux, Bordeaux Proteome, Bordeaux, France

caroline.teyssier@inrae.fr * equal contribution

The production of mature somatic embryos (SE), which closely resemble fully mature zygotic embryos, is hypothesized to enhance the likelihood of generating emblings comparable to seedlings. At the end of their maturation, SE exhibit a similar morphology to that of zygotic embryos, but retain a higher water content (WC). This suggests the absence of the late maturation stage, during which desiccation occurs. This desiccation is essential for drastically reduce water content. It could be crucial for triggering the biochemical changes that promote germination and the conversion to plants. In the presented study, we examined the biological, histological, biochemical, and molecular alterations in SEs of the *Larix eurolepis* subjected to desiccation under high relative humidity (RH), reduced RH, or a combination of both. Desiccation under high RH resulted in a slight reduction of WC, decreased starch production, reduced sucrose synthesis, and a lower abscisic acid content, with SE metabolism shifted towards the acquisition of desiccation tolerance and energy storage. These changes are known to be favourable for germination. In contrast, desiccation at low RH reduced WC to values similar to those of dried zygotic embryos. Among the activated biological processes, only defence mechanisms were observed, without any marker of the acquisition of desiccation tolerance. The sequential application of both desiccation treatments, which appeared to induce desiccation tolerance, had no negative impact on germination, but reduced SE WC. In summary, our findings provide novel insights into the processes involved in SE desiccation. In conifers, this represents the first successful desiccation of SE with a WC equivalent to that of dry zygotic embryos.

Keywords : desiccation tolerance; abscisic acid; carbohydrates; histology; phytohormones; proteome; *Larix x eurolepis*

P27 - Antioxidant systems and cytokinin balance during conifer regeneration *in vitro*: case study of spruce somatic embryogenesis and Saharan cypress micropropagation

Lukáš Ležal[1], Nikola Štěpánová[1], Karel Doležal[2], Helena Lipavská[1], Hana Konrádová[1]

[1]Department of Experimental Plant Biology, Faculty of Science, Charles University Viničná 5, 128 00, Prague 2, Czech Republic

[2]Laboratory of Growth Regulators, IEB CAS, Šlechtitelů 27, 783 71 Olomouc, Czech Republic

hana.konradova@natur.cuni.cz

Our project aims to contribute to clarification of the relationship between aromatic cytokinins, carbohydrates and antioxidant balance in the context of important morphogenic processes – somatic embryogenesis and micropropagation.

In Norway spruce somatic embryogenesis, auxin together with cytokinin are necessary medium components during initiation and proliferation. The cytokinin requirement is met usually by benzylaminopurine (BAP) and kinetin. There is, however, increasing number of reports showing negative effects of BAP in several species. Our study monitors the effect of substitution of BAP for meta-topolin (mT) during proliferation throughout the subsequent maturation and germination, when cytokinin is no longer present in the media. The substitution of BAP for mT caused increased yield of mature somatic embryos (SE), no signs of lowered SE quality (based on regular development markers and germination test), higher proportion of saccharides with protective potential (sucrose in mature SE and raffinose family oligosaccharides in desiccated SE), minor changes only in total soluble phenolics content and slight shift towards more reduced culture status.

Further, we have focused on critically endangered *Cupressus dupreziana*. Currently, no effective propagation method has been developed. In order to overcome its recalcitrance using knowledge-based approach, we analysed the cytokinin content and the endogenous carbohydrate spectrum.

Keywords: *Picea abies*, *Cupressus dupreziana*, micropropagatin, somatic embryogenesis, carbohydrate balance, meta-topolin

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P28 - Towards large scale production of Scots pine (*Pinus sylvestris* L.) via somatic embryos

Malin Abrahamsson, Magnus Hertzberg

SweTree Technologies AB, Skogsmarksgränd 7, SE-903 36 Umeå, Sweden

Malin.Abrahamsson@SweTree.com

Scots pine, compared to other pine species belong to the more difficult species to regenerate via somatic embryos, however, promising improvements of the protocols have been developed.

Challenges are still low initiation frequencies and few cell lines that work well at all stages up to seedlings and established plants. This means that the work to develop cell lines for Scots pine that will work in large-scale production will require more time and thereby be more expensive compared to for instance Norway spruce.

A Scots pine project was initiated at SweTree Technologies during 2024 with the overall goal to evaluate the possibility of large-scale production and deployment of Scots pine via somatic embryos and thereafter make tests and possible further development in SweTree SE-pilot facility. We will present data from our large-scale initiations as well as initial data from further evaluation of initiated cell lines.

SweTree have for many years been developing an automation solution for large scale production of conifer plants based on somatic embryogenesis (SE). A pilot facility has been built to test and further develop our automation solutions in preparation for a first large-scale production facility for Norway spruce.

Keywords: Scots pine, Somatic embryo, Initiation, Embryogenesis, Pilot facility

P29 - Novel antioxidant small molecules to improve somatic embryogenesis induction in European chestnut and holm oak

Cristina Buceta[1], María Teresa Martínez[1], Eduardo Piñas[1], Fátima Mosteiro[1], Elena Carneros[2], Carmen Gil[3], Ana Martínez[3], Pilar S.Testillano[2], Elena Corredoira[1]

[1]Misión Biológica de Galicia (MBG-CSIC), Sede de Santiago de Compostela, Avd. Vigo s/n 15705 Santiago de Compostela, La Coruña, Spain

[2]Pollen Biotechnology of Crop Plants Group, Margarita Salas Center of Biological Research, CIB-CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain

[3]Translational Medicinal and Biological Chemistry Group, Margarita Salas Center of Biological Research, CIB-CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain

elenac@mbg.csic.es; testillano@cib.csic.es

Somatic embryogenesis (SE) is widely regarded to be the most effective regeneration pathway in forest species. However, in many woody species, including European chestnut and holm oak, propagation via SE remains challenging, especially for cloning adult material. Increased oxidative stress and production of reactive oxygen species during induction of SE can significantly impair the process. Small antioxidant molecules are hence good candidates for improving SE induction. In this work, we evaluated novel small molecules with antioxidant activity, never used before in plants, as potential chemical enhancers of SE in holm oak and chestnut. The molecules were selected from the Medicinal and Biological Chemistry library (MBC, Ginex et al 2023, https://doi.org/10.3389/fphar.2023.1244317) and had showed neuroprotective effects in cell lines exposed to oxidative stress (Morales-García et al 2013, https://doi.org/10.1021/cn300182g). Leaves and shoot tips of chestnut and shoot tips of holm oak were used for SE induction experiments. The explants were excised from axillary shoot cultures previously established in vitro and cultured on induction media supplemented with 3 different antioxidant molecules. In chestnut, the SE induction rates were significantly improved when leaves were cultured with 25-50 µM CIB110. By contrast, in holm oak the best results were obtained by culturing with CIB123 and CIB113, being this positive effect only observed with explants derived from an adult tree.

Keywords: Antioxidants, *Castanea sativa*, cell reprogramming, *Quercus ilex*.

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P30 - Decoding somatic embryogenesis in woody species: Insights from cytological and multi-Omics analyses

Sandra Correia[1,2*], Daniela Cordeiro[1], André Caeiro[1], Mariana Neves[1], Miguel Rito[1], Ricardo Ferraz[1], 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, Estrada de Gil Vaz, 7350-999 Elvas, Portugal

sandraimc@uc.pt

In vitro-induced plant regeneration bridges plant biotechnology and development, showcasing pluripotency or totipotency through organogenesis or somatic embryogenesis (SE). SE is tightly regulated by auxin- and ethylene-dependent factors, influenced by non-coding RNAs and epigenetic mechanisms. However, insights from model species are often not transferable, necessitating integrative analyses to modulate SE and address recalcitrance in woody species. We have studied various woody dicot and gymnosperm species, generating cytological and multi-omics data to analyze embryogenic competence acquisition, maintenance, and expression. Focusing on indirect SE, we compared embryogenic (EC) and non-embryogenic (NEC) cells, as well as auxin-induced dedifferentiation (CD) and embryo development (ED). We found that cell fate stability and plasticity rely on spatiotemporal regulation of gene/protein expression, auxin distribution, and chromatin organization. Proteomic and metabolic analyses revealed EC's enhanced stress regulation and higher expression of energy metabolism-related proteins. Targeted transcriptomics of coding (mRNA) and non-coding RNAs (small RNAs, lncRNAs) showed NEC's abundant expression of miRNAs and lncRNAs targeting auxin- and ethylene-responsive genes, aligning with physiological data. These findings highlight the power of multi-scale approaches in understanding SE-associated cell reprogramming and recalcitrance in trees.

Keywords: Cell reprogramming, epigenetic regulation, non-coding RNAs, Somatic embryogenesis

P31 - Epigenetic memory of temperature sensed during somatic embryo maturation in 2-yr-old maritime pine trees

Jean-François Trontin[1,†], Mamadou Dia Sow[2,‡], Alain Delaunay[2,§], Ines Modesto[3], Caroline Teyssier[4], Isabelle Reymond[1], Francis Canlet[5], Nathalie Boizot[4], Claire Le Metté[4], Audrey Gibert[2], Cristian Chaparro[6], Christian Daviaud[7], Jörg Tost[7], Celia Miguel[3], Marie-Anne Lelu-Walter[4], Stéphane Maury[2]

[1]BioForBois, FCBA, Pôle Industrie Bois & Construction, Cestas 33610, France
[2]P2e, Université d'Orléans, INRAE, EA 1207 USC 1328, Orléans 45067, France
[3]Faculdade de Ciências, Biosystems and Integrative Sciences Institute, Universidade de Lisboa, Lisboa 1749-016, Portugal
[4]BioForA, INRAE, ONF, UMR 0588, Orléans 45075, France
[5]Sylviculture Avancée, FCBA, Pôle Ressources Forestières des Territoires, Cestas 33610, France
[6]IHPE, Université de Perpignan, UMR 5244, Perpignan 66100, France
[7]Laboratory for Epigenetics and Environment, Centre National de Recherche en Génomique Humaine, CEA—Institut de Biologie François Jacob, Université Paris Saclay, Evry 91000, France
[†]Present address: BEF, INRAE, UR 1138, 54280 Champenoux, France. [‡]Present address: GDEC, INRAE, UMR 1095, 63000 Clermont-Ferrand, France. [§]Deceased 7 August 2024.

stephane.maury@univ-orleans.fr

Embryogenesis is a brief but potentially critical phase in the life cycle of a tree for adaptive phenotypic plasticity. Using somatic embryogenesis in maritime pine (*Pinus pinaster* Ait.), we found that temperature during the maturation phase affects embryo development and postembryonic tree growth for up to 3 yr. We examined whether this somatic stress memory could stem from temperature- and/or development-induced changes in DNA methylation. For this, we developed a 200 Mb custom sequence capture bisulfite analysis of genes and promoters to identify differentially methylated cytosines (DMCs) between temperature treatments (18, 23, and 28 °C) and developmental stages (immature and cotyledonary embryos, shoot apical meristem of 2-yr-old plants) and investigate if these differences can be mitotically transmitted from embryonic to postembryonic development (epigenetic memory). We identified 10 genes involved in defense responses and adaptation, embryo development, and chromatin regulation that are candidates for the establishment of a persistent epigenetic memory of temperature sensed during embryo maturation in maritime pine. Here, we provide evidence that DNA methylation marks established during the embryonic phase are transmitted to the postembryonic plant development phase (Trontin et al, Plant Physiology 2024 https://doi.org/10.1093/plphys/kiae600).

Keywords: Somatic embryogenesis, ecological memory, temperature, epigenetics

P32 - Histone acetylation as a key epigenetic factor in cork oak somatic embryogenesis

De la Paz N. E, Carneros E., Testillano P. S.

Pollen Biotechnology of Crop Plants Group, Center of Biological Research Margarita Salas CIB-CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain.

testillano@cib.csic.es

Cork oak (*Quercus suber* L.) is a Mediterranean tree with remarkable economic and ecological value, but threatened by deforestation, diseases and limitations in natural regeneration. Somatic embryogenesis is a powerful *in vitro* regeneration process used in breeding, propagation, and conservation programs. Despite its great potential, low induction and unstable embryo production rates limit its use. Understanding the regulatory pathway of induced cellular reprogramming and *in vitro* embryogenesis development would help to enhance its efficiency. Epigenetic studies on somatic embryogenesis have focused on DNA methylation, but histone modifications are still poorly understood. In this study, we analyzed the dynamics of H4 acetylation, a relevant histone post-translational modification, during somatic embryogenesis in cork oak. Changes in H4 acetylation global levels and expression profiles of its regulating enzymes were analyzed, along with the effect of SAHA, a histone deacetylase inhibitor.

Our results showed that embryo development is associated with H4 acetylation, correlating with the upregulation of the histone acetyltransferase *QsHAM1-like* in developing embryos, whereas the histone deacetylases *QsHDA9*, *QsHDA19*, *QsHDA2* and *QsHDA15* were repressed. Additionally, SAHA treatments lead to an increase of H4 acetylation, the induction of embryogenesis marker genes, as *QsSERK1- like*, and to enhanced embryo production.

These insights contribute to understanding the role of histone epigenetic marks in forest species embryogenesis, where scarce information is available. Furthermore, open the door to new epigenetic strategies to improve *in vitro* regeneration for forest cloning and conservation.

Keywords: Cellular reprogramming, epigenetics, somatic embryogenesis, forest conservation

P33 - Genetic transformation of European chestnut somatic embryos using the putative anti-oomycete protein *Cast_Gnk2-like*

Susana Serrazina[1], María Teresa Martínez[2], Silvia Valladares[2*], Eduardo Piñas[2], Pablo Piñeiro[2], Marcelo Francisco[1], Rui Malhó[1], Rita Lourenço Costa[3], Elena Corredoira[2]

[1]Plant Functional Genomics Group (BioISI), Campus da Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal
[2]MBG-CSIC, Avda Vigo s/n, Campus Vida, Apartado 122, 15705 Santiago de Compostela, La Coruña, Spain
[3]Instituto Nacional de Investigação Agrária e Veterinária I.P. Avenida da República, Quinta do Marquês 2780-159 Oeiras- Portugal. Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, Lisboa, Portugal
[*] Current position at Agromillora Iberia, Barcelona, Spain

elenac@mbg.csic.es

The European chestnut is a widely distributed tree species that plays a crucial economic, social and ecological role across Europe. Chestnut trees are seriously threatened by ink disease/root rot mainly caused by *Phytophthora cinnamomi*. Overexpression of antifungal genes can be an alternative to conventional breeding efforts to generate tolerant genotypes. In the current study, a chestnut gene homologous to ginkbilobin-2 (*Cast_Gnk2-like*), which in *Ginkgo biloba* codifies an antifungal protein, was transferred into somatic embryos of two different embryogenic lines by *Agrobacterium* mediated transformation. The transformation efficiency (TE) was determined based on the fluorescence of surviving explants and was clearly genotype-dependent. The TE was 14.2% in CI-9 line whereas in the CI-3 line was 2.5%. A total of 12 independent transformed lines were obtained and the presence *Cast_Gnk2-like* gene in genomic DNA was confirmed by PCR. The number of transgene copies was estimated by qPCR in embryogenic lines with high proliferation ability. Also, the expression levels of *Cast_Gnk2-like* gene were determined in the embryogenic lines, with higher levels in lines CI-3-GIN1 and CI-9-GIN1. Transgenic plants were obtained from all transgenic lines evaluated after cold storage of the somatic embryos for 2 months and subsequent transfer to germination medium and in vitro tolerance assays are in progress.

Keywords: *Agrobacterium tumefaciens*, *Castanea sativa*, Cysteine-rich repeat secretory protein, Ink disease, *Phytophthora cinnamomi*

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P34 - Service for *in vitro* propagation and genetic transformation of plants. Engineering fruit trees

Nuria Alburquerque, Marina Martín-Valmaseda, Elena Yelo, Lorenzo Burgos

Group of Fruit Tree Biotechnology, Department of Plant Breeding, CEBAS-CSIC, Campus de Espinardo, 25, 30100 Murcia, Spain

burgos@cebas.csic.es

The service for *in vitro* propagation and genetic transformation of plants is established at CEBAS-CSIC (Murcia, Spain) and provides technical and scientific support to groups from research institutes or universities and private companies. Service staff have expertise in micropropagation and genetic transformation of recalcitrant woody species, such as apricot (*Prunus armeniaca* L.) and (*Prunus domestica* L.) or annual species such as melon (*Cucumis melo* L.). Additionally, the service offers the generation of transgenic lines in model species like *Arabidopsis thaliana*, *Nicotiana benthamiana*, *Nicotiana tabacum* and tomato (*Solanum lycopersicum*). The staff at the service is continuously developing new transformation protocols including innovative approaches such as *in planta* transformation, especially for recalcitrant woody species.

Sequencing platforms have generated a lot of information about the genome of various plant species. However, functional validation of the identified genes requires genetic transformation, which is increasingly demanded by researchers. Those interested in the service can provide their own molecular constructions or request customized designs according to their needs. The extensive expertise of the Fruit Biotechnology Group in plant transformation, particularly in recalcitrant species, ensures the successful production of the required plants, facilitating progress in applied genetic research.

Keywords: *Agrobacterium tumefaciens*, micropropagation, transgenic plants, recalcitrance, technical support

P35 - Protoplast and CRISPR technologies in woody plants: A case study on *Coffea arabica*

Sara Yasemin[1], Radisras Nkurunziza[2,4], Şükran Yıldız[3], Stefaan P.O. Werbrouck[4]

[1]Siirt University, Faculty of Agriculture, Department of Horticulture, 56100, Siirt, Türkiye
[2]Plant Breeding and Genetics Laboratory, International Atomic Energy Agency, Vienna, Austria
[3]Mersin University, Institute of Science, Department of Biotechnology, Mersin, Turkiye
[4]Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, 9000 Ghent, Belgium

sarayasemin1988@gmail.com

Protoplasts are widely used in plant biotechnology for various applications, including genome manipulations. In recent years, protoplast systems have become particularly important for validating the efficiency of gRNAs in CRISPR studies. However, factors such as the rigidity of the cell wall, the release of phenolic compounds, pose significant challenges in protoplast isolation from woody species such as *Coffee arabica*.

This study aims to determine the protoplast isolation efficiency, regeneration capacity, and transfection efficiency in *C. arabica*. Leaf tissues from in vivo and in vitro plants were used for protoplast isolation. Excessive leaf cutting, mannitol pre-treatment, and the use of very young leaves resulted in a high release of phenolic compounds. However, minimizing mechanical stress by using longitudinally cut leaves significantly reduced phenolic release. For protoplast isolation, different concentrations of mannitol (0.5, 0.6, and 0.7 M) and Cellulase-Viscosyme enzymes (1%, 2%, 3%) were tested. Although protoplast yields ($<2\times10^4$ cells/ml) were low, protoplasts were successfully obtained from all groups and microcalli were obtained from in vivo plants. The optimum isolation efficiency was achieved with 2% enzyme concentration. The protoplast density was $5x10^5$ cells/ml for in vitro plants. Viability of the protoplasts were detected with FDA staining.

Transfection efficiency was evaluated by introducing a GFP-containing plasmid into protoplasts via PEG-mediated transformation. Fluorescence microscopy analyses revealed a successful transfection. The findings indicate that protoplast-based genetic studies are feasible for *Coffee arabica*. This study provides a valuable foundation for future research in gene editing and regeneration studies particularly in woody plant species.

Keywords: gene editing, protoplast, regeneration, transfection

P36 - CRISPR/Cas9-mediated mutagenesis of the susceptibility gene *Cspmr4* in European chestnut

Vera Pavese[1], María Teresa Martínez[2], Lorenzo Antonio Marino[1], Ernesto González[2], Silvia Valladares[2*], Daniela Torello Marinoni[1], Andrea Moglia[1], Roberto Botta[1], Elena Corredoira[1]

[1]Dipartimento di Scienze Agrarie, Forestali ed Alimentari (DISAFA), Largo Paolo Braccini 2, 10095 Grugliasco, Torino

[2]Misión Biológica de Galicia (MBG-CSIC), Sede de Santiago de Compostela, Avd. Vigo s/n 15705, Santiago de Compostela, La Coruña

[*] Current position at Agromillora Iberia, Barcelona, Spain

elenac@mbg.csic.es

Castanea sativa L. is a highly versatile species of significant ecological, economic, and cultural importance across Europe. However, it faces severe threats from ink and canker diseases, caused by *Phytophthora cinnamomi* and *Cryphonectria parasitica*, with the severity of these diseases increasing in recent years due to climate change. The development of gene editing using the CRISPR/Cas9 technology has enabled targeted mutagenesis in an efficient and precise way. One promising gene-editing strategy for conferring tolerance to pathogens involves disabling plant susceptibility (S) genes. In the present work, the knock out of the *Cspmr4* susceptibility gene, which encodes a callose synthase, has been investigated using CRISPR/Cas9.

Somatic embryos from two lines (CI-9 and CI-3) were transformed using the *Agrobacterium tumefaciens* EHA105 nptII_Cas9_*pmr4*_gRNA1_gRNA2 strain. After eight weeks, kanamycin-resistant explants were observed exclusively in the CI-9 line, with the best results achieved after 5 days of co-cultivation, yielding a success rate of 6.7%. To assess the editing efficiency of the mutants and identify the types of mutations, Sanger sequencing was performed in conjunction with TIDE software. In five of the seven embryogenic lines, the editing event was confirmed, and four of them demonstrated high editing efficiency percentage (>93%). The tolerance assay of the edited somatic embryos and plant regeneration are currently under investigation.

Keywords: *Castanea sativa*, S genes, genome editing, *Phytophthora cinnamomi*, somatic embryogenesis

Funding: At MBG-CSIC, work was supported by Ministerio de Ciencia, Innovación y Universidades (MICIN, Spain) through the project PID2020-112627RB-C33 (AEI/10.13039/501100011033). At DIFASA, work was supported by Fondazione CRT, the BIORES project 2023.0374 and the project VALORE IN CAMPO MIPAAF-2022-0667521-Azioni di Valorizzazione e Recupero per le filiere italiane di Castagno, Mandorlo, Pistacchio e Carrubo

P37 - CRISPR-Cas technology to modify the flowering time of trees

Ashkan Hodaei, Stefaan P.O. Werbrouck

Laboratory for Applied In Vitro Plant Biotechnology, Department of Plants & Crops, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium.

ashkan.hodaei@ugent.be

Flowering time is critical to the reproductive success of trees, affecting biodiversity, ecosystem dynamics and economic outcomes. Modifying flowering time can increase yields, avoid early season frosts and reduce pest pressure. In fruit trees, reducing the juvenile phase allows earlier production, while adjusting flowering time can extend the harvest window. In addition, preventing alternate or biennial bearing ensures regular yields and consistent quality. In this context, the introduction of a new flowering-related trait into trees has great commercial value. However, traditional breeding methods are limited by the long seed-to-seed cycle and the lack of natural mutations in conserved genes available in the gene pool, making molecular approaches such as CRISPR essential for introducing new traits. The advent of CRISPR-based genome editing offers a unique opportunity to accelerate and refine these modifications. This poster explores the application of CRISPR/Cas9 to target key genes regulating flowering time in trees, examining flowering-related pathways, candidate genes for CRISPR knockout, and recent advances in CRISPR-mediated gene editing using morphogenic regulators for trees. We also discuss the potential for applying these techniques to both commercially valuable and ecologically important trees.

Keywords: fruit trees, morphogenic regulators, transformation capability, genome editing, juvenile phase, yield enhancement

P38 - Expression of flowering genes in *in vitro* propagated silver birch clones with differing flowering

Baiba Krivmane, Dainis Ruņģis, Ineta Samsone, Kaspars Liepiņš, Arnis Gailis

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

baiba.krivmane@silava.lv

Vegetatively propagated *in vitro* silver birch (*Betula pendula* Roth.) material is used for breeding and for the development of seed plantations. However, reduced flowering and seed formation is often observed, which delays breeding activities and hinders seed production in nurseries. This is probably related to the long juvenile phase in trees, which can last 10–20 years. *BpMADS4* is one of the major genes controlling initiation of flowering and inflorescence development in birch and in the transition from the vegetative to the reproductive phase. Overexpression of *BpMADS4* can cause very early flowering, and studies suggest that the expression of the *BpMADS4* gene alone is capable of turning the vegetative apical meristems into inflorescence meristems, but suppression of the gene inhibits inflorescence formation. The aim of this study was to assess expression of juvenility and maturity related target genes (*SPL1*, *SPL9*, *AP2*, *RAP2*) as well the flowering associated gene *BpMADS4* in 12-year-old silver birch trees with differing flowering. Several birch clones growing at the Norupe nursery were analysed, some of which regularly flowered, while other did not flower or had reduced flowering. In addition, three-year old non-flowering birch trees, which were planted in pots after microclonal propagation were analysed. Gene expression data were obtained for three genes – *BpMADS4*, *AP2* and *RAP2*. The highest gene activity of all three genes, both within clones and between clones, was observed in female flowers. When analysing the data obtained from leaf samples, it was observed that the lowest *BpMADS4* gene expression was observed in those clones that were not flowering at the time of sample collection. Unfortunately, we could not link the results obtained to juvenile or mature state in the 12-year-old trees, as higher expression of the *AP2* and *RAP2* genes, was observed in flowering inflorescences (indicating that the tree is entering the mature phase). This higher expression of the juvenility related genes could be related with tissues sampled (young flower buds) rather than with the maturity of the tree. However, in the case of the 3-year-old birch clones, a correlation was found between expression of the *AP2* and *RAP*2 genes and juvenility –lower *AP2* and *RAP2* gene expression was observed in the clone that was not flowering at the sample collection time, but flowered later. Higher *AP2* and *RAP2* expression was observed in the other clone that did not flower at all in the collection season (2024). Additional research is needed on a larger number of clones with differing flowering to further elucidate the role of these genes in the transition from the juvenile phase and initiation of flowering.

Keywords: *MADS4*, silver birch, *in vitro*, flowering, clones

P39 - Effect of volatile organic compounds emitted by *Serendipita indica* on plant growth

Hassiba Fraj, Stefaan Werbrouck

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

hassibaing@live.fr

Volatile organic compounds (VOCs) produced by microorganisms, particularly fungi, play a crucial role in promoting plant growth, enhancing stress tolerance, and improving overall plant health. Among these, the endophytic fungus *Serendipita indica* has gained significant attention for its ability to enhance plant growth through VOC emissions, both in field conditions and in vitro.

To investigate this, we utilized existing and newly developed in vitro setups to expose *Ocimum basilicum* (basil) seeds or explants to VOCs emitted by *S. indica*. Our findings revealed significant improvements in plant height, shoot number, and root length, even under salt stress conditions. At a salt concentration of 9 g/L NaCl, VOC-treated plants exhibited a more than threefold increase in chlorophyll content compared to non-treated plants, suggesting enhanced photosynthetic efficiency under saline conditions. Additionally, in basil plants grown in semi-solid medium under salt stress, VOC exposure positively influenced total phenolic content, total flavonoid content, and antioxidant activity. This suggests that *S. indica* VOCs contribute to strengthening the plant's antioxidant defense system.

Building on these promising results, we extended our study to tree species such as *Moringa oleifera* and *Populus* × *canadensis*. The results highlight the potential of fungal VOCs as new *in vitro* tools offering an added value to plant growth promoters.

Keywords: Volatile organic compounds, *Serendipita indica*, *in vitro* plant growth

P40 - Isolation and characterization of extracellular vesicles from *Juniperus communis* and *Pinus nigra.*

Ján Jurčík[1], Miroslav Perniš[1], Zuzana Kozovská[2], Katarína Klubicová[1]

[1]Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Akademická 2, P.O. Box 39A, 95007 Nitra, Slovakia

[2]Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovakia

jan.jurcik@savba.sk

Extracellular vesicles (EVs) are nano-sized particles naturally secreted by cells, including plants. EVs carry proteins, DNA, RNA, lipids, and secondary metabolites. These biologically essential cargos are involved in plant immunity and cell communication. Our laboratory is investigating somatic embryogenesis (SE) in selected conifers and initiating research on the potential role of EVs in SE of *Pinus nigra*. For this purpose, we optimized a protocol for isolating EVs from plant biological materials *Pinus nigra* (suspension culture) and *Juniperus communis* (mature berries). We homogenized biological material for EVs isolation and subsequently used differential centrifugation, ultrafiltration, and SEC chromatography. Our results demonstrated that the technique effectively isolated EVs from suspension culture and berries, yielding consistent and sufficient concentrations—the presence of EVs was confirmed by NTA analysis and TEM microscopy. Protein quantification revealed measurable protein levels in the isolated EVs fractions suitable for downstream analysis.

Keywords: extracellular vesicles, EVs, SEC, somatic embryogenesis, *Juniperus communis, Pinus nigra*

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Posters WG2

P41 - Optimizing micropropagation of *Prunus mahaleb* (L.) rootstock on semisolid media

Rumyana Valkova [1], Lilyana Nacheva [2], Mariyana Gozmanova [1], Ivaylo Tsvetkov[3]

[1]Department of Molecular Biology, Faculty of Biology, University of Plovdiv, 24, Tsar Assen Str. 4000 Plovdiv, Bulgaria,
[2]Fruit Growing Institute, Agricultural Academy, 12 Ostromila Str., 4004 Plovdiv, Bulgaria,
[3]Forest Research Institute – BAS, 132, Kliment Ohridski Blvd., Sofia 1756, Bulgaria

boncheva@uni-plovdiv.bg

Prunus mahaleb L. is a commonly used cherry rootstock, providing a suitable foundation for developing standard cherry trees adapted to various soil conditions. Cherry cultivars grafted onto this rootstock exhibit excellent compatibility, develop large canopies, and demonstrate stable fruiting, even in soils with high limestone content. Micropropagation of woody species is a method that combines the advantages of vegetative propagation while preserving unique genetic characteristics and multiplying selected plants in shortened periods. This study aims to investigate the efficiency of various culture media on the micropropagation of a mahaleb cherry genotype. Shoot tips were taken for explant initiation after surface sterilization and cultured in MS (Murashige and Skoog) medium with 30g/l of sucrose and 7g/l of agar. The inoculated shoots were subcultured on semisolid proliferation media, including MS and WPM (Woody Plant Medium), supplemented with 30g/l sucrose, 7g/l agar, 1 mg/l BAP (6-Benzylaminopurine) and 0,01mg/l NAA (α-naphthaleneacetic acid). A successful proliferation was observed on MS media, while WPM medium induced callus formation rather than shoot development. The experimental results also revealed a higher regeneration rate, as indicated by increased stem elongation and leaf formation, on MS medium. Future studies will focus on evaluating liquid MS and WPM media for large-scale multiplication using the PlantForm™ TIS bioreactor.

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P42 - Biotechnological approaches for *Thymus albicans* and *Thymus camphoratus* germplasm conservation: optimizing *in vitro* establishment protocols

Daniela Duarte[1*], Bernardo Onofre[1], Paula Castro[1], Jorge Canhoto[1], Mónica Zuzarte[2], Sandra Correia[1,3]

[1]Centre for Functional Ecology-Science for People & the Planet, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

[2]University of Coimbra, Faculty of Pharmacy, Azinhaga de S. Comba, Coimbra 3000-548, Portugal; University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Azinhaga de S. Comba, Coimbra 3000-548, Portugal; University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Coimbra 3030-790, Portugal

[3]InnovPlantProtect CoLAB, Estrada de Gil Vaz, 7350-999 Elvas, Portugal

daniela.d@sapo.pt

The conservation of endangered aromatic plant species is crucial for preserving biodiversity and their biotechnological potential. *Thymus albicans*, a vulnerable species endemic to the Iberian Peninsula, and *Thymus camphoratus*, a Portuguese endemic species classified as Least Concern by the IUCN, play crucial roles in their ecosystems and face constant human pressures. This study aimed to establish *in vitro* protocols for these two aromatic species' germplasm conservation and metabolite production. For *T. albicans, in vitro* culture establishment from seedlings was effectively achieved, with success rates up to 62.5%. Milder surface disinfection conditions were more effective in maintaining seed viability while reducing contaminations. Additionally, callus induction was optimized by adjusting auxin-to-cytokinin ratios. The best results were obtained using a combination of 1 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid) and 1 mg/L KIN (kinetin). Hairy-root cultures are currently being optimized using *Agrobacterium rhizogenes*-mediated transformation. For *T. camphoratus*, shoots were collected from selected mother plants and surface disinfected (ethanol 70%, 3% commercial bleach, sterile water rinses) before establishment in MS medium, with 18.6% of the explants successfully established. The results obtained so far provide insights into a sustainable approach for the conservation and utilization of these species, supporting both ecological preservation and biotechnological advancements.

Keywords: Conservation, endemic species, *in vitro* establishment, *Thymus* spp.

P43 - Optimizing micropropagation protocols for the *ex situ* conservation of *Vaccinium myrtillus* L. from Albanian flora

Yllka Lala[1], Efigjeni Kongjika[2], Valbona Sota[1,2]

[1]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Boulevard "Zog I" Tirana 1001, Albania

[2]Biotechnology & Genetics Scientific Research Unit, Section of Natural and Technical Sciences, Academy of Sciences of Albania, "Murat Toptani" Promenade, Tirana 1000, Albania

valbona.sota@fshn.edu.al

This study aimed to develop an efficient micropropagation protocol for Vaccinium myrtillus L. (bilberry), a wild species native to the Albanian flora, with many uses in the food industry and for medical purposes. Due to the increasing impact of climate change, several valuable genetic resources are at risk of extinction. Therefore, optimizing biotechnological methods for ex situ conservation of important native bilberry varieties is considered an emergency. In order to determine the most effective basal medium, lateral buds were initially selected as explants, which were then individually cultured in Murashige and Skoog medium (MS) and McCown's woody plant medium (WPM) supplemented with three different concentrations of cytokinin: 0, 1, 1.5 and 2 mg/l of 6-Benzylaminopurine (BAP), 0.1 mg/l of 1-Naphthaleneacetic acid (NAA). The results indicated that WPM was the most effective basal medium, promoting superior plant regeneration. The high concentration of cytokinins stimulated extensive shoot proliferation, favoring the early formation of multiple microshoots. Regarding shoot multiplication, the most optimal cytokinin concentration resulted in 2 mg/l BAP, followed by 1.5 mg/l concentration, referring to the monitored biometric parameters. Optimization of micropropagation protocols will enable the sustainable use of bilberry genetic resources for future stages of long-term preservation through cryopreservation methods.

Keywords: bilberry, micropropagation, cytokinin, *ex situ* conservation, cryopreservation

P44 - Comparison of two *in vitro* multiplication methods for phenotyping experiments in Croatian breeding program

Iva Šikuten, Zvjezdana Marković, Darko Preiner
Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, Zagreb, Croatia

isikuten@agr.hr

Maintaining a consistent supply of plant material for *in vitro* research is a crucial step, and in vitro propagation is essential in achieving that goal. This study compared two propagation techniques, nodal segment culture and meristematic bulk (MBT) culture, using two growing mediums, Murashige and Skoog (MS) and Chee and Pool (CP). The experiment was conducted on two grapevine genotypes developed through the breeding program, GRP-33 and DRP-7, along with the Chardonnay cultivar.

The initial *in vitro* culture of all three genotypes was successfully established, with no differences observed between the two tested growing mediums. Multiplication rates were evaluated over two propagation cycles, revealing that both methods exhibited similar multiplication rates. The MBT method showed a slightly higher multiplication rate for GRP-33 and Chardonnay, while the nodal segment method was more effective for DRP-7 cultivar. However, neither the propagation method nor the growing medium had statistically significant impact on multiplication success. These results indicate that the selection of propagation method should be guided by specific reasearch needs and experimental requirements.

Key words: *in vitro* culture, grapevine, multiplication methods, breeding program

P45 - Metabolic profiling of zygotic and somatic embryos of *Aesculus flava*

Snežana Zdravković-Korać, Uroš Gašić, Branka Uzelac, Slađana Jevremović, Maja Belić, Dušica Ćalić, Jelena Milojević

Department of Plant Physiology, Institute for Biological Research Siniša Stanković – National Institute of the Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11108, Belgrade, Serbia

sladja@ibiss.bg.ac.rs

The zygotic embryos (ZE) of *Aesculus* species are a rich source of health-promoting phytochemicals used in the pharmaceutical industry and traditional medicine. However, the yield of the seeds decreases due to various stresses and the seeds contain high levels of heavy metals as they are mostly collected from trees grown as ornamentals in urban environments. Plant tissue culture is a promising alternative for the production of plant specialized metabolites, thus somatic embryos (SE) could serve as a suitable source of these phytochemicals. Therefore, the aim of the present study was to establish a metabolic profile of *A. flava* ZE (isolated from ripe fruits) and SE at different developmental stages. The SE were obtained from embryogenic suspensions with high proliferation and SE regeneration rates. LC/MS characterisation of the ethanolic extracts revealed 117 metabolites: benzoic and cinnamic acid derivatives, flavan-3-ols, procyanidins, flavonoids and saponins. Whereas the SE at early developmental stages contained mainly flavonoids and ZE saponins, the SE at the cotyledonary stage (CSE) contained both, but also a considerable amount of flavan-3-ols and procyanidins. In addition, new saponins and aescin derivatives were detected in ZE and CSE. Thus, CSE obtained from suspension cultures are a good source of a number of valuable phytochemicals, including the most appreciated aescin derivatives. This is the first report on the metabolic profiling of *A. flava* ZE and SE.

Keywords: yellow buckeye, LC/MS, embryogenic suspensions, secondary metabolites, somatic embryos

P46 - Development of synthetic seed technology of silver birch (*Betula pendula* Roth.)

Ineta Samsone, Ieva Bērziņa

Latvian State Forest Research Institute "Silava", Riga street 111, Salaspils LV-2169, Latvia

ineta.samsone@silava.lv

Synthetic seeds are artificially encapsulated embryonic or non-embryonic plant tissues. They represent a promising biotehnology-based solution for the conservation of genetic resources. This method can aid in the preservation and propagation of genetically improved reproductive material with high forestry value. Synthetic seeds are small in size making them easier to store, transport, and planting. Encapsulation is crucial for reducing plantlet costs and extending storage times. The aim of this study was to develop a shoot tip encapsulation method for long-term storage of *in vitro* silver birch genetic material. The study demonstrated that encapsulated silver birch shoot tips could be stored at low positive temperatures for at least seven months, using 3% sodium alginate and 100 mM calcium chloride for capsule formation. The regrowth rates of encapsulated shoots after seven months of storage at +4°C ranged from 73.4% to 100 %, depending on the composition of the capsules and storage conditions. The highest shoot regrowth ability was observed in capsules containing MS medium, growth regulators, and sugar. The composition of the culture medium used before encapsulation did not affect the plant storage and regrowth parameters. Direct regrowth of encapsulated shoots is also possible in a non-sterile peat substrate.

Keywords: encapsulation, sodium alginate, shoot tips

P47 - Detection of bacterial blight caused by *Xanthomonas campestris* in Georgian hazelnuts

I. Megrelishvili[1], T. Elbakidze[2], Stefaan P.O. Werbrouck[3], Z. Khidesheli[1], N. Maziashvili[1], A. Rabaaoui[3], L. Ujmajuridze[1]

[1]LEPL Scientific-Research Center of Agriculture, 36b Marshal Gelovani ave, 0159, Tbilisi, Georgia

[2]LEPL State Laboratory of Agriculture, 49 V. Godziashvili str., 0159, Tbilisi, Georgia

[3]Dept. of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, B-9000 Ghent, Belgium

ivetameg@yahoo.com

In 2022-2023, hazelnut plants in nurseries across western Georgia were observed with symptoms of bacterial blight.538 symptomatic samples both 'Anakliuri' (Georgian) and 'Tonda di Giffoni' (Italian) hazelnut cultivars were collected from the Samegrelo and Guria regions in Georgia. DAS-ELISA with polyclonal antibodies identified 34 potentially positive for *X. campestris.* Additionally, samples were cultured on YPGA and King's medium B for presumptive *X. campestris* identification based on yellow colonies with subsequent confirmation of 28 samples by a TaqMan triplex real-time PCR assay using species-specific primers. Sanger sequencing of the 16S rRNA gene, performed on 24 of these, with further BLAST analysis revealed four isolates as *X. campestris* (GenBank Accession Numbers:PP437082.1, PP434581.1, PP434578.1, PP434556.1) showing up to 100% nucleotide identity to *X. campestris pv. campestris* strains isolated from different Countries and sources.

Pathogenicity was confirmed by inoculating young shoots (20-30 cm long) of 2-year-old potted hazelnut plants (‘Hall's Giant’) with a bacterial suspension (10^{-8} CFU/ml) of each isolate. Bacterial cultures were re-isolated, fulfilling Koch's postulates.

The discovery of *Xanthomonas campestris* in Georgian hazelnuts necessitates regular control to protect hazelnut production in Georgia and beyond.

Keywords: Bacterial blight, biosecurity, epidemiology, Georgia, nursery

P48 - Phylogeny and mycotoxin profiles of pathogenic *Alternaria* and *Curvularia* species isolated from date palm, in South Tunisia

Amal Rabaaoui[1↓], Mario Masiello[2], Stefania Somma[2], Chiara Dall'Asta[3], Laura Righetti[3], Antonia Susca[2], Antonio Francesco Logrieco[2], Ahmed Namsi [5], Radhouane Gdoura,[4] Stefaan P.O Werbrouck [1], Antonio Moretti[2]

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

[2]National Research Council, Institute of Sciences of Food Production, Bari, Italy

[3]Department of Food and Drug, University of Parma, Parma

[4]Laboratory of Toxicology-Microbiology and Environmental Health, Department of Biology, University Sfax, Sfax, Tunisia;

[5]Laboratoire de Phytopathologie, Centre Régional de Recherches en Agriculture Oasienne, Degache, Tunisia.

amal.rabaaoui@ugent.be

Date palm in Tunisia is threatened by fungal pathogens, notably *Alternaria*. Samples from 'Deglet Nour' palms yielded 50 fungal strains: 45 *Alternaria* and 5 *Curvularia*. Phylogeny, mycotoxin analysis, and pathogenicity tests were used for identification. Gene sequencing (allergen-alt-a1, gpd, calmodulin) grouped 35 strains as *Alternaria arborescens*, *Alternaria tenuissima*, *Alternaria alternata* (*Alternaria* Section), and 10 as *Alternaria consortialis* (Ulocladioides Section). *Curvularia* strains were identified as *Curvularia spicifera* using ITS, gpd, and elongation factor sequencing.

Pathogenicity tests on date palm plantlets confirmed all species were pathogenic, with *Alternaria arborescens* being most aggressive and *Curvularia spicifera* least. Mycotoxin production was assessed. *Alternaria consortialis* produced none. Over 80% of Alternaria Section strains produced alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), tenuazonic acid (TA), and 40% produced altenuene (ALT). *Curvularia spicifera* showed trace fumonisins B. This study is the first comprehensive analysis of mycotoxigenic *Alternaria* and *Curvularia spicifera* linked to date palm leaf spot.

Keywords: Date palm, *Phoenix dactylifera*, mycotoxins, pathogenicity, phylogenetic analysis

P49 - Quantification of immunohistochemistry fluorescence of plant cell wall glycan epitopes – A semiautomated workflow design

Maria João Ferreira[1], Martin Vojtek[2], Carmen Diniz[2], Ieva Lelenaite[3], William G. T. Willats[3], Sílvia Coimbra[1], Ricardo M. F. da Costa[4,5]

[1]LAQV/REQUIMTE, Biology Department, Faculty of Sciences, University of Porto, Porto, Portugal
[2]LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3]School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, UK
[4]University of Coimbra, Molecular Physical-Chemistry R&D Unit, Department of Chemistry, LAQV/REQUIMTE, Coimbra, Portugal
[5]Centre for Functional Ecology – Science for People and the Planet (CFE), Department of Life Sciences, University of Coimbra, Coimbra, Portugal

dacosta.rmf@gmail.com

Automating plant cell wall (CW) glycan epitope quantification in immunohistochemistry is hindered by plant growth plasticity, glycan dynamics and monoclonal antibody (mAb) cross-reactivity. To address this, we designed an experimental pipeline to compare labeling patterns and fluorescence intensities across: (1) mAbs targeting different CW glycans in the same tissues, (2) KOH-treated vs. untreated sections, (3) plant species with distinct CW compositions/structures – *Arabidopsis thaliana* (dicot), *Zea mays* (commelinid monocot), and *Pinus pinaster* (conifer). Thin LR-White resin cross-sections were incubated with mAbs against CW matrix glycans: xyloglucan (LM15), xylan (CCRC-M144, LM10), and pectin (JIM7). Some sections were pretreated with 0.1M KOH and all were stained with 0.01% (w/v) calcofluor-white. Specimens were imaged using a Lionheart FX automated fluorescence microscope for uniform acquisition. DAPI and GFP channels were captured and analyzed with a custom Fiji (ImageJ) macro, which pairs images, defines regions of interest (ROIs) via DAPI thresholding, and transfers them to the GFP channel for fluorescence quantification. Processed images with ROI overlays were saved for validation, and fluorescence data stored for statistical analysis. The produced distinct immunolabelling patterns and signal intensities allowed reliable detection of differences based on recognized epitope, KOH-treatment, and plant species. The dataset generated during the developed workflow allows semiautomated, computer-assisted image analysis of bulk immunolabelling data with reduced bias and increased speed. Several applications may benefit from this tool, namely the elucidation of CW roles in plant-pathogen interactions in *Pinus* spp. infected by *Bursaphelenchus xylophilus*, the pinewooMd nematode – the broader focus of our project.

Keywords: plant cell wall glycans, immunohistochemistry, automated fluorescence quantification

Posters WG3

P50 - Establishment of willow (*Salix* spp.) *in vitro* culture

Ivaylo Tsvetkov[1], Lilyana Nacheva[2], Mariyana Gozmanova[3], Rumyana Valkova[3]

[1]Forest Research Institute – BAS, 132, Kliment Ohridski Blvd., Sofia 1756, Bulgaria
[2]Fruit Growing Institute, Agricultural Academy, 12 Ostromila Str., Plovdiv 4004, Bulgaria
[3]Department of Molecular Biology, University of Plovdiv, Plovdiv 4000, 24, Tsar Assen Str., Bulgaria

tsvet_i@yahoo.com

The experiment was set up to study the nutrient media effect on the multiplication rate of *Salix in vitro* cultures with an idea to use the technique for providing plant material in further bioreactor cultivation. Cuttings from white willow (*Salix alba* L.) and a highly productive variety ('Tora', Orm x *S. schwerini*) served as source material. Forced buds of sterilized cuttings kept for 10 days at room temperature were used as initial explants. The sterilization was done following a routine procedure (two-step treatment with 70% ethanol for 30 s and 5% calcium hypochlorite for 4 min). Three nutrient media (MS, DKW and WPM) supplemented with 2.5 µM BAP, 0.005 µM IBA, 30 g/l sucrose and 6.5 g/l Phyto agar, were tested. The media was adjusted to pH 5.6 before autoclaving at 121°C for 20 min. Initially, the explants were inoculated in tubes (5ml medium). Due to some visible symptoms of vitrification during the first week of cultivation, the explants were transferred on the same media variants with a reduced in half amount of BAP (1.25 µM). At this stage, five explants (1 cm long segments with two pairs of leaves) were cultivated in glass jars (190ml, 25ml medium) providing at least 10 jars per variant. After three passages of 4-weeks each, a bunch of plant biometric parameters were recorded (number of shots, length of the main shoot, length of the lateral shoots, number of leaves, fresh/dry weight). The DKW medium was found to be superior for both types of plant material in providing statistically significant better values of the observed parameters. As the only exception, MS medium ensured significantly better fresh/ dry weights of the shoots originating from 'Tora'. Shoots rooted spontaneously in the course of the experiment, with frequencies varying from 20 to 90%.

Keywords: Micropropagation, nutrient media, *Salix*

Acknowledgements: This study was financially supported by COST Action CA21157 and the Bulgarian National Science Fund, (KP-06-COST/17 "CopyFruitTree: Innovative approaches for woody fruit species cloning")

P51 - Optimizing micropropagation of *Prunus mahaleb* (L.) rootstock on semisolid media

Rumyana Valkova [1], Lilyana Nacheva [2], Mariyana Gozmanova [1], Ivaylo Tsvetkov[3]

[1]Department of Molecular Biology, Faculty of Biology, University of Plovdiv, 24, Tsar Assen, Str. 4000 Plovdiv, Bulgaria,
[2]Fruit Growing Institute, Agricultural Academy, 12 Ostromila Str., 4004 Plovdiv, Bulgaria,
[3]Forest Research Institute – BAS, 132, Kliment Ohridski Blvd., Sofia 1756, Bulgaria

boncheva@uni-plovdiv.bg

Prunus mahaleb L. is a commonly used cherry rootstock, providing a suitable foundation for developing standard cherry trees adapted to various soil conditions. Cherry cultivars grafted onto this rootstock exhibit excellent compatibility, develop large canopies, and demonstrate stable fruiting, even in soils with high limestone content. Micropropagation of woody species is a method that combines the advantages of vegetative propagation while preserving unique genetic characteristics and multiplying selected plants in shortened periods. This study aims to investigate the efficiency of various culture media on the micropropagation of a mahaleb cherry genotype. Shoot tips were taken for explant initiation after surface sterilization and cultured in MS (Murashige and Skoog) medium with 30g/l of sucrose and 7g/l of agar. The inoculated shoots were subcultured on semisolid proliferation media, including MS and WPM (Woody Plant Medium), supplemented with 30g/l sucrose, 7g/l agar, 1 mg/l BAP (6-Benzylaminopurine) and 0,01mg/l NAA (α-naphthaleneacetic acid). A successful proliferation was observed on MS media, while WPM medium induced callus formation rather than shoot development. The experimental results also revealed a higher regeneration rate in terms of stem elongation and leaf formation on MS medium. Future studies will focus on evaluating liquid MS and WPM media for large-scale multiplication using the PlantForm™ TIS bioreactor.

Acknowledgementss: This study was financially supported by COST Action CA21157 and the Bulgarian National Science Fund (projects КП-08-КОСТ/17 (12.08.2024) “Optimizing micropropagation of valuable tree species through innovative PlantForm™ TIS bioreactors”)

P52 - Micropropagation of *Hibiscus* hybrids through axillary shoot proliferation

Jéssica Batista, Mariana Neves, Jorge Canhoto

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

jorgecan@ci.uc.pt

Among the approximately 250 species of *Hibiscus*, the most popular ornamental one is *Hibiscus rosa-sinensis*, a shrub used both as an indoor and outdoor plant in pots and landscaping, with a variety of shapes, flower colors and growth forms. Seedlings growing in vitro from crosses between individuals displaying flowers of different colors were used for in vitro establishment. The seeds were germinated in vitro in MS basal medium and apices and nodes transferred to the proliferation media consisting of MS media containing different concentrations of the cytokinin benzyladenine (BA). After 1 month of culture four parameters were evaluated, namely 1) shoot height, 2) number of phytomers, 3) root number and 4) length of the largest root. Whenever roots were not formed in the proliferation media, the shoots were transferred to media with different concentrations of IBA (0.5 – 2.0 mg/l) for 2-4 weeks followed by transfer to hormone-free media for root elongation. Best results for shoot proliferation were obtained when 0.2 mg/l BA were used, giving the longest shoots as well as the highest number of phytomers. Usually, shoots rooted well in the proliferation medium, especially if 0.2 mg/l BA was used. Treatments with IBA usually inhibited root formation and elongation in comparison with the control, promoting also callus formation at the shoot basis. Adult plants resulting from these crosses and further micropropagation acclimatized well. Some adult shrubs are already growing in the filed display new flower colors. A second generation of lines resulting from the crosses was already micropropagated and plantlets transferred to soil to further analyse their phenotypes.

Key words: Benzyladenine, flowering, *Hibiscus rosa-sinensis,* hybridization, rooting

P53 - Micropropagation of cork oak

Isa Ramalho

Amorim Florestal, SA., Lugar de Salteiros - Longomel, 7400-909 Ponte de Sor, Portugal

irramalho2@gmail.com

Cork Oak (*Quercus suber*) is a species native to Portugal being found all over the country; however, it also tends to exist in other locations with a Mediterranean climate. Its principal use is cork, mainly for the production of stoppers, but it is also used, for instance, in insulation, flooring, and clothing.

Due to the climate changes that have been taking place in Portugal, it is necessary to produce quality plants that are tolerant to these changes in order to guarantee new adapted plantations. Therefore, there is a need to produce clones.

After phenotypic selection, the species has been introduced *in vitro* using the somatic embryogenesis technique. Organogenesis trials have been carried out, testing culture media and growth regulators. In addition, a protocol for rooting and acclimatization of the plants has been developed, as well as maintenance in a greenhouse for subsequent planting in the field.

Keywords: Cork Oak; Micropropagation; Somatic embryogenesis

P54 - Enhancing forest resilience through hybrid larch clonal production *("LarchForFlexibility")*

Andrea Rupps[1], Emma Ehmke[1], Madlen Walther[1], Juliane Raschke[1], Matthias Meyer[2], Ute Tröber[2], Ricardo Joffe[2], Silvan Liebsch[2], Janine Otte[3], Christin Müller[3], Birgit Reiche[4], Sophie Maiwald[5], Tony Heitkam[5]

[1]Dept. of Plant Evolution and Biodiversity, Späth-Arboretum, Institute of Biology, Humboldt-Universität zu Berlin, Späthstr. 80/81, 12437 Berlin, Germany
[2]Staatsbetrieb Sachsenforst, Dept. 42 Forest Genetics and Forest Plant Breeding, Bonnewitzer Str. 42, 01796 Pirna OT Graupa, Germany
[3]Baumschulen Oberdorla GmbH, Burgstr. 57, 99986 Vogtei OT Oberdorla, Germany
[4]TTU Dresden, Institut für Forstbotanik und -zoologie, Pienner Str. 19, 01737 Tharandt, Germany
[5]RWTH Aachen University, Institute of Biology I, 52074 Aachen, Germany

andrea.rupps@hu-berlin.de

Climate change poses significant challenges to forestry and forest management, necessitating the development of resilient, mixed-species forests. Our objective is to improve silvicultural flexibility by providing characterized and certified propagation material - independent of seed availability. Our species of interest is hybrid larch (*Larix x eurolepis*), which is becoming increasingly attractive for forestry due to its advantages in establishing pioneer forests, rapid juvenile growth, robustness, and drought resistance. Our approach combines traditional breeding methods with modern *in vitro* culture technologies, specifically somatic embryogenesis (SE), to select and produce high-quality hybrid larch genotypes. We have successfully generated and cryopreserved 256 hybrid larch genotypes through SE, with 70 showing promising characteristics *in vitro* as well as in the field suitable for commercial or scalable applications, which we are aiming to expand further.
Based on this collection, we have established a productive system for cultivating *in vitro* propagated plant material and integrated it as a routine procedure into nursery operations. At this point, our partners from industry were able to offer our optimized strategy and propagation material to the market. The launch goes along with further economic tasks, including streamlining the SE process, particularly the maturation phase, and automating labor-intensive steps to ensure high production volumes and competitive pricing.
Concurrently, we are establishing clone trials at diverse plots in Eastern Germany, which are necessary to secure approval for the propagation material. As a prerequisite for the complex and long approval procedure, we are working on the genetic characterization of our material and developing a molecular marker system for reliable genotype identification. These markers will be stored in a database, ensuring that the entire clone spectrum is documented and can be expanded and supplemented with phenotypic characteristics obtained during the in vitro phase as well as subsequent growth characteristics from the field. In this regard, we are placing special emphasis on drought tolerance of the respective genotypes. To this end, we are conducting in vitro and ex vitro drought tests to categorize the reactions and resistance to water withdrawal.
This research contributes to sustainable forestry by enabling the production of climate-resilient planting material, supporting rapid reforestation of calamity areas, and enhancing forest productivity and resilience against the impacts of climate change.

Keywords: somatic embryogenesis, nursery integration, clone trials, approval, genetic characterization, drought tolerance

P55 - *In vitro* propagation of *Quercus canariensis* Willd.: the most endangered tree species in Portugal

José C. Gonçalves[1,2]; Joana Domingues[1]; Alice Almeida[1]; Carlos Vila-Viçosa[3,4,5]; João Nunes[3,4]; Iuri Frias[5]; Sara Freitas[3,4,6]; Carmen Correia[7,8]; Nuno Rico[7]; Bruno Marcos[3,4]; João Gonçalves[3,4,9]; Herlander Azevedo[3,4,6]

[1] CBPBI—Centro de Biotecnologia de Plantas da Beira Interior, Quinta da Senhora de Mércules, 6000-098 Castelo Branco, Portugal
[2] CERNAS-IPCB—Centro de Estudos em Recursos Naturais, Ambiente e Sociedade, Instituto Politécnico de Castelo Branco, 6000-084 Castelo Branco, Portugal
[3] CIBIO (Research Center in Biodiversity and Genetic Resources)—InBIO (Research Network in Biodiversity and Evolutionary Biology), University of Porto. Campus Agrário de Vairão; Rua Padre Armando Quintas; 4485-661 Vairão; Portugal
[4] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO. Campus de Vairão, 4485-661Vairão, Portugal
[5] MHNC-UP — Museu de História Natural e da Ciência da Universidade do Porto – Herbário PO, Universidade do Porto. Praça Gomes Teixeira, 4099-002, Porto, Portugal
[6] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto. Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal
[7] The Navigator Company
[8] Viveiros Aliança, Herdade de Espirra, 2985-270 Pegões, Portugal
[9] proMetheus—Research Unit in Materials, Energy and Environment for Sustainability, Instituto Politécnico de Viana do Castelo (IPVC), Avenida do Atlântico, No. 644, 4900-348 Viana do Castelo, Portugal

jcgoncalves@ipcb.pt

Quercus canariensis is a rare tree on the Iberian Peninsula whose distribution in Portugal is restricted to the Serra de Monchique and the surrounding area. It is currently in critical danger of extinction with the status of Critically Endangered (CR) according to the Red List of the Flora of Portugal and it is estimated that only 250 adult individuals are concentrated in that area. Considering the potential of micropropagation techniques for preserving plant material *in vitro*, a multiplication protocol was developed from young plants, from seeds collected in the Serra de Monchique. The results obtained in the different phases - establishment, multiplication, rooting and acclimatisation will be presented.

These studies are funded by the project 'TRANSFORM - Digital transformation of the forestry sector for a resilient and low-carbon economy'. Project PRR no. 02/C05-i01/2022

P56 - Influence of light quality on the in vitro propagation of sycamore maple (*Acer pseudoplatanus* L.)

Vitalina Karfik[1], Marvin Mohlfeld[2]

[1]Department of Forest Genetic Resources, Northwest German Forest Research Institute, Professor-Oelkers-Straße 6, 34346 Hann. Münden, Germany
[2]Formerly at Institute of Horticultural Production Systems, Section Woody Plant and Propagation Physiology, Leibniz Universität Hannover, Hannover, Germany

vitalina.karfik@nw-fva.de

The propagation of woody plants through in-vitro culture requires precise control of environmental conditions, with light quality playing a crucial role in shoot growth and overall plant vitality. Adjusting light quality can influence the efficiency of micropropagation in Sycamore Maple (*Acer pseudoplatanus* L.), though the response appears to be genotype-dependent. This study investigates the effects of different light spectra on shoot development under controlled in-vitro conditions. Using an LED lighting system, we analyzed the impact of monochromatic and combined light sources on shoot elongation, biomass accumulation, and physiological plant quality. Preliminary results indicate that pure red light promotes shoot elongation but reduces shoot quality compared to the control group. Blue light resulted in increased leaf biomass. A combination of red and blue light led to increased biomass production in some cases but did not consistently enhance overall plant growth and was strongly genotype-dependent.

Keywords: In vitro propagation, Sycamore Maple, light quality, LED, micropropagation

P57 - The use of photoautotrophic systems in the in vitro propagation of some ornamental plants

Bora Onur Hallac[1]*, Soner Yağ [2], Yeşim Yalçin Mendi[3]

[1]Bolu Abant İzzet Baysal University, Postgraduate Education Seed Science and Technology, Bolu

[2]Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana

[3]Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana

ymendi@gmail.com

Plant tissue culture techniques are commonly used methods for in-vitro propagation of ornamental plants. However, traditional micropropagation culture systems often face issues during the acclimatization process, such as high losses, phenolic oxidation, bacterial contamination, and vitrification. To overcome these problems, new techniques are continuously being developed to create highly efficient and sustainable propagation systems. Among these techniques, temporary immersion bioreactors, as an alternative to semi-solid systems, have gained significant attention. However, some plants still encounter problems in these systems, such as vitrification and rapid spread of contamination. In a system where multiple plants are fed in the same liquid medium, especially the solution to contamination problems spreading throughout the plant clusters without affecting the entire system does not seem possible with current technology in temporary immersion systems.
In this study, a photoautotrophic micropropagation method has been proposed as an alternative to bioreactors and semi-solid culture systems for some ornamental plants. The photoautotrophic micropropagation method is a system in which plants can be fed in parallel with their natural growth processes, using common plant cultivation media such as rock wool, peat, and perlite instead of solidifying agents like agar or gellan gum. It also uses CO_2, the natural carbon source for photosynthesis, instead of sucrose sources, and it can even be controlled. This study investigates the effectiveness of photoautotrophic systems for in-vitro propagation of *Syngonium*, *Lyratta*, and *Spathiphyllum* species. The challenges in the stem thickening process and the difficulties in acclimatization under greenhouse conditions for *Lyratta* are expected to be minimized with the photoautotrophic system. In Syngonium, the goal is to increase the acclimatization rate. For *Spathiphyllum*, this method has been proposed as an alternative solution to common problems such as bacterial contamination and weak stem structure. In a study by Pruski et al. (2002), it was found that the photoautotrophic micropropagation method, where CO_2 is introduced and removed and sucrose is not present, resulted in stronger plant structures compared to traditional micropropagation methods, and no issues were encountered during the acclimatization phase. In a study by Silve et al. (2006), it was determined that plants grown under high CO_2 (3000 ppm) enrichment and low light intensity in a sugar-free liquid medium showed the best growth for the photoautotrophic micropropagation of *Spathiphyllum*. Similar results were observed in the studies conducted on *Spathiphyllum*, and the expected commercial objectives were also achieved for *Lyratta* and *Syngonium*.

P58 - Temporary Immersion System: a valid tool for *Humulus lupulus* L., cv Columbus, *in vitro* proliferation

Valeria Gianguzzi[1], Leandra Leto[2], Anna Agosti[2], Andrea Di Fazio[2], Francesco Paolo Marra[1], Martina Cirlini[2], Benedetta Chiancone [2,3]

[1]Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, 90128 Palermo, Italy
[2]Department of Food and Drug, University of Parma, Viale Parco Area delle Scienze 27/A, 43124 Parma, Italy
[3]Institute of Biophysics, National Research Council (CNR), Via Ugo La Malfa 153, 90146 Palermo, Italy

valeria.gianguzzi@unipa.it

Temporary Immersion System (TIS) has been successfully applied for large-scale propagation in various plant species, representing a valid alternative to traditional *in vitro* culture method on solid medium. In this study, the influence of culture system, solid medium vs. TIS, on the in *vitro* proliferation of hop (*Humulus lupulus* L.), cv. Columbus, was evaluated. Moreover, the effect of sucrose in the culture medium (both solid and liquid) on plantlet response was investigated. The morpho-physiological parameters, the total (poly)phenol content (TPC) and antioxidant capacity (AOC) of extracts obtained from *vitro*-derived hop plantlets were analysed. After four weeks of culture, explants produced a higher number of shoots (+12%) and longer roots (+33%) in plantlets grown in TIS compared to those in solid medium. Finally, extracts from plantlets cultured in TIS exhibited significantly higher levels of TPC and AOC compared to those derived from plantlets grown on solid medium.
This study confirms the efficacy of the TIS as a hop propagation method and as a valid tool for producing biomass to be used as a source of bioactive compounds.

Keywords: Bioactive compounds, bioreactors, hop, liquid culture, micropropagation

P59 - Proliferation of axillary shoots of selected berry and fruit tree cultivars in bioreactors

Tatjana Anđelić[1], Tatjana Vujović[1], Conchi Sánchez[2], Anxela Aldrey[2], Bruce Christie[3], Nieves Vidal[2]

[1] Fruit Research Institute (FRI), Department of Fruit Physiology. Kralja Petra I 9, 32000 Čačak, Serbia
[2] Misión Biológica de Galicia (MBG), CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
[3] The Greenplant Company, Palmerston North, New Zealand

nieves@mbg.csic.es

This study aimed to develop efficient protocols for the proliferation of berry and fruit tree cultivars in temporary immersion bioreactors. Plant material consisted in axillary shoots of 3 cultivars of genus *Rubus* (*R. idaeus* cv Meeker and Willamette, *R.* subg. *Rubus* Watson cv Cacanska Bestrna) and 2 cultivars of genus *Prunus* (*P. domestica* cv Crvena Ranka and *P. cerasus* cv. Oblacinska). These cultivars were selected because of their importance in Serbian horticulture. They were established in vitro in the Serbian FRI and transferred to the MBG in a COPYTREE collaboration through a STSM.

Factors investigated included bioreactor type, immersion frequency, cytokinin concentration and the use of physical supports to hold explants in a vertical position.

After six weeks data were collected on shoot number per explant -differentiating between normal and hyperhydric shoots-, the shoot length and multiplication coefficient, defined as the number of nodal segments useful for multiplication obtained per initial explant. Vigorous shoots cultivated in liquid medium were rooted in medium with auxin either in jars or in bioreactors using temporary or continuous immersion. The five cultivars grew well in bioreactors, with Meeker, Willamette and Cacanska Bestrna being easier to manage as they didn't show hyperhydricity. Experiments to evaluate the effect of cytokinin oxidase inhibitors on proliferation capacity are currently in progress.

Keywords: blackberry, plum, raspberry, sour cherry, temporary immersion

P60 - *In vitro* introduction and evaluation of temporary immersion systems for fruit tree species

Alois Bilavcik[1], Jiri Zamecnik[1], Barbora Tunklova[1], Olena Bobrova[1,2], Milos Faltus[1]

[1]Plant Physiology and Cryobiology, Czech Agrifood Research Center, Drnovska 507, 16100 Prague 6, Czech Republic

[2]Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine

alois.bilavcik@carc.cz

The aim of the National Cryobank at the Czech Agrifood Research Center is to serve as a safe backup facility for the preservation of vegetatively propagated plants. One of its primary objectives is to preserve healthy plant; therefore, establishing *in vitro* stock plant material is a crucial step. However, introducing woody crops into *in vitro* conditions presents challenges due to potential contamination from surface-associated pathogens. This study aimed to evaluate the feasibility of initiating *in vitro* cultures of selected fruit tree species using two different sources: sprouting shoots collected from an orchard and dormant buds harvested during the winter period. Surface sterilization was performed using chlorine-based disinfectants. Subsequently, the growth and multiplication of the cultures in bioreactors utilizing a temporary immersion system (TIS) were monitored. Experiments were conducted with specific varieties of stone fruits and berry fruits. The study assessed the development of *in vitro* cultures and the response of different fruit tree species within the bioreactor system. The proposed approach highlights the potential of integrating TIS into the *in vitro* cultivation of selected fruit tree species.

Keywords: micropropagation; fruit trees; bioreactor

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P61 - Effect of the substrate and culture system on the rooting performance of micropropagated *Bougainvillea spectabilis* shoots

Conchi Sánchez [1], Anxela Aldrey [1], Bruce Christie [2], María José Cernadas [1], Nieves Vidal [1]

[1] Misión Biológica de Galicia (MBG), CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
[2] The Greenplant Company, Palmerston North, New Zealand

brucechristie101@gmail.com

Bougainvilleas are perennial ornamental shrubs very popular in tropical, subtropical and temperate gardens because of their colorful bracts. They differ in colors, temperature requirements and in the capacity to be vegetative propagated. Within Bougainvilleas, *Bougainvillea spectabilis* is considered a difficult to root species in natural conditions.

In this study we investigated the rooting step of *in vitro* cultured *B. spectabilis*. For in vitro establishment we used axillary buds collected from an adult plant (at least 40 years old) grown in a private garden. Shoots were proliferated in jars with MS BA 0.8 mg/l and IBA 0.5 mg/l in a four- week subculture regime. For rooting we used vigorous shoots treated with half-strength MS with IBA 1-4 mg/l in jars with agar or in bioreactors with forced ventilation. Bioreactors consisted of 10 L rectangular polypropylene containers adapted in our laboratory from food containers and equipped with 0.2 µm air filters. Cultures were aerated for 1 min every 4 h and were grown under standard conditions.

To evaluate the effect of substrate on rooting, the bioreactor was distributed in sections so that the basal part of the shoots was inserted into agar, rock wool cubes or directly into liquid medium using various types of plastic supports to avoid total immersion. The use of rockwool cubes increased rooting percentages compared with the rest of treatments, suggesting that a certain gas exchange in the rooting zone is beneficial for root formation.

Keywords: bioreactor, forced ventilation, porous substrates, root zone

P62 - Influence of different wavelengths of LED lights and auxins addiction on the induction of rhizogenesis in *Myrtus communis* L. (Myrtaceae)

Dawid Kocot

Dept. of Ornamental Plants and Garden Art, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Krakow, Poland

dawid.kocot@urk.edu.pl

Root induction is a critical stage in the micropropagation process, as it determines the success of plant regeneration. In this study, were investigated the effects of different auxins and LED light wavelengths on rhizogenesis induction in *Myrtus communis* L. The auxins tested were indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), while the LED light wavelengths included 100% Blue, 100% Red, 70% Red + 30% Blue (RB), and Far-Red. Control conditions involved shoots illuminated with fluorescent lamps. The experiment was carried out in a phytotron, using MS medium as the control. The tested media contained 1 mg/L of either IAA or IBA. Shoot segments of *Myrtus communis*, approximately 2 cm in length and containing at least two nodes, were defoliated and used as explants. The hypothesis was that auxin addition would promote root induction, and that different wavelengths of light would influence this process. The first roots were observed after 5 weeks of cultivation. The best root induction results were observed on MS medium with IAA supplementation, regardless of the light used. The highest number of rooted shoots (40%) was obtained on MS medium with IAA added under 70% Red + 30% Blue light. Additionally, it was observed that the present roots were longer. On the other media variants, a very low degree of shoot rooting was observed (max. 10%). The results indicated that both auxin type and light wavelength affected rhizogenesis.

Keywords: myrtle, in vitro rooting, light spectrum, auxin application, plant regeneration

P63 - Prospects of using surfactants for cryopreservation of peach shoot tips

Olena Bobrova[1,2], Alois Bilavcik[1], Jiri Zamecnik[1], Milos Faltus[1]

[1]Plant Physiology and Cryobiology, Czech Agrifood Research Center, Drnovska 507, 16100 Prague 6, Czech Republic

[2]Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Pereyaslavska 23, 61016, Kharkiv, Ukraine

olena.bobrova@carc.cz

Cryopreservation is a critical method for preserving plant genetic resources, particularly for species such as peach (*Prunus persica*), which cannot be effectively stored as seeds. However, the sensitivity of peach shoot tips to cryogenic stress often results in low viability and poor regrowth due to dehydration, oxidative stress, and cellular damage. This study evaluates the potential of the surfactant Pluronic F-68 to enhance the viability and regrowth of cryopreserved shoot tips from four peach genotypes: Flame Prince, Vate 1543, Guglielmina, and Nectared 4. A droplet-vitrification approach was used, incorporating Pluronic F-68 at various stages of the process, including loading, vitrification, and post-thaw recovery solutions. Long-term exposure to PVS3 in the control group (without surfactant) significantly reduced viability, highlighting the cytotoxic effects of vitrification solutions. Compared to the control, the inclusion of Pluronic F-68 resulted in a twofold increase in shoot tip regrowth. The improved outcomes suggest that the surfactant helps stabilize membranes, reduce osmotic stress, and mitigate dehydration damage during cryopreservation. These findings underscore the surfactant's potential as a valuable additive for optimizing cryopreservation protocols, facilitating the efficient conservation of peach germplasm for breeding and preservation programs. This research was funded through the MSCA4Ukraine project, supported by the European Union.

Keywords: *Prunus persica*, Pluronic F-68, vitrification

P64 - Histochemical studies on regeneration of date palm (*Siwy* cv.) *via* direct organogenesis

Karam Mostafa[1,2], Abdel-Halim S. Aly[2], Amal FM Zein El Din [2], Ola Abd Elbar [3].

[1]Faculty of Agriculture, Department of Agricultural Biotechnology, Ondokuz Mayis University, 55200 Samsun, Türkiye.

[2]The Central Laboratory for Date Palm Research and Development, Agricultural Research Center (ARC), 12619 Giza, Egypt.

[3]Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo 11566, Egypt.

Karamkrush1@gmail.com, Karam.ali@arc.sci.eg

The date palm (*Phoenix dactylifera* L.) is a historically cultivated fruit crop native to the arid regions of the Middle East and North Africa. Due to the limitations of seed and offshoot propagation, micropropagation provides a reliable method for producing uniform, high-quality date palm plants for large-scale cultivation. This study aims to elucidate the effects of different plant growth regulators on the initiation stage of direct organogenesis in date palm, focusing on morphological, chemical, and histological aspects. This research investigates the effects of NAA combined with different cytokinin (BA, Kin, and 2iP) at 1 mg l^{-1}, as well as the impact of low concentrations of 2,4-D (0.1, 0.5, and 1 mg/L) on morphogenesis of date palm through direct organogenesis. The results showed that the highest explant responsiveness (36.36%) was achieved on a medium with 1 mg l^{-1} NAA combined with 1 mg l^{-1} each of BA, Kin, and 2iP, producing 9.20 shoots per explant after 32 weeks. In contrast, a medium with 1 mg l^{-1} 2,4-D resulted in 22.22% responsiveness and 7.3 shoots per explant. Biochemical analysis revealed that the auxin/cytokinin ratio, influenced by total indoles, phenols, and the activities of peroxidase and polyphenol oxidase, directly impacted bud formation from shoot tips. Histological observation showed that shoots originated from the ground tissue, mainly from tissue surrounding the vascular tissue in the explant. Then the vascular cylinder of the newly shoots connected with the neighboring vascular bundle of the explant by developing some tracheal elements, and thereafter shoots were elongated in the following sixteen weeks. This study advances knowledge of direct organogenesis, aiding in the enhanced propagation of climate-resilient date palms.

Key words: Histochemical, Regeneration, Organogenesis, Date Palm

P65 - Determination of the effects of strawberry tree endophytic bacteria

Mariana Correia[1], Mónica Zuzarte[2,3,4], Sandra Correia[1,5], Jorge Canhoto[1]

[1]University of Coimbra, Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, 3000-456 Coimbra, Portugal
[2]University of Coimbra, Faculty of Pharmacy Azinhaga de S. Comba, Coimbra 3000-548, Portugal
[3]University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal
[4]University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Coimbra 3030-790, Portugal
[5]InnovPlantProtect CoLab, Estrada de Gil Vaz, Elvas, Portugal

mjcorreia324@gmail.com

Plants must adapt to ever-changing environments, facing stresses that affect their performance. Bacterial biostimulants offer a promising solution to alleviate these stresses and reduce reliance on synthetic fertilizers and pesticides. Among them, endophytic bacteria can enhance plant growth, improve nutrient uptake, and boost stress tolerance.

This study aims to characterize and evaluate endophytic bacteria from the strawberry tree (*Arbutus unedo* L.) as biostimulants for horticultural and ecological applications. The research involves screening bacterial isolates using *Arabidopsis thaliana* as a model system to identify effective strains, followed by testing on the strawberry tree, valued for its edible fruits and role in soil erosion prevention.

The experimental approach includes *in vitro* establishment and multiplication analyses of strawberry tree under different light conditions, and bacteria maintenance, as well as assays where plants will be inoculated with bacterial cultures. Phenotypic assessments, including growth rate, multiplication, and plant performance, will be conducted. This study aims to uncover the mechanisms by which endophytic bacteria influence their host plants and identify promising strains for future use in sustainable crop management.

Keywords: *Arabidopsis thaliana*; biostimulants; endophytic bacteria; strawberry tree

P66 - Towards a stronger network: advancing *Paulownia* research and phytomanagement in Ukraine

Oksana V. Pasat[1,2], Vadym I. Pasat[2], Ihor Ya Shyndak[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

Our work with Paulownia began in 2013, when we discovered a Paulownia tomentosa tree in the Dniester Canyon, Western Ukraine, and were impressed by its biology. One plantlet was planted in our home garden near Ivano-Frankivsk city. The first two years it grew well but died after winter two years later, likely due to high frost sensitivity and fluctuating groundwater levels. In 2016 we sourced plantlets from various suppliers in Ukraine to get more tolerant lines. Some plants thrived but suffered winter damage to different extents mainly in the form of top damage and winter scars. To propagate tolerant trees we established a microclonal propagation system laboratory. We also tested Paulownia's tolerance to pollutants, particularly heavy metals, using hydroponic culture, and found it highly resistant. In 2018, we planted several hectares of Paulownia on fertile black soil. The first three years were highly successful: by the second year after planting, after technical cutting most trees reached 4–6 m to the end of growth season; by the third year after planting, many trees grew up to 10 m. In the fourth year, severe winter frost eventually destroyed nearly the entire plantation. Paulownia trees planted in Ivano-Frankivsk city thrived and even bloomed. Now, we restore our field plantation, leveraging the well-developed root system and potential mycorrhizal associations in the soil. We are looking for partners to cooperate to improve Paulownia's overwintering ability to be used in phytomanagement.

Keywords: *Paulownia*, growth, frost resistance, field plantation, overwintering

P67 - Effects of drought stress during somatic embryogenesis and germination of hybrid and European larch

Ahmed Sadkhan, Juliane Raschke, Andrea Rupps

Dept. of Plant Evolution and Biodiversity, Späth-Arboretum, Institute of Biology, Humboldt-Universität zu Berlin, Späthstr. 80/81, 12437 Berlin, Germany

andrea.rupps@hu-berlin.de

Hybrid and European larch (*Larix x eurolepis* and *L. decidua*) are economically and ecologically important forest trees, known for their adaptability to various environmental conditions, like drought. Current challenges, such as climate change necessitate further understanding of the stress impact and adaptation, including the enhancement of drought tolerance. Our study aimed to assess the developmental impact and the potential of early mild sublethal stress in potentially enhancing tolerance to subsequent severe drought conditions. To simulate drought stress, the nutrition media was supplemented with sorbitol (4 and 8%) during various phases of somatic embryogenesis and germination.

Results showed that moderate stress (4%) enhanced certain growth parameters, while high-intensity stress (8%) negatively affected physiology, survival and growth. The study also revealed that mild stress (4%) during maintenance resulted in higher drought tolerance. In comparison, high-intensity priming (8%) showed minimal or negative effects.

This study provides valuable information on larch responses to drought stress and demonstrates that early managed stress can facilitate adaptive responses that enhance drought tolerance.

Keywords: drought stress in vitro, stress tolerance, priming, somatic embryogenesis, *Larix* spec

P68 - Attempts to improve somatic embryo yield and quality in the myrtaceous fruit tree *Feijoa sellowiana* (O.Berg) O.Berg

Miguel Batalha, Jorge Canhoto

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

jorgecan@ci.uc.pt

Feijoa (*Feijoa sellowiana*) is a Myrtaceae from South America growing in different world regions due to the quality of its fruits. Protocols for somatic embryogenesis induction have been described. However, the number of abnormal somatic embryos is high (around 40%) and poor germination rates occur, thus impairing regeneration. Previous works showed that in MS medium containing high sucrose levels (9%) and 1.0 mg/l of 2,4-D, percentages of induction above 90% were obtained and the number of somatic embryos per explant often reached a hundred. In spite of these data, the number of plantlets after germination rarely exceeds ten per induced explant. In this work several conditions aiming to increase the quality of the embryos and further germination were attempted, namely induction through physical or chemical stresses other than auxin, auxinic shocks (50 – 500 mg/l) for reduced periods of time (1 – 24 h), lower concentrations of 2,4-D (0.01 to 0.2 mg/l) and other auxins (NAA, Dicamba and Picloram). The results showed that from all physical treatments tested, only treatments of the explants for 2 weeks at 4 ºC were able to stimulate somatic embryo formation in the apical zone of the cultured mature zygotic embryos. However, the percentage of induction was lower than 1% and the number of somatic embryos equally reduced, with only one or two embryos arising. However, when shock treatments were applied followed by culture of the somatic embryos on media with reduced levels of 2,4-D (0,1 mg/l) and sucrose (3%), the number of somatic embryos displaying anomalous phenotypes sharply decreased (24.5%) and the average number of plantlets that could be obtained also increased from 6.7 to 9.8 per induced explant. Phenotypic analysis showed that embryos obtained in continuous cultures with high dosages of 2,4-D (1.0 mg/l) and sucrose (9%) presented abnormalities similar to those observed in *Arabidopsis* mutants that affect the development of large parts of the seedling body along the apical–basal axis, whereas embryos induced by auxinic shocks and developed in low (0.1 mg/l or less) are mostly normal.

Keywords: auxins, germination, phenoype, sucrose, stress conditions

P69 - Cyclic somatic embryogenesis in *Theobroma cacao* L.

Mirni Ulfa Bustami[1,2], Stefaan P. O. Werbrouck[1]

[1]Department of Applied Bioscience, Faculty of Bioscience Engineering, Ghent University, 9000 Gent, Belgium; meetmot@yahoo.com

[2]Department of Agrotechnology, Faculty of Agriculture, Tadulako University, Central Sulawesi Province, 94148 Palu, Indonesia

meetmot@yahoo.com

In vitro somatic embryogenesis is crucial for cocoa mass propagation. This study optimized somatic embryogenesis in Indonesian cocoa clones SUL1 and SUL2, examining both primary and cyclic secondary embryogenesis. Staminode explants, particularly from SUL2, yielded significantly more primary somatic embryos (SEs) than petal explants when cultured in a liquid medium with 2 mg/L 2,4-D and 0.25 mg/L kinetin. Fragmenting SUL2 cotyledons and hypocotyls on a medium with 2,4,5-T and proline induced high yields of secondary SEs. This cyclic process, where SEs remain attached, enabled continuous production. Proline enhanced SE quality, resulting in well-organized embryos with defined meristems. These findings provide a promising protocol for cocoa SE production that is beneficial for plant transformation and gene editing.

Keywords: Cocoa; vegetative propagation; tissue culture, cyclic embryogenesis, regeneration

P70 - A five-year study on storage of sugi (Japanese cedar, *Cryptomeria japonica*) somatic embryos at temperatures above freezing point

Tsuyoshi E. Maruyama

Department of Forest Molecular Genetics and Biotechnology, Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki, 305-8687 JAPAN

tsumaruy@ffpri.affrc.go.jp

Sugi-pollinosis, one of the world's most severe allergies, affects around 40% of the Japan's population. This allergy is considered a national affliction, causing economic losses due to absenteeism estimated at more than USD 1.5 billion per day. In this context, we developed a simplified methodology for the efficient propagation of pollen-free plants as an alternative to counteract pollinosis, combining the marker-assisted selection and somatic embryogenesis for the mass propagation of somatic seedlings for reforestation. Our results showed that somatic embryo production from selected genotypes could exceed 1,000 embryos per gram of fresh weigh embryogenic mass. Subsequently, high plantlet conversion and survival rates were achieved after *ex vitro* acclimatization (>80%). In addition, we included short to medium-term storage of somatic embryos to make the plant production schedule more flexible and to allow easy regulation of the optimal time for transferring somatic seedlings to the field. The results of our five-year study showed that although germination capacity decreased after three years of storage, somatic embryos of sugi can be stored at 25°C, 15°C, and 5°C for at least six, twelve, and twenty-four months, respectively, without a significant decrease in the germination rate or plant conversion capacity. The developed methodology will facilitate the large-scale production of pollen-free sugi plants.

Keywords: Cupressaceae, Emblings, Pollen-free plants, Pollinosis, Somatic embryogenesis

P71 - Towards a Norway spruce SE plant lot in the six-figure scale using robotics

Sakari Välimäki[1], Saila Varis[1], Jaakko Salminen[1], Elmar Bernhardt[2], Elina Havia[2], Tuija Aronen[1], Mikko Tikkinen[1]

[1]Natural Resources Institute Finland (Luke), Vipusenkuja 5, FI-57200 Savonlinna, Finland

[2]Southeastern Finland University of Applied Sciences (Xamk), Electronics 3K Factory, Laitaatsillantie 3, FI-57170 Savonlinna, Finland

saila.varis@luke.fi

Using somatic embryogenesis (SE), many conifers can be propagated using a zygotic embryos as explants. In forestry, SE can be used to propagate the progeny of elite trees, and the resulting plants can be subjected to field testing to find the clones best suited for forestry. The main advantage of SE to other vegetative propagation methods is its scaling up potential. However, SE is laborious to carry out, and automation is needed to make it commercially relevant for producing forest regeneration material. Therefore, robotics have been developed by Luke and Xamk to automate the screening and transferring somatic embryos into germination.

The commercialization of Finnish SE pipeline and related innovations are being prepared in Laukansaari Giants project, funded by Business Finland. In spring 2024, over 60 000 SE plants from 44 cell lines were germinated and transplanted in a single lot as a proof of concept (PoC). The selection of cell lines was done based on laboratory performance and early (5 growing seasons) height growth results from a single breeding trial. Over 90% of the plants were transferred to germination using robotics. In a comparison test, 97% of the manually transferred germinants and 86% of those transferred by robot were fit for transplanting from Vivi plugs into larger container plugs.

In PoC 2025, the target is a lot of 100 000 SE plants, and the lines are selected based on series of breeding trials, laboratory performance and their suitability for the robot. The robots have been improved based on user experiences and machine learning models will be utilized and tested in practice to improve the selection accuracy.

P72 - Response of *Arbutus unedo* L. genotypes to water stress: Phenolic profile and priming in vitro

Beatriz Figueiredo[1], Jorge Canhoto[1], João Martins[1,2]

[1]Centre for Functional Ecology, Laboratory Associate TERRA, Department of Life Sciences, University of Coimbra, Coimbra, 3000-456, Portugal

[2]Molecular Physical-Chemistry R&D Unit, Department of Chemistry, University of Coimbra, Rua Larga, 3004-535 Coimbra, Portugal

joao.martins@uc.pt

Water stress is affecting the productivity of crops. *Arbutus unedo* (strawberry tree), an Ericaceae shrub/tree of the Mediterranean flora, is an isohydric species that can deal with water stress, but given the more severe droughts, it is necessary to invest in plants more tolerant to water stress. Phenolic compounds can be used as selection markers for tolerant genotypes to water stress, thus predicting phenotypes and their performance under stress, with a view on future breeding programs. In this study, the role of secondary metabolites in water stress tolerance in strawberry tree was evaluated, through two experiments: 1) targeted metabolomics analysis of phenolic compounds, particularly arbutin, to access the influence of genotype, stress and plant age, and identify selection markers and 2) a preliminary experiment for in vitro priming in strawberry tree under water stress. Leaves of micropropagated plants (2-years-old, four clones) were used. The results showed that genotype, treatment and plant age play a key role in the phenolic profiles of stressed plants, and some phenolics might be used as selection markers. In the priming assay, a significant decrease in flavonoids and an increase in proline were observed under water stress. Future experiments will be necessary to evaluate the capacity of rooting, acclimatization and development of the primed plants and then to induce stress in the plants again and carry out physiological studies to determine the occurrence of priming in the strawberry tree.

Keywords: arbutin, metabolomics, phenolic compounds, strawberry tree, water stress

P73 - Boosting stilbene biosynthesis in peanut cells: An emerging approach with metabolic regulators

Hajer Ben Ghozlen[1], Sven Mangelinckx [2], Stefaan P.O. Werbrouck [1]

[1]Laboratory for Applied *In Vitro* Plant Biotechnology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, B-9000 Ghent, 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

The biotechnological production of specialized metabolites using in vitro plant cultures offers a sustainable alternative to traditional plant-based methods. Stilbenes, including resveratrol, piceatannol, and piceid, exhibit valuable bioactive properties but are often limited in terms of bioproduction yields. Advances in plant cell culture techniques, particularly callus and suspension cultures, provide a promising approach for large-scale stilbene production. This study explores the potential of L-phenylalanine, sodium malonate dibasic, and cerulenin as metabolic modulators to enhance stilbene biosynthesis in *Arachis hypogaea* cell cultures. These modulators were tested at different concentrations and time points across growth phases, and their effects on cell growth and metabolite production were evaluated. Our findings showed that metabolic modulators significantly impact the production patterns of resveratrol, piceid, and piceatannol, with distinct responses for each treatment. Among them, 0.5 mM sodium malonate dibasic exhibited the strongest effect, enhancing resveratrol production during the lag phase, while piceatannol and piceid were more prominent in the stationary phase. This enhancement surpassed that observed with phenylalanine and cerulenin. This study highlights the potential of metabolic modulation in peanut cells, establishing them as a viable platform for stilbene production.

Keywords: Stilbene, peanut cells, metabolic modulators, resveratrol, bioproduction

P74 - *Arbutus unedo* for phytostabilization in lead- and nickel-contaminated soils

João Martins[1,2], Jorge Ascenção[1], Pedro Monteiro[3], Glória Pinto[3], João Pratas[4], Jorge Canhoto[1]

[1]Centre for Functional Ecology, Laboratory Associate TERRA, Department of Life Sciences, University of Coimbra, Coimbra, 3000-456, Portugal

[2]Molecular Physical-Chemistry R&D Unit, Department of Chemistry, University of Coimbra, Rua Larga, 3004-535 Coimbra, Portugal

[3]Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, Campus Universitário de Santiago, Aveiro 3810-193, Portugal

[4]Geosciences Center, Department of Earth Sciences, University of Coimbra, Coimbra, 3030-790, Portugal

joao.martins@uc.pt

Heavy metal contamination of soil constitutes a challenge for the environment and agriculture and needs to be addressed through sustainable approaches. The use of plants able to immobilize and reduce the bioavailability of metal pollutants is a promising approach to mitigate this problem. Due to its ability to grow on marginal lands and resilience to harsh environmental conditions, *Arbutus unedo* (strawberry tree) has been identified as a potential candidate for bioremediation. This study investigates the ability of *A. unedo* to tolerate and accumulate two common pollutants in soil: copper (Cu) and nickel (Ni). *A. unedo* plants were cultivated under controlled conditions and exposed to increasing concentrations of Cu and Ni (0-1000 ppm). Preliminary assays were carried out in vitro but results were inconclusive. Further studies were carried in pots and physiological and biochemical parameters were analyzed to assess plant responses, and metal accumulation in roots and leaves was quantified using a flame atomic absorption spectrophotometer. Preliminary results indicate *A. unedo* exhibits a high tolerance to Cu and Ni, without compromising physiological traits. However, its ability to primarily sequester metals in roots (bioconcentration factor ≈2) and very low translocation factor (<1), suggests its potential for stabilization rather than accumulation, without compromising the food chain. This study highlights *A. unedo* as a potential tool for heavy metal remediation, reinforcing the importance of native species in ecological restoration.

Keywords: ecological restoration, heavy metals, native species, translocation

P75 - Machine learning-based assessment of cadmium stress impact on Aronia (*Aronia melanocarpa*) micropropagation

Tolga İzgü[1], Özhan Şimşek[2]

[1]National Research Council of Italy (CNR), Institute of BioEconomy, 50019 Florence, Italy

[2]Horticulture Department, Agriculture Faculty, Erciyes University, 38030 Kayseri, Türkiye

tolgaizgu01@gmail.com

This study explores the impact of cadmium (Cd) stress on the micropropagation of Aronia (*Aronia melanocarpa*) across three distinct genotypes, utilizing various machine learning (ML) algorithms, including Multilayer Perceptron (MLP), Support Vector Machines (SVM), Random Forest (RF), Gaussian Process (GP), and Extreme Gradient Boosting (XGBoost). The research aims to understand genotype-specific responses to Cd stress, which poses significant threats to agricultural productivity and food safety. By examining the effects of different Cd concentrations on plant growth parameters such as proliferation, shoot and root lengths, and root numbers, predictive models were developed to optimize plant growth under stress conditions. The ML models identified complex interactions between Cd exposure and plant physiological responses, with certain models exhibiting strong predictive capabilities. These findings enhance the understanding of plant tolerance mechanisms under heavy metal stress and highlight the potential of ML-based approaches in improving plant tissue culture techniques and promoting sustainable agricultural practices.

Keywords: heavy metal tolerance; plant stress; genotypic variation; artificial intelligence in horticulture

P76 - Assessing cadmium stress resilience in Myrtle genotypes using machine learning predictive models: A comparative in vitro analysis

Mehmet Tütüncü[1], Musab A. Isak[2], Tolga İzgü[3], Dicle Dönmez[2], Yıldız Aka Kaçar[5], Özhan Şimşek[6]

[1]Department of Horticulture, University of Ondokuz Mayıs, 55270 Samsun, Türkiye
[2]Agricultural Sciences and Technology Department, Graduate School of Natural and Applied Sciences, Erciyes University, 38280 Kayseri, Türkiye
[3]National Research Council of Italy (CNR), Institute of BioEconomy, 50019 Florence, Italy
[4]Biotechnology Research and Application Center, Çukurova Univ., 01330 Adana, Türkiye
[5]Horticulture Department, Agriculture Faculty, Çukurova University, 01330 Adana, Türkiye
[6]Horticulture Department, Agriculture Faculty, Erciyes University, 38030 Kayseri, Türkiye

tolgaizgu01@gmail.com

This study investigated the effects of cadmium (Cd) stress on the micropropagation and rooting dynamics of two myrtle (*Myrtus communis* L.) genotypes with different fruit colors under controlled in vitro conditions. We evaluated the response of these genotypes to varying concentrations of Cd (0, 100, 200, 300, 400, and 500 µM) to determine dose-dependent effects on plantlet multiplication and root formation. Our results demonstrate that the white-fruited (WF) genotype exhibits greater resilience than the black-fruited (BF) genotype across all concentrations, maintaining higher multiplication rates and shoot heights. For instance, the multiplication rate at 100 µM Cd was highest for WF at 6.73, whereas BF showed the lowest rate of 1.94 at 500 µM. Similarly, increasing Cd levels significantly impaired root length and the number of roots for both genotypes, illustrating the detrimental impact of Cd on root system development. Additionally, this study incorporated machine learning (ML) models to predict growth outcomes. The multilayer perceptron (MLP) model, including random forest (RF) and XGBoost, was used to analyze the data. The MLP model performed notably well, demonstrating the potential of advanced computational tools in accurately predicting plant responses to environmental stress. For example, the MLP model accurately predicted shoot height with an R^2 value of 0.87 and root length with an R^2 of 0.99, indicating high predictive accuracy. Overall, our findings provide significant insights into the genotypic differences in Cd tolerance and the utility of ML models in plant science. These results underscore the importance of developing targeted strategies to enhance plant resilience in contaminated environments.

Keywords: heavy metal tolerance; plant stress; genotypic variation; toxic metal accumulation; artificial intelligence in horticulture

Posters WG4

P77 - Methyl jasmonate: A key agent in drought stress mitigation and phenolic elicitation in *Thymus lotocephalus* López and Morales *in vitro* cultures

Inês Mansinhos[1], Sandra Gonçalves[1], Raquel Rodríguez-Solana[1,2], Anabela Romano[1]

[1]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[2]Department of Agroindustry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Rancho de la Merced Center, Carretera Cañada de la Loba (CA-3102) Km 3.1., SN, 11471 Jerez de la Frontera, Cádiz, Spain.

aromano@ualg.pt

The aim of this work was to investigate the impact of drought stress induced by polyethylene glycol (PEG) on the morphological, physiological, biochemical, phytochemical and biological traits of shoot cultures of *Thymus lotocephalus* López and Morales, an endangered Portuguese aromatic species. Additionally, the potential of methyl jasmonate (MeJA), salicylic acid and iron oxide nanoparticles in mitigating the effects of drought stress was also investigated. The findings indicate that drought induces oxidative stress in a concentration-dependent manner, resulting in an increased production of reactive oxygen species, particularly hydrogen peroxide. This has a detrimental impact on shoot growth, chlorophyll and carotenoid production, as well as the synthesis of secondary metabolites and their associated biological activity. Fourier-transform infrared spectroscopy (FTIR) demonstrated that drought induced alterations in the chemical composition of the shoots, promoting terpenoid biosynthesis. The results of the high-performance liquid chromatography-high-resolution mass spectrometry (HPLC-HRMS) analysis demonstrated that rosmarinic acid, the most prevalent phenolic compound in this species, was the most adversely affected by drought stress. Furthermore, this stressor resulted in a reduction in the biological activities, including antioxidant, anti-melanogenic, and photoprotective properties, of the eco-friendly Natural Deep Eutectic Solvent-based extracts. Of the three agents tested, MeJA was the most effective in combating oxidative damage, promoting the production of proline – an essential plant osmoprotectant – and enhancing the antioxidant activity of the extracts. Moreover, under non-stress control conditions, MeJA was identified as an effective elicitor, enhancing the biosynthesis of phenolic compounds, including rosmarinic acid, salvianolic acid I, caffeic acid, and protocatechuic aldehyde, in *T. lotocephalus* cultures. Furthermore, MeJA-related extracts demonstrated exceptional potential for incorporation into skincare products, exhibiting the most pronounced antioxidant, anti-melanogenic, and photoprotective activities. In conclusion, this study demonstrated the detrimental impact of drought on *T. lotocephalus* shoots and emphasized the effectiveness of MeJA as a drought stress mitigator and elicitor.

Keywords: Mediterranean plant; abiotic stress, secondary metabolites; mitigating agents; elicitation; cosmetics

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P78 - How to improve survival of strawberry tree plants under water stress

Inês Moço[1,3], Jorge Canhoto[1], Filomena Gomes[2,3]

[1]Centre for Functional Ecology, Laboratory Associate TERRA, Department of Life Sciences, University of Coimbra, Coimbra, 3000-456, Portugal

[2]Instituto Politécnico de Coimbra, Escola Superior Agrária de Coimbra, Bencanta, 3045-601 Coimbra, Portugal, Centro de Estudos de Recursos Naturais Ambiente e Sociedade (CERNAS), Instituto Politécnico de Coimbra, Bencanta, 3045-601 Coimbra, Portugal

[3]Centro de Estudos de Recursos Naturais Ambiente e Sociedade (CERNAS), Instituto Politécnico de Coimbra, Bencanta, 3045-601 Coimbra, Portugal

uc2010162426@student.uc.pt

The strawberry tree (*Arbutus unedo* L.) is a Mediterranean species that is active resistant to forest fires, a characteristic that makes it relevant for afforestation programs, and is particularly used in buffer zones at the forest-village interface. The species adapts well to degraded soils, contributing to their recovery, which, together with fruit production, contributes to an increase in the income of landowners. Due to climate change, the planting period is not only uncertain but also becoming shorter. The objective of this study was to evaluate the effect of mycorrhizae, the use of water-retaining agents and its application method on the survival of strawberry tree plants under water stress conditions simulated in a greenhouse (without irrigation and shade nets). Micropropagated plants of the same clone were used to reduce the genotype effect as a response to the treatments. Plants were planted in 2.8 L pots using a sandy texture substrate. Two distinct conditions were tested: moist substrate (simulating the occurrence of rainfall) *vs* substrate taken to field capacity (simulating irrigation of 2 L/plant). The water-retaining agents were applied (2g/plant) on dry *vs* hydrated (2g/600 ml). The addition of fertilizer was compared to *Pisolithus tinctorius* spore broth or a mixture of algae and vitamins (a total of 225 plants in 3 blocks of 15 treatments, each with 5 plants, randomly arranged). The best survival results (> 80%, after 98 days under water stress) were achieved when it was tested substrate taken to field capacity, using dry water-retaining agent added with *Pisolithus tinctorius* spore broth.

Keywords: *Arbutus unedo* L., climate change, mycorrhizae, water retention agents

P79 - Effects of Rhizopon AA in *ex vitro* rooting of different genotypes of almond (*Prunus amygdalus)* micropropagated shoots

Tércia Lopes[1], Mariana Correia[1], Elsa Baltazar[1], Cláudia Marinho[2], Mónica Zuzarte, Liliana Marum[2,4], Sandra Correia[1,5], Jorge Canhoto[1]

[1]Centre for Functional Ecology, TERRA Associate Laboratory, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

[2]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal

[3]QualityPlant – Investigation and production in Plant Biotechnology, Lda. Coimbra, Portugal

[4]MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, CEBAL, 7801-908 Beja, Portugal

[5]InnovPlantProtect CoLAB, 7350-999 Elvas, Portugal

tercia.lopes@student.uc.pt

The increasing health awareness has led to a rising consumption of dried nuts, including almonds. This growing trend drives the need for the production of more almond trees, which can be addressed through micropropagation as a viable solution for large-scale propagation of high-quality disease-free plants. However, almond tree in vitro propagation faces significant challenges in the regeneration of proper plants. A major bottleneck, common to many other woody species is rooting, which is a limiting step due to recalcitrant mechanisms that prevent functional adventitious root formation in micropropagated shoots. This is highly influenced by several factors, including auxin balances, environmental conditions and genotype that play key roles in root induction and development. This work aimed to assess ex vitro rooting of different genotypes of bitter almond tree (AA15, AA16 and AA21) using 0.5% w/v and 1% w/v Rhizopon AA powder (RAAp) composed with IBA (n=5 in triplicate). Rooting percentages were registered after 30 days after treatment. AA16 genotype showed the best results (control: 40.00%; RAAp 0.5%:73.33%; RAAp 1%: 80.00%) in contrast with AA15 (control: 6.67%; RAAp 0.5%: 20.00%; RAAp 1%: 26.67%); AA21 also presented good results (control: 13.33%; RAAp 0.5%: 60.00%; RAAp 1%: 20.00%). RAAp 0.5% appears to be the most promising treatment for ex vitro rooting in almond as it significantly improved rooting in two of the genotypes. Further studies will be conducted to evaluate the acclimatization and plant survival.

Key-words: auxin, *ex vitro* rooting, micropropagation, *Prunus amygdalus*

P80 - Use of somatic embryogenesis in the genetic improvement program of forestal ARAUCO

Yenny Lineros, Paulina Nuñez, Ximena Muñoz

Forestal ARAUCO S.A, Centro de Investigación Bioforest, Camino a Coronel km 15, Chile

Yenny.Lineros@arauco.com

Genetic improvement of forest species is essential to optimize productivity and sustainability in the forestry industry. In this context, Forestal ARAUCO, one of the leading companies in the sector in Chile, has promoted the development of improved *Pinus radiata* genotypes using the somatic embryogenesis technique. Somatic embryogenesis is a biotechnological process that enables plant regeneration from somatic cells, facilitating the clonal propagation of trees with superior characteristics. Furthermore, when combined with cryopreservation, it creates a robust platform for evaluating improved genotypes under field conditions and ensures the long-term availability of plant material. The implementation of this technology has allowed the development of a precision clonal forestry program, which facilitates the mass production of clones with desired characteristics, contributing to increased productivity, reduced resource use, and improved sustainability in wood production.

A key aspect of Forestal ARAUCO's genetic improvement and clonal forestry is traceability and the implementation of molecular certification using microsatellites. These molecular markers, repetitive DNA sequences with high variability between individuals, allow for confirmation of the genetic identity of clonal genotypes and ensure that desired characteristics are maintained across generations.

In conclusion, the adoption of these technologies has allowed Forestal ARAUCO not only to improve desired characteristics in *Pinus radiata* genotypes, but also to conserve them long-term and produce them efficiently on a large scale.

Keywords: somatic embryogenesis, cryopreservation, molecular certification, clonal forestry

P81 - Micropropagation of *Eucalyptus camaldulensis* Dehn. using organogenesis

Yeşim Yalçın Mendi[1], Recep Yıldız[2], Meslihna Karayel[1], Senem Uğur[1]

[1]Department of Horticulture, Faculty of Agriculture, Cukurova University

[2]Eastern Mediterranean Forestry Research Institute, General Directiorate of Forestry

ymendi@gmail.com

Eucalyptus camaldulensis is of great importance among industrial forest species due to its rapid growth, strong cellulose source and wide ecological tolerance. However, vegetative propagation and cuttings production suffer losses due to biotic and abiotic factors. In addition, rooting difficulty limits seedling production. The aim of this study was to develop *in vitro* tissue culture protocols for mass production of *E. camaldulensis*. The focus was on the optimization of sterilization, basic media and plant growth regulators (PGRs). Different sterilization protocols were applied and 70% ethyl alcohol (3 min.), 20% NaClO (20 min.) solution was determined as the optimum sterilization protocol. This protocol provided 80% success. NaClO concentration and retention time were found to be critical to avoid contamination and damage to shoots. The study also examined the effects of different basic media (MS, WPM, B5) and different PGR concentrations (KIN, GA_3, NAA, 2IP, BAP). The combination of MS medium + 0.5 mg/L BAP was found to be the most productive combination in terms of shoot formation time, shoot number and plant height in all elite 3 clones (C-188, C-201, and C-298). The average shoot formation time was 25 days, the number of shoots was 2.36 and plant height was 1.4 cm. These findings contribute to the development of effective tissue culture protocols for the mass production of *E. camaldulensis.*

Keywords: *Eucalyptus camaldulensis*, Micropropagation, Tissue Culture, Sterilization, *in vitro*

Posters WG5

P82 - The phytotoxicity of titanium dioxide nanoparticles on Blueberry plantlets in *in vitro* culture

Viktor Husak[1], Ulyana Kilchytska[1], Ihor Mykytyn[2], Volodymyr I. Lushchak[1,3]

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

volodymyr.lushchak@pnu.edu.ua

The increasing use of titanium dioxide nanoparticles (TiO_2NPs) in agriculture raises concerns about their accumulation in soil and potential phytotoxic effects. This study examined the impact of TiO_2NPs at concentrations of 30, 60, 90, and 150 mg/L on the morphophysiological and biochemical responses of micropropagated blueberry (*Vaccinium corymbosum* L.) plantlets *in vitro*. Plant exposure to TiO_2NP significantly inhibited plantlet growth, as indicated by reduced shoot length and stem biomass. It also caused a decline in chlorophyll, carotenoid, and anthocyanin content, while inducing leaf necrosis at 60–150 mg/L in a dose-dependent manner. Additionally, TiO_2NPs increased polyphenol and flavonoid levels, suggesting a stress-induced secondary metabolite response and elevated activity of antioxidant enzymes—superoxide dismutase and ascorbate peroxidase indicated oxidative stress development. Histological analysis revealed significant tissue damage, further confirming the cytotoxic effects of TiO_2NPs on blueberry plantlets. These findings provide valuable insights into the phytotoxicity of TiO_2NPs and underscore the need for careful regulation of nanoparticle applications in agricultural systems.

Keywords: titanium dioxide nanoparticles, *Vaccinium corymbosum* L., blueberry, biochemical characteristics, oxidative stress

P83 - CopyTreewebinar series

Tobias Bruegmann

Thünen Institute of Forest Genetics, Sieker Landstrasse 2, 22927 Grosshansdorf

tobias.bruegmann@thuenen.de

A webinar series for the COST Action CopyTree was launched in the second half of 2024. This webinar series is intended to foster a closer-knit community, stimulate cooperative ideas, promote frequent exchange – in addition to the annual conferences – and enrich the members of the Cost Action with new technical aspects. By May 2025, five webinars had been held.

The webinars were evaluated using an online questionnaire. The evaluation results show that the webinars are perceived as interesting and provide new information for the audience. Both speakers' presentations and moderation were highly rated. However, the evaluation had a relatively low participation rate.

This interactive poster aims to gather a wide range of opinions on the webinars from conference attendees. Participants are invited to answer several questions and suggest new topics for future webinars.

Keywords: Science communication, webinar, community, network, interaction

P84 - Approaching the clone communication challenge

Tobias Bruegmann

Thünen Institute of Forest Genetics, Sieker Landstrasse 2, 22927 Grosshansdorf

tobias.bruegmann@thuenen.de

The specialized language used by scientists is often not understood by the general public, possibly leading to misconceptions. A prime example is the term “clone” when referring to vegetatively propagated plants, which is often mistakenly associated with genetic engineering. This highlights the need for science communication, which informs the public about scientific topics in an accessible manner.

Public lectures, podcasts, and books are well-known formats for science communication. However, these formats often reach people already interested in science. Social media allows scientists to reach a wider audience by delivering information directly to users' timelines. For one week in the summer of 2023, I took over the German Twitter account “Real Scientists DE.” Each day was dedicated to specific subjects such as forests and climate change, plant biotechnology, and genome editing in trees. During this experience, I observed that explaining “clone” in the context of vegetative propagation was well-received. The use of simple comparisons and the transparent presentation of research were helpful.

Science communication is increasingly being centralized by institutions. Direct communication from scientists offers an additional level to reach people. In my view, entertaining and authentic formats are key to effective communication. The time investment must be weighed between communication and research activities, although public engagement can also be enriching for scientists.

Keywords: Biotechnology, misconceptions, science communication, scientific language, social media

P85 - The Application of Next-Generation Sequencing in Agricultural Genomics

Norbert Hidvégi[1], Andrea Gulyás[1], Eszter Gaál[1], Klaudia Kruppa[1], Edina Türkösi[1], Éva Szakács[1], András Farkas[1], László Ivanizs[1], Péter Kovács[1], Péter Mikó[1], István Molnár[1]

[1]HUN-REN Centre for Agricultural Research, Agriculture Institute, Martonvásár, Hungary

norbert.hidvegi@atk.hun-ren.hu

The application of next-generation sequencing (NGS) technologies in agricultural genomics has brought significant advancements in understanding the molecular basis of plant traits and increasing the efficiency of breeding programs. Genotyping platforms capable of SNP (single nucleotide polymorphism)-based genotyping, enable high-resolution characterization of plant genomes and investigation of genetic diversity.
Through genome-based selection and marker-assisted selection, breeders can identify and select plants with desired traits, thereby accelerating breeding processes. DNA polymorphisms in the genome, such as deletions, insertions, translocations, inversions, and SNPs, play important roles in shaping plant traits and maintaining genetic variability. In genome-based selection, plants' genomes are characterized with high-resolution marker systems, and a large number of lines are examined. This technique is particularly useful in diversity studies, QTL analysis (bi-parental populations), association mapping (GWAS), marker-assisted selection, and gene bank research.
Gene expression studies (transcriptomics) examine the expression of interacting gene groups, offering the opportunity to identify new functions of genes. These studies help understand how genes regulate protein quantity and structure and how they influence disease susceptibility and genetic variability. Transcriptomic studies often employ whole transcriptome sequencing, a reproducible and sensitive method. This technique allows sequencing of all transcripts, including those new to science.

Keywords: Genotyping by sequencing, GWAS, diversity

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P86 - The potential applications of micropropagation and in vitro conservation of olive genetic resources: OMIBREED project

Waed Tarraf, Anna De Carlo

Institute of BioEconomy (IBE), National Research Council (CNR) of Italy, 50019 Sesto Fiorentino, Florence, Italy

waed.tarraf@ibe.cnr.it

ABSTRACT

The conservation of plant biodiversity is crucial worldwide, not only for threatened species but also for those with important cultural and economic values, such as *Olea europaea* L. In this context, the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF) has funded the project "OMIBREED - Characterization and Valorization of Agrobiodiversity through Multiomic and Next-Generation Breeding Approaches for Resistance to *Xylella fastidiosa*". This research aims to identify genetic traits that confer resistance to *Xylella fastidiosa* (Xf) and to adopt advanced techniques such as micropropagation, slow-growth storage, and cryopreservation, for the sustainable management of olive tree genetic resources. Once effective protocols have been developed, this will enable rapid clonal propagation, conservation, and enhancement of valuable olive biodiversity, including the genotypes selected by the project and centuries-old plants threatened by Xf in olive groves in the Apulia region. Preliminary results have shown effective micropropagation protocols and in vitro storage at low temperatures for short periods for some varieties of olive. Furthermore, due to the lack of optimized cryogenic protocols, studies to verify how different olive genotypes respond to the cryopreservation process are currently in progress. These findings could contribute to protecting and propagating this vital genetic material through clonal methods, ensuring maximum genetic and phytosanitary safety.

Keywords: *Olea europaea*, in vitro culture, slow-growth storage, cryopreservation, *Xylella fastidiosa*

P87 - Innovative small synthetic molecules for enhancing somatic embryogenesis induction in European chestnut and holm oak

María Teresa Martínez[1], Eduardo Piñas[1], Cristina Buceta[1], Fátima Mosteiro[1], Pablo Piñeiro[1], Elena Carneros[2], Carmen Gil[3], Ana Martínez[3], Pilar S. Testillano[2], Elena Corredoira[1]

[1]Misión Biológica de Galicia (MBG-CSIC), Sede de Santiago de Compostela, Avd. Vigo s/n 15705 Santiago de Compostela, La Coruña, Spain
[2]Pollen Biotechnology of Crop Plants group, Margarita Salas Center of Biological Research, CIB-CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain
[3]Translational Medicinal and Biological Chemistry group, Margarita Salas Center of Biological Research, CIB-CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain

elenac@mbg.csic.es; testillano@cib.csic.es

ABSTRACT

Somatic embryogenesis (SE) is considered the most appropriate plant regeneration pathway for cloning forest species. However, in many woody species, including European chestnut and holm oak, in vitro clonal propagation by SE remains problematic, particularly for cloning adult specimens. The general aim of the research reported here is to discover new chemical promoters, by screening existing chemical libraries (used in the pharmaceutical field), and to test their ability to improve SE induction in European chestnut and in holm oak. Recent reports indicate that small molecule inhibitors of mammalian glycogen synthase kinase 3 β (GSK3 β) and leucine rich repeat kinase 2 (LRRK2) can enhance initiation of plant embryogenesis (Berenguer et al. 2021, J. Exp. Bot., 72, 7808-7825. http://doi.org/10.1093/jxb/erab365; Carneros et al. 2024, J Plant Physiol 303, 154334. http://doi.org/10.1016/j.jplph.2024.154334). Other small molecules are the inhibitors of phosphodiesterases (PDEs), enzymes that inactivate cAMP, which can promote mammalian cell reprogramming. Leaves from chestnut and shoot tips from both chestnut and holm oak, excised from axillary shoot cultures, were used as the initial explants for SE induction. These explants were cultured on induction media supplemented with various kinase inhibitors (GSK3β or LRRK) or PDE inhibitors. In chestnut, the embryogenic competence of leaves was significantly higher than that of shoot tips. Evaluation of different small molecules showed that SE rates in chestnut were enhanced by two LRRK or GSKβ inhibitors, while the PDE inhibitors did not yield any positive response. By contrast, in holm oak, SE was improved by molecules of all three inhibitor types.

Keywords: *Castanea sativa*, cell reprogramming, *Quercus ilex*, small molecule inhibitors.

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List of participants

Abdel Rahman Mohammad Said Al-Tawaha
Al Hussein Bin Talal University, Maan, Jordan
abdeltawaha74@gmail.com
Afonso Melo
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
ajcldemelo@gmail.com
Afonso Zambela
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
afonsojzambela@gmail.com
Alexander Fendel
Thünen Institute of Forest Genetics, Grosshansdorf, Germany
alexander.fendel@thuenen.de
Alois Bilavcik
Czech Agrifood Research Center, Prague, Czech Republic
alois.bilavcik@carc.cz
Amal Rabaaoui
Faculty of Bioscience Engineering, Ghent, Belgium
amal.rabaaoui@ugent.be
Ana Pedrosa
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
anasimoespedrosa@gmail.com
Ana Isabel Coelho
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
acoelho@student.uc.pt
Anabela Romano
Universidade do Algarve, Faro, Portugal
aromano@ualg.pt
André Caeiro
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
andrecaeiro91@gmail.com
Andrea Gulyás
HUN-REN Centre for Agri. Research Agricultural Institute, Martonvasar, Hungary
gulyas.andrea@atk.hun-ren.hu
Andrea Rupps

Humboldt-Universität zu Berlin, Berlin, Germany
andrea.rupps@hu-berlin.de
Ángeles Meira
University of Vigo, Vigo, Spain
mariaangeles.meira@uvigo.es
Arianna Cesarini
Universita Degli Studi Di Perugia, Perugia, Italy
arianna.cesarini@collaboratori.unipg.it
Ashkan Hodaei
Ghent University, Ghent, Belgium
ashkan.hodaei@ugent.be
Baiba Krivmane
Latvijas Valsts Mezzinatnes Instituts Silava, Salaspils, Latvia
baiba.krivmane@silava.lv
Barbara Ruffoni
Consiglio per la Ricerca in Agri. e l'Analisi dell'Economia Agraria, Sanremo, Italy
barbara.ruffoni@crea.gov.it
Ben Bubner
Thuenen Insitute of Forest Genetics, Waldsieversdorf, Germany
ben.bubner@thuenen.de
Benedetta Chiancone
Department of Food and Drug, University of Parma, Parma, Italy
benedetta.chiancone@unipr.it
Bernardo Onofre
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
bapvaonofre@gmail.com
Bianca Gonçalves
ARALAB, Rio de Mouro – Portugal
aralab@aralab.pt
Bogomil Breznik
Triglavski Narodni Park, Bled, Slovenia
breznik.bogomil@gmail.com
Bruce Christie
The GreenPlant Company Ltd, Palmerston North, New Zealand
brucechristie101@gmail.com
Brunilda Çuko
University of Tirana, Tirana, Albania
cuko.brunilda@gmail.com
Buhara Yücesan
Bolu Abant İzzet Baysal University, Bolu, Turkey
ibuhara@yahoo.com
Carmen Diaz-Sala
University of Alcalá de Henares, Alcalá de Henares, Spain
carmen.diazsala@uah.es
Caroline Teyssier
INRAE, Orléans, France
caroline.teyssier@inrae.fr
Chaima Ben Othmen
Ghent University, Ghent, Belgium
chaimabenothmen11@yahoo.fr
Claudia Ruta
University of Bari Aldo Moro, Bari, Italy
claudia.ruta@uniba.it
Conchi Sánchez

CSIC - Misión Biológica de Galicia, Santiago de Compostela, Spain
conchi@mbg.csic.es
Cristina Marques
RAIZ - Instituto de Investigação da floresta e Papel, Eixo, Portugal
cristina.marques@thenavigatorcompany.com
Daniela Cordeiro
University of Alcalá de Henares, Alcalá de Henares, Spain
danielacordeiro@outlook.pt
Daniela Duarte
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
daniela.d@sapo.pt
Dawid Kocot
University of Agriculture, Kraków, Poland
dawid.kocot@urk.edu.pl
Dicle Dönmez
University of Cukurova, Adana, Turkey
dicledonmez4@gmail.com
Dirk Stubbe
Sac02, Veldeken 29, 9850 Deinze, Belgium
info@saco2.com
Doaa Elkassas
Assiut University - Faculty of Agriculture, Assiut, Egypt
doaa.elkassas@agr.au.edu.eg
Elektra Papakosta
Center of Agricultural Tecnology Transfer (ATTC), Vlore, Albania
elektrapapakosta@gmail.com
Elena Carneros
Biological Research Center Margarita Salas (CIB-CSIC), Madrid, Spain
ecarneros@gmail.com
Elena Corredoira
CSIC - Misión Biológica de Galicia, Santiago de Compostela, Spain
elenac@mbg.csic.es
Eleni Abraham
Aristotle University of Thessaloniki, Thessaloniki, Greece
eabraham@for.auth.gr
Elif Aylin Ozudogru
İstinye University Istanbul, Istanbul, Turkey
elifaylinozudogru@yahoo.it
Elnara Sadigova
Ghent University - Campus Coupure, Ghent, Belgium
elnara.sadigova@ugent.be
Elsa Baltazar
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
e.c.s.baltazar@gmail.com
Emma Ehmke
Humboldt University of Berlin, Berlin, Germany
emma.ehmke@hu-berlin.de
Fabiano Gattabria
Vivai Piante Battistini Società Agricola s.s., Cesena, Italy
fgattabria@battistinivivai.com
Filip Vandenbussche
Deroose Plants, Evergem, Belgium
Filip.Vandenbussche@derooseplants.com
Francisco Javier Alfosea-Simón

Agencia Estatal Consejo Superior de Investigaciones Cientificas, Murcia, Spain
fjalfosea@cebas.csic.es
Francy Burgos
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
jhoannacevallosb@gmail.com
Giovanni Broggini
ETH Zürich, Zurich, Switzerland
giovanni.broggini@usys.ethz.ch
Giuseppe Natale Basile
University of Bari Aldo Moro, Bari, Italy
giuseppe.basile@uniba.it
Glória Pinto
University of Aveiro, Aveiro, Portugal
gpinto@ua.pt
Hajer Ben Ghozlen
Ghent University, Ghent, Belgium
hajer.benghozlen@ugent.be
Hajer Darouez
Faculty of Bioscience Engineering Ghent University, Ghent, Belgium
hajerdarouez@yahoo.fr
Hana Konradova
Charles University, Faculty of Science, Prague, Czech Republic
hana.konradova@natur.cuni.cz
Hannes Wilms
Instituut voor natuur en bosonderzoek (INBO), Geraardsbergen, Belgium
hannes.wilms@inbo.be
Hans Bethge
Gottfried Wilhelm Leibniz Universitaet Hannover, Hannover, Germany
bethge@baum.uni-hannover.de
Hassiba Fraj
Ghent University, Evergem, Belgium
hassibaing@live.fr
Inês Moço
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
uc2010162426@student.uc.pt
Ineta Samsone
Latvia Forest Research Institute "Silava", Salaspils, Latvia
ineta.samsone@silava.lv
Irum Saadia Khan
Walter Blom Plants, Hillegom, The Netherlands
irumsaadia73@gmail.com
Isa Ramalho
Amorim Florestal, AS, Ponte de Sôr, Portugal
irramalho2@gmail.com
Itziar Montalbán
NEIKER - Campus Agroalimentario de Arkaute, Álava, Spain
imontalban@neiker.eus
Iva Sikuten
University of Zagreb Faculty of Agriculture, Zagreb, Croatia
isikuten@agr.hr
Ivana Gribaudo
Consiglio Nazionale delle Ricerche, Torino, Italy
ivana.gribaudo@gmail.com
Ivaylo Tsvetkov

Forest Research Institute (BAS), Sofia, Bulgaria
tsvet_i@yahoo.com
Iveta Megrelishvili
LEPL Scientific Research Center of Agriculture, Tbilisi, Georgia
ivetameg@yahoo.com
Ján Jurčík
Inst of Plant Genet. and Biotech., Plant Sci. & Biodiv. Center, Nitra, Slovakia
jan.jurcik@savba.sk
Jana Krajnakova
New Zealand Forest Research Institute LTD, Rotorua, New Zealand
jana.krajnakova@scionresearch.com
Jana Šedivá
Research Inst. for Landscape and Ornamental Gardening, Průhonice, Czech Republic
sediva@vukoz.cz
Jaroslav Nisler
Institute of Experimental Botany of CAS, Prague, Czech Republic
jaroslav.nisler@gmail.com
Jesús Mª Vielba
Misión Biológica de Galicia (MBG-CSIC), Santiago de Compostela, Spain
jmvielba@mbg.csic.es
João Martins
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
joao.martins@uc.pt
Jorge Sofia
Instituto Nacional de Investigação Agrária e Veterinária, Dois Portos, Portugal
jorge.sofia@iniav.pt
Jorge Canhoto
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
jorgecan@ci.uc.pt
José Carlos Gonçalves
Escola Superior Agrária de Castelo Branco – IPCB, Castelo Branco, Portugal
jcgoncalves@ipcb.pt
Juan José Silva Pupo
Universidad de Granma, Bayamo, Cuba
jsilvapupo@gmail.com
Justine Perrotte
Invenio, Bordeaux, France
j.perrotte@invenio-fl.fr
Karam Mostafa
Ondokuz Mayis University, Samsun, Turkey
karamkrush1@gmail.com
Karel Dolezal
Institute of Experimental Botany AS CR, Olomouc, Czech Republic
karel.dolezal@upol.cz
Katarina Klubicová
Inst of Plant Genet. and Biotech., Plant Sci. & Biodiv. Center, Nitra, Slovakia
katarina.klubicova@savba.sk
Katchamon Nimprang
COST Association
Katchamon.Nimprang@cost.eu
Kateřina Eliášová
Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic
eliasova@ueb.cas.cz
Kubra Yazici

Yozgat Bozok University, Yozgat, Turkey
kubra.yazici@yobu.edu.tr
Ladislav Havel
Mendel University in Brno, Brno, Czech Republic
ladislav.havel@mendelu.cz
Lilyana Nacheva
Agricultural Academy - Fruitgrowing Institute Plovdiv, Plovdiv, Bulgaria
lilyn@abv.bg
Liva Purmale-Trasune
Bulduri Technical School, Jurmala, Latvia
liva.purmale@bulduri.lv
Lorenzo Burgos
Consejo Superior de Investigaciones Científicas (CSIC), Murcia, Spain
burgos@cebas.csic.es
Luca Regni
Universita Degli Studi Di Perugia, Perugia, Italy
luca.regni@unipg.it
Lucie Fischerová
Institute of Experimental Botany of CAS, Prague, Czech Republic
fischerova@ueb.cas.cz
Luís Fontes
Altri Florestal AS, Olho Marinho, Portugal
luis.fontes@altri.pt
Lukáš Ležal
Charles University, Prague, Czech Republic
lukas.lezal@natur.cuni.cz
Magnus Hertzberg
SweTree Technologies AB, Umea, Sweden
Magnus.hertzberg@SweTree.com
Maia Kukhaleishvili
Georgian Technical University, Biotechnology Center, Tbilisi, Georgia
maia.kukh@gmail.com
Malin Abrahamsson
SweTree Technologies AB, Umea, Sweden
malin.abrahamsson@swetree.com
Marga Fraga Ares
Fundación Paideia Galicia, Coruña, Spain
contacto@cultigar.es
Mariana Correia
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
mjcorreia324@gmail.com
Mariana Neves
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
mariananevespt@gmail.com
Mariana Sottomayor
Faculty of Sciences of University of Porto, Porto, Portugal
mssottom@fc.up.pt
Marina Martín de Valmaseda Sánchez
CSIC - Centro de Edafologia y Biologia Aplicada del Segura, Murcia, Spain
mmartin@cebas.csic.es
Mariyana Gozmanova
University of Plovdiv, Plovdiv, Bulgaria
mgozmanova@gmail.com
Maroua Grira

Ghent University, Ghent, Belgium
grira.maroua@gmail.com
Marta Cruz
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
martacruz016@gmail.com
Marzieh Mohri
Institute of Exp. Botany of the Czech Academy of Sciences, Prague, Czech Republic
mohri.m@ueb.cas.cz
Maria Teresa Martínez
Misión Biológica de Galicia (MBG-CSIC), Santiago de Compostela, Spain
temar@mbg.csic.es
Marten Elsinga
Iribov R&D Tissue Culture, The Netherlands
marten.elsinga@iribov.com
Maurizio Lambardi
National Research Council (CNR), IBE-Institute of BioEconomy, Firenze, Italy
maurizio.lambardi@ibe.cnr.it
Michaela Pekařová
Landscape Research Institute, Průhonice, Czech Republic
pekarova@vukoz.cz
Michiel van Bennekom
Iribov R&D Tissue Culture, The Netherlands
m.vanbennekom@iribov.com
Miguel Pedro Guerra
Federal University of Santa Catarina, Florianopolis, Brazil
miguel.guerra@ufsc.br
Miguel Rito
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
miguel9_rito@hotmail.com
Mirni Ulfa Bustami
Tadulako University, Palu, Indonesia
meetmot@yahoo.com
Miroslav Perniš
Slovak Academy of Sciences, Nitra, Slovakia
miroslav.pernis@savba.sk
Monika Höfer
Julius Kuehn-Institute, Inst. for Breeding Res. on Fruit Crops, Dresden Germany
monika.hoefer@julius-kuehn.de
Muhammad Asim
Sivas University of Science and Technology, Sivas, Turkey
mshazim@gmail.com
Natália Expósito de la Paz
Biological Research Center - Margarita Salas, Madrid, Spain
natelenaexpo@gmail.com
Neslihan Yeşim Yalçın Mendi
Cukurova University, Adana, Turkey
ymendi@gmail.com
Nieves Vidal
MBG-CSIC, Agencia Est. Cons. Sup. de Invest. Cient., Santiago de Compostela, Spain
nieves@mbg.csic.es
Norbert Hidvégi
HUN-REN Center for Agricultural Res. Agricultural Institute, Martonvasar, Hungary
norbert.hidvegi@atk.hun-ren.hu
Nourhene Jouini

University of Palermo, Palermo, Italy
jn.nourhen@gmail.com
Nur Koyuncu
Field Crops Central Research Institute, Ankara, Turkey
nur.koyuncu@tarim.gov.tr
Nuria Albuquerque
CEBAS-CSIC, Murcia, Spain
nalbur@cebas.csic.es
Olena Bobrova
Vyzkumny Ustav Rostlinne Vyroby Vvi, Prague, Czech Republic
helen.bobrova.77@gmail.com
Pablo Piñeiro Insua
Misión Biológica de Galicia (MBG-CSIC), Santiago de Compostela, Spain
pablomanuel.pineiro@mbg.csic.es
Paula Oros
University of Agric Sci. and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania
paula.oros@usamvcluj.ro
Pawel Chmielarz
Polish Academy of Sciences, Institute of Dendrology, Kórnik, Poland
pach@man.poznan.pl
Pedro Pablo Gallego
Vigo University, Vigo, Spain
pgallego@uvigo.es
Pilar Sánchez Testillano
Biological Research Center Margarita Salas (CIB-CSIC), Madrid, Spain
testillano@cib.csic.es
Ralph Stüebner
COST Association
ralph.stuebner@cost.eu
Rakefet David-Schwartz
Stubbe (ARO), Volcani Center, Bet-Dagan, Israel
rakefetd@volcani.agri.gov.il
Ricardo Castro-Camba
Consejo Sup de Invest. Cientificas - Mision Biol. de Galicia. St. de Compostela, Spain
ricardo.castro@mbg.csic.es
Ricardo da Costa
University of Coimbra, Coimbra, Portugal
dacosta.rmf@gmail.com
Ricardo Ferraz
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
rikayferr@hotmail.com
Ruben Melo
Escola Superior Agrária de Coimbra, Portugal
ruben-b-melo@hotmail.com
Rumyana Valkova
University of Plovdiv, Plovdiv, Bulgaria
boncheva@uni-plovdiv.bg
Saba Taheri
Ghent University, Ghent, Belgium
s.taheri14@gmail.com
Saila Varis
Natural Resources Institute Finland, Savonlinna, Finland
saila.varis@luke.fi
Saleta Rico

MBG-CSIC, Santiago de Compostela, Spain
saleta@mbg.csic.es
Sandra Correia
InnovPlantProtect, Elvas, Portugal
sandra.correia@iplantprotect.pt
Sanja Bogunović
Hrvatski Sumarski Institut, Jastrebarsko, Croatia
sanjam@sumins.hr
Sara Yasemin
Siirt University, Siirt, Turkey
sara.yasemin@siirt.edu.tr
Slađana Jevremović
Inst. for Biol. Research "Sinisa Stankovic" - Nat Inst of Rep. Serbia, Belgrade, Serbia
sladja@ibiss.bg.ac.rs
Soner Yağ
FSB biotech ltd. sti., Cukurova University, Adana Turkey
soneryag@gmail.com
Stefaan Werbrouck
Ghent University, Ghent, Belgium
stefaan.werbrouck@ugent.be
Stéphane Maury
University Orléans, Orléans, France
stephane.maury@univ-orleans.fr
Şükran Yıldız
Mersin University, Turkey
yldzskran@gmail.com
Susana Neves
ARALAB, Rio de Mouro – Portugal
aralab@aralab.pt
Tatjana Vujovic
Fruit Research Institute, Čačak, Serbia
tvujovic@institut-cacak.org
Tércia Lopes
Centre for Functional Ecology, Dep. of Life Sciences, Univ. of Coimbra, Portugal
tercia.lopes.95@gmail.com
Teresa Hazubska-Przybył
Institute of Dendrology, Polish Academy of Sciences, Kórnik, Poland
hazubska@o2.pl
Tobias Bruegmann
Thünen Institute of Forest Genetics, Grosshansdorf, Germany
tobias.bruegmann@thuenen.de
Tolga İzgü
CNR, Sesto Fiorentino, Fizenze, Italy
tolgaizgu01@gmail.com
Tomas Arteta
Vigo University, Vigo, Spain
tarteta@uvigo.gal
Tsuyoshi Maruyama
Forestry and Forest Products Research Institute, Tsukuba, Japan
tsumaruy@ffpri.affrc.go.jp
Tujia Aronen
Natural Resources Institute Finland, Punkaharju, Finland
tuija.aronen@luke.fi
Valbona Sota

University of Tirana, Tirana, Albania
valbona.sota@fshn.edu.al
Vanja Danicic
University of Banja Luka, Faculty of Forestry, Banja Luka, Bosnia & Herzegovina
vanja.danicic@sf.unibl.org
Virginia Zahn
Thuenen Institute of Forest Genetics, Grosshansdorf, Germany
virginia.zahn@thuenen.de
Vitalina Karfik
Northwest German Forest Research Institute, Hann Muenden, Germany
vitalina.karfik@nw-fva.de
Vladislava Galović
Institute of Lowland Forestry and Environment, Novi Sad, Serbia
vladislava.galovic@gmail.com
Volodymyr Lushchak
Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukania
volodymyr.lushchak@pnu.edu.ua
Waed Tarraf
Institute of BioEconomy, Nat. Res. Council, Sesto Fiorentino, Firenze, Italy
waed.tarraf@ibe.cnr.it
Xuan Xu
Luxembourg Institute of Science And Technology, Hautcharage, Luxembourg
xuan.xu@list.lu
Yenny Lineros
Forestal Arauco S.A, Concepción, Chile
yenny.lineros@arauco.com
Zhibo Hamborg
Nibio - Norsk Institutt For Biookonomi, Aas, Norway
zhibo.hamborg@nibio.no

List of authors

	Pages
Abdel R. M. Said Al-Tawaha	119
Ahmed Namsi	103
Ahmed Sadkhan	122
Alain Delaunay	23,86
Alberto Pizarro	13
Alexander Fendel	21,22
Alexandre Mary	23
Alice Almeida	110
Alice-Jeannine Sievers	21
Alois Bilavcik	15,118
Amal F.M. Zein El Din	119
Amal Rabaaoui	102,103
Ana Martínez	84
Ana Reis	16
Anabela Romano	132
Ander Castander-Olarieta	41
André Caeiro	43,85
Andre Skirtach	25
Andrea Di Fazio	113
Andrea Gulyás	140
Andrea Moglia	91
Andrea Rupps	109,122
Ángeles Meira	55
Anna Agosti	113
Anna Tagarelli	45
Antonia Maiara M. Nascimento	41
Antonia Sofianou	56
Antonia Susca	103
Antonio Francesco Logrieco	103
Antonio Moretti	103
Anxela Aldrey	76,114,116
Arianna Cesarini	27
Armelle Delile	81
Arnis Gailis	93
Ashkan Hodaei	92
Asta Žukauskaitė	79
Audrey Gibert	86
Baiba Krivmane	93
Barbora Tunklova	115
Bart Panis	32
Beatriz César	48
Beatriz Cuenca	35,66
Beatriz Figueiredo	127
Ben Bubner	21
Benedetta Chiancone	113
Bernardo Onofre	97
Birgit Reiche	109
Bogdan Parakhonskiy	25
Bora Onur Hallaç	50,112
Branka Uzelac	100
Bruce Christie	53,114,116
Brunilda Çuko	44
Bruno Marcos	110
Buhara Yücesan	54
Carla Benelli	51

	Pages
Carlos Cação	20
Carlos Vila-Viçosa	110
Carmen Correia	110
Carmen Díaz-Sala	13
Carmen Diniz	104
Carmen Gil	84
Caroline Teyssier	81,86
Cathie Reeves	38
Célia Miguel	86
Celine Mercier	38
Céline Ridel	80
Chaima Ben Othmen	69
Chiara Dall'Asta	103
Christian Daviaud	86
Christin Müller	109
Claire Le Metté	86
Cláudia Marinho	134
Claudia Ruta	36,45
Conchi Sánchez	35,67,71,76,114,116
Corina Cătană	26
Cristian Chaparro	86
Cristian Pérez-Caselles	34,47
Cristina Buceta	66,84
Cristina M. Marques	17
Cynthia Marbun	62
Dainis Ruņģis	93
Daniel Zahradník	74
Daniela Cordeiro	13,85
Daniela Duarte	97
Daniela Torello Marinoni	91
Dardan Klos	79
Daria Penzina	78
Darko Preiner	99
David Fernández	58
Dawid Kocot	117
Dicle Dönmez	80,131
Diego Fansa	58
Diyana Aleksandrova	70
Doaa Elazab	36,45
Dušica Ćalić	33,100
Eduardo Piñas	66,84,88
Efigjeni Kongjika	44,98
Elektra Papakosta	31
Elena Carneros	23,84,87
Elena Corredoira	57,66,84,88,91
Elena Yelo	34,47,89
Eleni Abraham	56
Elif Aylin Ozudogru	36
Elina Havia	126
Elmar Bernhardt	38,126
Elnara Sadigova	65
Els Prinsen	19,64
Elsa Baltazar	29,46,134
Emma Ehmke	109
Emma López	66
Erea Garcés	55
Ernesto González	91
Ewa Kazimierczak-Grygiel	63

	Pages
Fátima Mosteiro	84
Felipe Pérez	66
Filip Vandenbussche	62
Filomena Gomes	133
Florin Huiban Adrian	75
Francesco Paolo Marra	113
Francis Canlet	86
Francisco Cotta	43
Francisco J. Alfosea-Simón	30,47
Franka Thiesen	21
Franklin Back	41
Gederts Ievinsh	72
Giuseppe De Mastro	45
Giuseppe Natale Basile	45
Glória Pinto	129
Guillermo Salvatierra	38
Hajer Ben Ghozlen	128
Hajer Darouez	67,68
Hana Konrádová	82
Hannes Wilms	32
Hassiba Fraj	94
Helena Lipavská	82
Herlander Azevedo	110
Hojjat Ataee	71
İ. Halil Yiğit	24
I. Megrelishvili	102
Ieva Bērziņa	101
Ieva Lelenaite	104
Ihor Mykytyn	137
Ihor Ya Shyndak	121
Inés Castejón	58
Inês Mansinhos	132
Inês Moço	133
Inês Modesto	86
Ineta Samsone	93,101
Isa Ramalho	108
Isabelle Le Jan	23
Isabelle Reymond	86
Itziar Aurora Montalbán	41
Iuri Frias	110
Iva Šikuten	99
Ivana Gribaudo	14
Ivaylo Tsvetkov	70,96,105,106
Iveta Megrelishvili	59
Jaakko Salminen	126
Ján Jurčík	28,95
Jana Hanzal Šedivá	47
Jana Krajnakova	38
Jana Pavlíčková	78
Janine Otte	109
Jaroslav Nisler	74
Javier Sampedro	57
Jean-François Trontin	86
Jelena Milojević	33,100
Jéssica Batista	107
Jesús Vielba	35,53,67,71,76
Jiri Zamecnik	115,118
Joana Domingues	110

	Pages
Joana Ferreira	20
João F. Martins	46,127,129
João Gonçalves	110
João Nunes	110
João P. Martins	52
João Pratas	129
Jörg Tost	86
Jorge Ascenção	129
Jorge Canhoto	20,29,43,46,48,77,85,97,107,120,123,127,129,133,134
Jorge L. Pérez	60
Jorge Terrados	58
José Carlos Gonçalves	110
Juan J. Pupo	60
Juan M. Ley-López	52
Juliane Raschke	109,122
Justyna Wiland-Szymańska	63
Karam Mostafa	119
Karel Chala Arias	60
Karel Doležal	78,79,82
Kaspars Liepiņš	93
Kassiani Papadopoulou	56
Katarína Klubicová	28,95
Kateřina Eliášová	78,81
Kourosh Vahdati	71
Kristýna Bieleszová	79
L. Ujmajuridze	102
Laura Guerrero-Meseguer	58
Laura Marconi	27
Laura Righetti	103
Leandra Leto	113
Liliana Marum	134
Lilyana Nacheva	70,96,105,106
Liva Purmale-Trasune	30,72
Lorenzo Antonio Marino	91
Lorenzo Burgos	30,34,47,89
Luca Regni	27
Lucas Vanhaelewyn	62
Lucie Fischerová	78
Lucie Plíhalová	79
Luís Ferreira	16
Luís Fontes	16
Luís Leal	16
Luiza Giacomolli Polesi	41
Lukáš Ležal	82
Madlen Walther	109
Magdalena Bryksová	79
Magnus Hertzberg	49, 83
Mahdi Alizadeh	71
Maia Kukhaleishvili	59
Maja Belić	33,100
Malin Abrahamsson	49,83
Mamadou Dia Sow	86
Marcelo Francisco	88
Marek Zatloukal	79
Maria João Ferreira	104
María José Cernadas	67,76,116
María Rubio	58
María Teresa Martínez	57,66,84,88,91

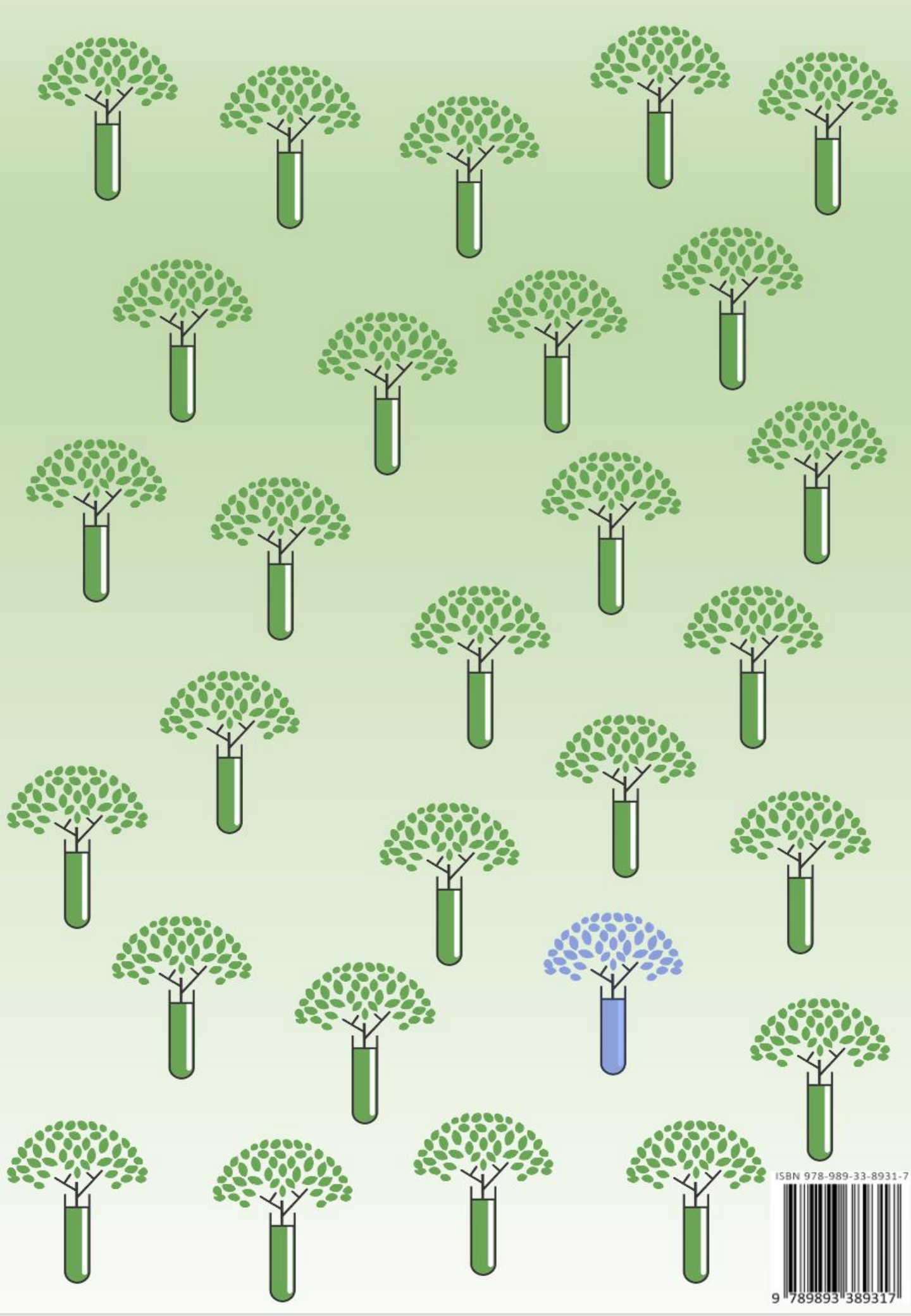
	Pages
Mariana Correia	46,120,134
Mariana Neves	77,85,107
Marie-Anne Lelu-Walter	81,86
Marina M. Valmaseda Sánchez	34,89
Mário Louro	16
Mario Masiello	103
Mariyana Gozmanova	96,105,106
Maroua Grira	19,64
Marten Elsinga	39
Martin Vojtek	104
Martina Cirlini	113
Marvin Mohlfeld	111
Marzenna Guzicka	63
Marzieh Mohri	78
Matthias Fladung	21,22
Matthias Meyer	109
Maurizio Lambardi	15,36,44,45
Mehmet Tütüncü	131
Meslihna Karayel	136
Michaela Pekařová	74
Michal Karady	77
Michiel van Bennekom	39
Miguel Batalha	123
Miguel Pedro Guerra	18,41
Miguel Rito	28,48,85
Mikko Tikkinen	38,42,126
Mikołaj Krzysztof Wawrzyniak	52
Milos Faltus	115,118
Mirjana Mićević	61
Mirni Ulfa Bustami	124
Miroslav Perniš	28,95
Miroslav Strnad	79
Mónica Zuzarte	97,120,134
Muhammad Asim	40
Musab A. Isak	131
N. Maziashvili	102
Natália Expósito de la Paz	87
Natalia Fanega Sleziak	32
Nataliya Dimitrova	70
Nathalie Boizot	81
Nathalie Boizot	86
Neusa Steiner	41
Nieves Vidal	35,53,67,76,114,116
Nikola Štěpánová	82
Norbert Hidvégi	140
Nourhene Jouini	29
Nuno Rico	110
Nunzia Cicco	51
Nur Koyuncu	24
Nuria Alburquerque	30,34,47,89
Odile Rogier	81
Oksana V. Pasat	121
Ola Abd Elbar	119
Olena Bobrova	115,118
Ondřej Novák	79
Orges Cara	31
Özhan Şimşek	51,130,131

	Pages
Pablo Piñeiro Insua	57,88
Paloma Moncaleán	41
Parisa Savane	81
Patricia Fernández-Calvo	35
Paula Castro	97
Paula Oros	26
Paulina Nuñez	135
Paulo Gameiro	20
Paulo R. Rocha	43
Paweł Chmielarz	21,52
Pedro Monteiro	129
Pedro Pablo Gallego	37,55
Petre I. Dobrev	81
Pilar S. Testillano	23,84,87
Puri Covelo	76
Purificación Covelo	53
Radhouane Gdoura	103
Radisras Nkurunziza	90
Raffaella Brigante	27
Raquel Rodríguez-Solana	132
Recep Yıldız	136
Reinout Impens	62
Ricardo Bittencourt	18
Ricardo Castro-Camba	58,76
Ricardo Ferraz	20,85
Ricardo Joffe	109
Ricardo M. da Costa	104
Rita Lourenço Costa	88
Roberto Botta	91
Rui Malhó	88
Rumyana Valkova	96,105,106
Saba Taheri	25,29
Saila Varis	42,126
Sakari Valimaki	38,42,126
Saleta Rico	35,67,71,76
Sam Davidson	38
Sandra Correia	20,28,46,48,77,85,97,120,134
Sandra Gonçalves	132
Sanja Bogunović	73
Sara Freitas	110
Sara Yasemin	90
Sara Zollini	27
Sara Zwolenkiewicz	63
Saša Orlović	75
Senem Uğur	136
Silvan Liebsch	109
Silvia Calisti	27
Sílvia Coimbra	20,104
Silvia Valladares	88,91
Slađana Jevremović	33,100
Snežana Zdravković-Korać	33,100
Sofie Van Gijseghem	62
Soner Yağ	50,112
Sophie Maiwald	109
Stefaan Werbrouck	19,25,29,44,60,64,65,67,68,69,80,90,92,94,102,103,124,128
Stefania Somma	103
Stéphane Claverol	81
Stéphane Maury	23,86

	Pages
Şükran Yıldız	90
Susana Serrazina	88
Sven Mangelinckx	128
T. Elbakidze	102
Taoufik Bettaieb	69
Taryn Saggese	38
Tatjana Anđelić	61,114
Tatjana Vujović	61,75,114
Tércia Lopes	46,134
Teresa Hazubska-Przyby	63
Tiziano Caruso	29
Tobias Bruegmann	21,22,138,139
Tolga İzgü	51,130,131
Tomas A. Arteta	37,55
Tony Heitkam	109
Tsuyoshi E. Maruyama	125
Tuija Aronen	38,42,126
Ulyana Kilchytska	137
Uroš Gašić	100
Ute Tröber	109
Václav Motyka	81
Vadym Pasat	121
Valbona Sota	44,98
Valeria Gianguzzi	113
Vera Pavese	91
Viktor Husak	137
Virginia Zahn	21
Vitalina Karfik	111
Vladislava Galović	75
Vlasta Matušková	79
Volodymyr I. Lushchak	121,137
Waed Tarraf	51, 141
William G. Willats	104
Ximena Muñoz	135
Yaprak Bölük	24
Yenny Lineros	135
Yeşim Yalçın Mendi	50,112, 136
Yıldız Aka Kaçar	131
Yllka Lala	98
Z. Khidesheli	102
Zuzana Kozovská	78,95
Zuzana Vondráková	81
Zvjezdana Marković	99



Strawberry tree (*Arbutus unedo L.*), Ericaceae



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