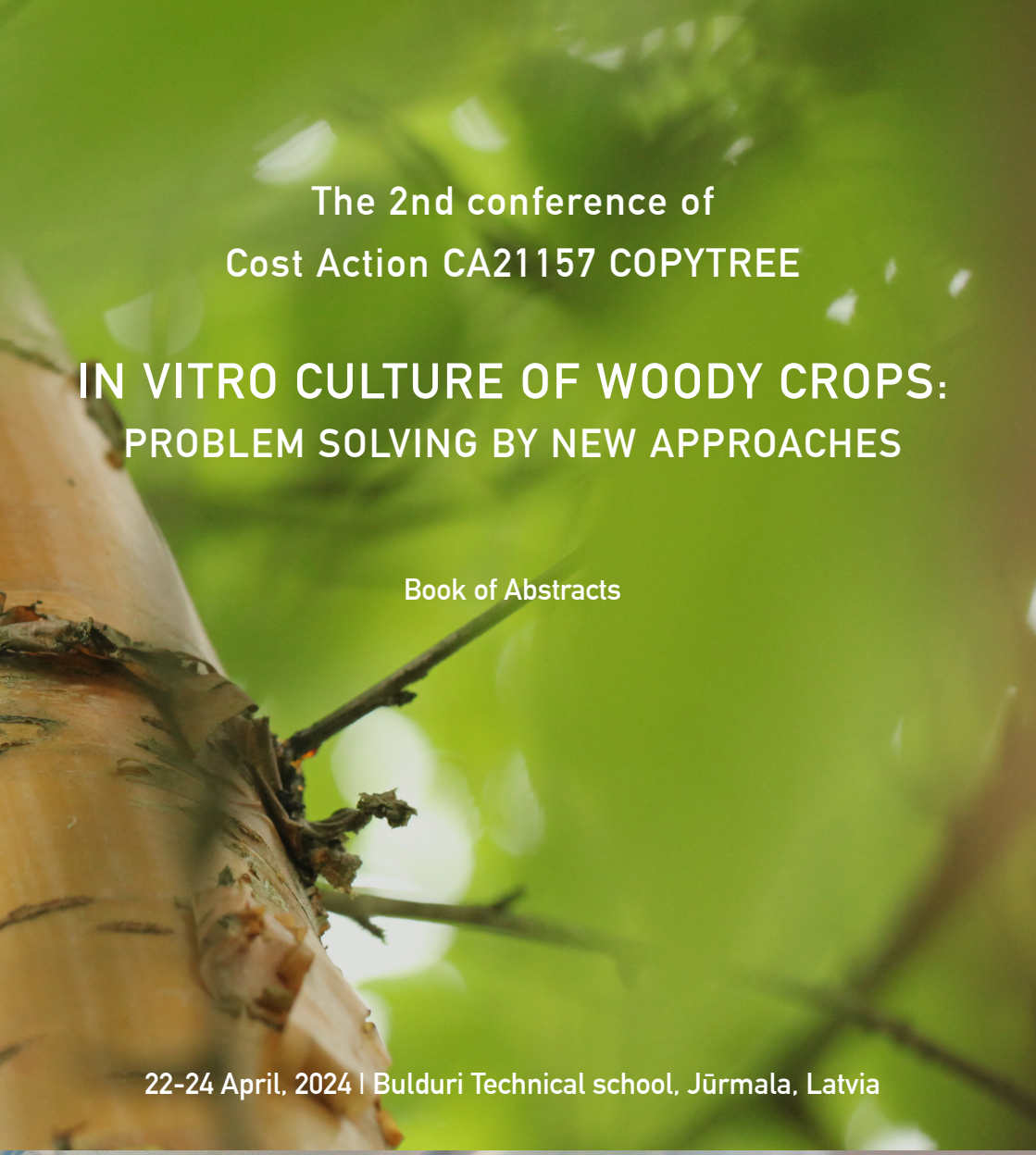

The 2nd conference of
Cost Action CA21157 COPYTREE

IN VITRO CULTURE OF WOODY CROPS:
PROBLEM SOLVING BY NEW APPROACHES

Book of Abstracts

22-24 April, 2024 | Bulduri Technical school, Jūrmala, Latvia

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

Bulduru Tehnikums

ISBN 978-9934-9252-2-1

Local Organising Comitee

[1,2]Līva Purmale, [2]Rafaels Joffe, [1,2]Anna Korica,
[1,2]Anta Sparinska, [1,2]Kārlis Shvirksts,
[1,2]Roberts Krūmiņš, [1,2]Daiga Birzleja, [1,2]Elga Ence
[1]Bulduri Biotechnology center, [2]Bulduri Technical school

Scientific Comitee

Stefaan Werbrouck
University of Ghent, Belgium

Maurizio Lambardi
National Research Council (CNR),
IBE-Institute of BioEconomy, Italy

Sandra Correia
InnovPlantProtect CoLAB, Portugal

Elif Aylin Ozudogru
İstinye University Istanbul, Turkey

Nieves Vidal
Misión Biológica de Galicia (MBG-CSIC), Spain

Neslihan Yeşim Yalçin Mendi
Cukurova University, Turkey

Valbona Sota
University of Tirana, Albania

Tuija Aronen
Natural Resources Institute Finland, Finland

Lucie Fischerová
Institute of Experimental Botany of CAS, Czech Republic

Acknowledgements

We gratefully appreciate the assistance and encouragement of the following persons, institutions and companies, who were always willing to help and support us:

Mafalda Quintas and Katchamon Nimprang
COST headquarters in Brussels

Labassociates
The Netherlands

Vita Morica, Dagnija Aditaja, Olga Veismane, Judite Emsina, Sarmite Vajeika, Edgars Logins, Aleksandrs Kozlovs
Bulduri Technical School

Semarah Hotel Lielupe

Kristine Talberga
Manager "Impro travel"

The staff and students of Bulduri Technical School

We are grateful to everyone who offered practical suggestions and helpful advice; you were instrumental in making this conference a success.

Welcome

Dear colleagues,

Imagine a world where we can easily save and replant threatened tree species *in vitro*, where virus-free orchards flourish and multi-clonal forests thrive sustainably. That's the potential of our work here at the COPYTREE conference, a major milestone in our European COST Action.

This Action addresses a fundamental challenge: how to meet the world's growing demand for trees and woody plants in a sustainable way. They are cornerstones of our ecosystems, providing timber, food, medicine and materials essential to our lives. Micropropagation is a powerful tool, but challenges remain.

That's why we're here. Our action unites us to solve these problems, from overcoming recalcitrant *in vitro* growth to providing clean supplies, streamlining production and building public trust. These efforts will have an impact not only on forestry, but also on global food security and the health of our planet.

This conference promises to be a dynamic exchange of ideas. We'll hear from leading researchers, industry experts and stakeholders. Poster sessions will stimulate discussion, and informal moments - coffee breaks, our conference dinner, the post-conference excursions - will provide vital space for networking and collaboration. We'll have the chance to forge relationships, arrange short-term scientific missions and plant the seeds for exciting new projects.

I'd like to thank Liva Purmale and her dedicated organizing team, as well as our invaluable core group. Their tireless work has made this event possible. A special thanks goes to Valbona Sota for maintaining our website and communication platform - a lifeline for our Action.

I'd also like to thank our Grant Holder Scientific Officer, Lucie Fischerova, for her financial expertise, and of course our COST Scientific Officer, Mafalda Quintas, and Administrative Officer, Katchamon Nimprang, for their unwavering support. This conference would not be possible without your contributions.

Most of all, thank you to each and every one of you. Your research, knowledge and passion will drive these next few days. Together, let's plant the seeds for a future where woody plants meet the needs of people and the planet, driven by innovation in micropropagation.

Let the discussions begin!

Stefaan Werbrouck
Chair COPYTREE

PROGRAMME

FIRST DAY - April 22, 2024	
08.30 – 09.15	Registration of participants and posters' installation
09.15 – 9.30	CONFERENCE OPENING - Welcome speech
WORKING GROUP 1 - RECALCITRANCE **Moderators: Sandra Correia, Itziar A. Montalbán**	
09.30 – 10.15	**Keynote speaker: Jaroslav Nisler** "Potential and mode of action of novel CKX inhibitors in *in vitro* cultures"
10.15 – 11.00	***Coffee break***
11.00 – 11.15	**Giovanni Broggini** "Strategies to understand and overcome recalcitrance to tissue culture in apple"
11.15 – 11.30	**Anne-Mareen Eisold** "The project "Wood of value" (Wertholz) – a forest tree breeding story"
11.30 – 11.45	**Hannes Wilms** "The impact of translocation mechanisms and plant architecture on the success or failure of propagation, a thidiazuron case study"
11.45 – 12.00	**Loredana Moffa** "Advancements in *in vitro* culture techniques and genetic transformation for grapevine improvement"
12.00– 12.45	**Posters' session**
12.45 – 14.15	***Lunch Break***
14.15-14.30	**Caroline Teyssier** "Does infrared spectrometry help in the study of somatic embryos maturation?"
14.30-14.45	**Mariana Neves** "The effect of ethylene modulation on *Solanum betaceum* Cav. *in vitro* regeneration: from meristem propagation to somatic embryogenesis"

14.45-15.00	**Paula Oros** "Improvement of *in vitro* regeneration in *Passiflora quadrangularis* - a recalcitrant species"
15.00-15.15	**Dhekra Abdouli** "Topolin cytokinins enhanced shoot proliferation, reduced hyperhidrocity and altered cytokinin metabolism in *Pistacia vera* L. seedling explants"
15.15-15.30	**Saila Varis** "Plant Growth regulators in somatic embryogenesis of Norway spruce and Scots pine - testing the effect of cytokinin oxidase inhibitors"
15.30 – 16.00	***Coffee break***
WORKING GROUP 5 - COMMUNICATION **Moderators: Valbona Sota; Branislav Cvjetkovic**	
16.00 – 16.15	**Bruce Christie** "Using micropropagated trees in a community-owned revegetation project on neglected land"
16.15– 16.30	**Andrea Rupps** "PInK-net: Resuming the network idea of *in vitro* tree labs in Germany"
16.30 – 16.45	**Monika Hoffer** "'Exploiting the untapped potential of fruit tree wild diversity for sustainable agriculture"
16.45 – 17.00	**Stéphane Maury** "Epigenetics as a regulator of tree specialized metabolites *in vitro* production"
17.00-17.15	**Valbona Sota** "Present and future perspective on knowledge-sharing and stakeholder engagement strategies on CopyTree"
17.15	**WG1 and WG2 meetings**

PROGRAMME

SECOND DAY - April 23, 2024	
WORKING GROUP 3 - AUTOMATION **Moderators: Nieves Vidal; Līva Purmale**	
09.00 – 09.45	**Keynote speaker: Ander Castander-Olarieta** "Somatic embryogenesis in pines: from mass propagation to stress response and adaptation"
09.45 – 10.00	**Xuan Xu** "Production of high-value molecules using apple cell suspension cultures"
10.00 – 10.15	**Lorenzo Burgos** "Temporary immersion system and addition of silver nanoparticles to eliminate pathogens in apricot *Prunus armeniaca* L."
10.15 – 11.00	***Coffee break***
11.00 – 11.15	**Brunilda Çuko** "Overcoming challenges for micropropagation of *Prunus domestica* cv. 'Tropojane' in various TIS bioreactor systems"
11.15 – 11.30	**Sladjana Jevremovic** „Recent advances in somatic embryogenesis induction in *Aesculus* species"
11.30 – 11.45	**Mariana Correia** "Morpho-physiological evaluation of *Solanum betaceum* Cav. *in vitro* cloned plants: a comparison of different micropropagation methods"
11.45 – 12.00	**Yildiz Aka Kacar** "Somaclonal variation in *in vitro* culture"
12.00 – 12.30	**Posters' session**
12.30 – 14.15	***Lunch break***

14.15-14.30	**Pierre Videau** "Vineyard evolution: advancing with embryogenic callus technology"
14.30-14.45	**Faheem Shehzad Baloch** "Genetics, genomics, and machine learning applications in the propagation of woody plants: case studies on Laurel and Peruvian Rosewood"
WORKING GROUP 4 - RISK ASSESSMENT **Moderators: Yesim Yalçin Mendi; Pilar S. Testillano**	
14.45 – 15.30	**Keynote speaker: Selim Çetiner** "The impact of regulatory oversight on the development and adoption of plant biotechnology"
15.30 – 16.00	***Coffee break***
16.15 – 16.30	**Madlen Walther** "Somatic embryogenesis in conifers: present state and application in Germany"
16.30 – 16.45	**Bora Onur Hallaç** "Micropropagation of *Phalaenopsis* using temporary immersion system"
16.45 – 17.00	**Buhara Yucesan** "Strategic innovations in *in vitro* woody crop production: balancing technical excellence with market and stakeholder dynamics"
17.00-17.15	**Branislav Cvjetkovic** "Addressing forest seed challenges: exploring somatic embryogenesis and tissue culture for tree propagation"
17.15 – 18.30	**WG3, WG4 and WG5 meetings**
19.30	***SOCIAL DINNER***

PROGRAMME

THIRD DAY - April 24, 2024	
WORKING GROUP 2 - SANITATION and CONSERVATION **Moderators: Elif Aylin Ozudogru; Claudia Ruta**	
09.00 – 09.45	**Keynote speaker: Bart Panis** "Cryotherapy, breeding instrument, commercial stock deposit; Cryopreservation is more than a long-term conservation tool for of plant genetic resources"
09.45 – 10.00	**Corina Catana** "3D plant imaging in woody tissue culture practice"
10.00 – 10.15	**Conchi Sánchez** "Chestnut gene soldiers against *Phytophthora cinnamomi* infection"
10.15 – 11.00	***Coffee break***
11.00 – 11.15	**Ine Dewitte** "Detection of plant viruses with high-throughput sequencing and *in vitro* elimination of detected viruses"
11.15 – 11.30	**Vera Pavese** "Susceptibility genes: the new frontier of improving plant tolerance to pathogen"
11.30 – 11.45	**Volodymyr I. Lushchak** "Enhancement of medicinal bioactive compounds of *Gynura procumbens* by silver nitrate and phytohormones: perspective in phytotherapy for diabetes and cancer"
11.45 – 12.00	**Tuija Aronen** "Dormant bud cryopreservation to supplement elm genetic resources conservation in Finland"
12.00 – 12.45	**Posters' session**
12.45 – 14.15	***Lunch break***

14.15-14.30	**Daniela Cordeiro** "CopyAlderTrees - propagation of selected genotypes tolerant to *Phytophthora*"
14.30-14.45	**Sandra Correia** "Micropropagation tools for the characterization and conservation of valuable agri-food germplasm: case studies from the CULTIVAR Project in Portugal"
14.45-15.00	**Doaa Elazab** "Micropropagation and *in vitro* conservation of *Zizyphus spina-christi* L. germplasm by using abscisic acid"
15.00 – 15.15	**Closing talk**
15.15-15.30	***Break***
15.30 – 17.30	**Management Committee meeting**

Oral presentations

WG 1 - RECALCITRANCE

WG 2 - SANITATION and CONSERVATION

WG 5 - COMMUNICATION

WG 3 - AUTOMATION

WG 4 - RISK ASSESSMENT

ORAL PRESENTATIONS

WG1

Potential and mode of action of novel CKX inhibitors in *in vitro* cultures

Jaroslav Nisler

Isotope Laboratory

Institute of Experimental Botany of the Czech Acad. Sci.

jaroslav.nisler@gmail.com

In the last few years, we have developed over 100 urea-derived compounds (JXB - Nisler et al., 2021; 2022, 2024 - submitted) which inhibit various isoforms of Cytokinin oxidase/dehydrogenase (CKX) from maize and *Arabidopsis*. The compounds also protected exogenously applied cytokinin iP in tissue cultures of some other plant species, which was used to improve shoot regeneration of e.g. tobacco, Canadian poplar and lobelia as well as to induce direct somatic embryogenesis in *Coffea arabica*. However, our further investigation of the biological activity of the key compounds suggests that they are not only inhibitors of CKX. For example, their application caused greater root branching and overall root growth of wheat and oilseed rape, which contradicts current knowledge about cytokinins' effect on root development. Our RNAseq and hormone content analysis further indicates that the key compounds can modulate auxin activity and jasmonic acid content, both species-dependent. This opens up new possibilities for testing of these urea derivatives in different plant species and for different types of applications.

Strategies to understand and overcome recalcitrance to tissue culture in apple

[1]Giovanni A. L. Broggini, [2]Célia Baroux and [1]Bruno Studer

[1]Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Universitaetstrasse 2, 8092 Zurich, Switzerland

[2]Institute of Plant and Microbial Biology, University of Zürich, Zollikerstrasse 107, 8008 Zurich, Switzerland

giovanni.broggini@usys.eth.ch

Keywords: apple, organogenesis, Malus domestica Borkh., new genomic techniques

The production of apples (*Malus domestica* Borkh.) has a large environmental footprint, and new genomic techniques were successfully applied to improve disease resistance of established cultivars by, e.g., cisgenesis or genome editing. However, these improved apple cultivars may contain stretches of plasmid-derived sequences and depending on national regulations, are regulated as genetically modified organisms. Achieving improvement of established apple cultivars through so-called DNA-free methods requires the transfection of individual apple cells, primarily protoplasts, with the requisite genome editing components, followed by plantlet regeneration. Despite several publications reporting such regeneration from apple protoplasts, these protocols could not be replicated efficiently in recent times. Thus, understanding recalcitrance and identifying molecular mechanisms that could overcome it is crucial for the adoption of new genomic techniques in apple. For this, we assessed shoot regeneration from leaf disks of approximately sixty diverse apple accessions genotyped with a 50K SNP array. This allows quantifying genotypic effects on organogenesis, performing initial steps towards association studies and identifying genetic determinants of recalcitrance. Further, we tested different media and approaches to successfully achieve plant regeneration from single apple cells. Overcoming apple recalcitrance will allow to apply state-of-the-art new genomic techniques in a more diverse set of apple cultivars, contributing to more sustainable apple production in the future.

ORAL PRESENTATIONS

WG1

The project "Wood of value" (Wertholz) – a forest tree breeding story

Anne-Mareen E. Eisold, Cornelia Bäucker, Volker Schneck

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

anne-mareen.eisold@thuenen.de

Wavy grain is a rare wood anatomical characteristic that increases the value of the wood tremendously. The phenomenon occurs in several species, such as *Acer pseudoplatanus* (wavy grain maple) or *Fraxinus excelsior* (wavy grain ash). The wavy effect is caused by undulation of wood fibres in the tree ring and visible as "washboard" structure when splitting wood. Detecting wavy grain on living trees is extremely difficult as the phenomenon is restricted only to the radial plane of wood and no external symptoms of wavy grain exist. Therefore, trees with wavy-grained wood are identified rarely and it is assumed that depending on the location only up to 7% of the trees show this specific wood anatomy. An earlier knowledge about quantity and availability of trees with wavy grain would be appreciated by forest owners and other players involved in timber production.

Here, our project aims to establish *in vitro* clones of wavy-grained trees in tissue culture in order to propagate it for commercial and scientific purpose. Therefore, wavy-grained material from several locations in Germany has been collected and integrated in the clone collection by tissue culture and grafting, respectively. Tissue culture techniques play a crucial role as tools for breeding purposes. The effectiveness of the establishment of *Acer* sp. clones in tissue culture depends on the kind of material as well as on the colonization status of the material by endophytes. Several tests have been conducted to find the optimal establishment conditions and optimized media were developed for long-term cultivation. Nevertheless, some clones suffered from losses of vitality and finally have been lost after years of stable *in vitro* cultivation. Moreover, the stability of the wavy grain characteristics after a period of *in vitro* cultivation will be proved by planting plantlets of 11 wavy grained maple clones in the field, followed by wood anatomical analyses. Additionally, the clonal material has been characterized genetically by the use of microsatellite markers for subsequent clone identification.

The project continues long-term investigations and reveals the importance of tissue culture methods in combination with progeny trials for forestry purpose. It is funded by the German Federal Ministry of Food and Agriculture.

The impact of translocation mechanisms and plant architecture on the success or failure of propagation, a thidiazuron case study

[1,2]Hannes Wilms, [2,3]Bart Panis

[1]Research Institute for Nature and Forest (INBO), Herman Teirlinckgebouw, Havenlaan 88 bus 73, 1000, Brussel, Belgium

[2]Department of biosystems, KU Leuven, Willem de croylaan 42, 3001, Leuven, Belgium

[3]The alliance of Bioversity international and CIAT (Belgian office), Willem de croylaan 42, 3001, Leuven, Belgium

hannes.wilms@inbo.be

Keywords: thidiazuron, coconut, meristem culture, translocation

While developing a propagation method for coconut (*Cocos nucifera*), we observed that the addition of thidiazuron (TDZ), a phenylurea based cytokinin, to the medium, did not elicit a reaction. We hypothesized that the apical dominance was too strong, preventing the TDZ from initiating shoot proliferation. To test this, we broke the apical dominance by cutting the plantlets vertically in two, removing the apical meristem from one piece. Both pieces were then placed in a medium containing TDZ. While this resulted in proliferation, more questions arose as it was the apical meristem that reacted. In other plants such as banana, this cut was not necessary, as there the axillary meristems on the outside of the explant proliferated. These observations lead us to hypothesize that TDZ might not be translocated through the plant the same way as adenine type cytokinins. To test this hypothesis, we placed a stem cutting of cactus fig containing many meristem along the stem, vertically in a medium containing BAP or TDZ. In the BAP medium this resulted in shoot formation of meristems on top of the plant, whilst in the case of the TDZ medium, the shoot formation originated from meristems submerged in the medium. With these observations, we can conclude that not all cytokinins are translocated in the same way. When developing proliferation protocols, both plant architecture and translocation of the PGR thus need to be taken into account, as these parameters can determine success or failure.

ORAL PRESENTATIONS

WG1

Advancements in *in vitro* culture techniques and genetic transformation for grapevine improvement

[1]Loredana Moffa, [1,2]Luca Nerva, [1,3]Ivan Bevilacqua, [1,3]Anna Narduzzo, [2]Irene Perrone, [2]Chiara Pagliarani, [1]Riccardo Velasco, [2]Giorgio Gambino, [1,2]Walter Chitarra

[1]Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (TV), Italy

[2]Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Strada delle Cacce 73, 10135 Torino, Italy

[3]University of Padua, Department of Agronomy, Food, Natural Resources, Animals and Environment, Agripolis, Viale dell'Università 16 - 35020 Legnaro (PD), Italy

Grapevine (*Vitis vinifera* L.) stands as a pivotal fruit crop with immense economic significance globally, contributing substantially to the agricultural sector. In-vitro culture methodologies have emerged as indispensable tools for the propagation, preservation, and genetic enhancement of grapevine, particularly through the induction and maintenance of embryogenic calli, which hold considerable promise for biotechnological applications, including NPBT.

Despite the potential of NPBT to augment grapevines by introducing desirable traits such as disease resistance, tolerance to environmental stress its present application is limited by technical and biological issues. These challenges encompass the considerable heterozygosity of the grapevine genome, resistance to transformation, the requisite presence of embryogenic calli specific for genotype of interest, and the intricacies associated with regenerating embryos post-transformation.

In our investigation, we developed a novel system incorporating Growth-Regulating Factors (GRFs) and their associated proteins, the GRF-Interacting Factors (GIFs), aimed to enhance the regenerative potential of genetically transformed calli, notably within the Glera genotype, which holds particular importance in Prosecco winemaking. To evaluate the efficacy of this system, many experiments were conducted on recalcitrant calli derived from the Glera genotype. Six months following genetic transformation, the regeneration efficiency was evaluated.

Does infrared spectrometry help in the study of somatic embryos maturation?

[1,2]Parisa Savane, [1]Nassim Belmokhtar, [1]Armelle Delile, [1]Nathalie Boizot, [1]Céline Ridel, [1]Marie-Anne Lelu-Walter, [1]Caroline Teyssier

[1]INRAE, ONF, BioForA, UMR 0588, F-45075 Orléans, France
[2]Curent affiliation: Amatera Biosciences, 4 rue Pierre Fontaine, F-91000 Evry, France
caroline.teyssier@inrae.fr

Keywords: infrared spectrometry, biochemical composition, Larix eurolepis, prediction, chemiometrics

Evaluating the physiological state of somatic embryos throughout their maturation is of great importance to determine their quality. This state is given by morphological parameters and their biochemical composition. The latter throughout the development of somatic embryos allows us to better understand their maturation and opens new perspectives for comparing embryogenesis protocols. However, biochemical analyses are time-consuming and material-intensive. Evaluating the relevance of using an alternative, rapid and economical method, which is infrared spectrometry, become therefore our challenge. We characterized hybrid larch somatic embryos throughout their maturation with determinations of their carbohydrate, lipid, protein, and water content (Savane et al., 2023). These results were then used to develop prediction models based on infrared spectrometry and chemometrics. These models were quantitative prediction of all these biochemical parameters, or qualitative models to evaluate the physiological state of somatic embryos. All these prediction models showed good prediction accuracies, between 65% and 89%.

In conclusion, infrared spectrometry combined with chemometric tools offers a new, fast and sample-saving method for monitoring the maturation of somatic embryos by accessing a set of information on their composition and physiological state contained in infrared spectra.

Savane P, Belmokhtar N, Delile C, Boizot N, Ridel C, Lelu-Walter M-A, Teyssier C (2023). Characterisation of hybrid larch somatic embryo maturation by biochemical analyses and by a novel, fast mid-infrared method. *Physiologia Plantarum*, DOI : 10.1111/ppl.13966.

ORAL PRESENTATIONS
WG1

The effect of ethylene modulation on *Solanum betaceum* Cav. *in vitro* regeneration: from meristem propagation to somatic embryogenesis

[1]Mariana Neves, [1,2]Sandra Correia and [1]Jorge Canhoto

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

[2]InnovPlantProtect CoLab, 7350-478 Elvas, Portugal

mariananevespt@gmail.com

Keywords: ACC, cytokinins, micropropagation, silver nitrate, woody species

Despite its importance in micropropagation, the effect of ethylene (ET) on the regeneration of woody species has not yet been thoroughly investigated. Here, we induced several regeneration processes in the presence of ET modulators such as $AgNO_3$ (action inhibitor) and ACC (metabolic precursor) in *Solanum betaceum*. We showed that ET is essential for de novo shoot organogenesis, with marked differences in the expression of regeneration-related genes, such as *STM, WUS,* and *PIN1*. In axillary shoot proliferation, the inhibition of ET action significantly reduced the explant response capacity (%). Exogenous CK application reverted the negative effect of $AgNO_3$ in regeneration response (%) but is insufficient to revert the negative impact in plant height and phytomer number. For the somatic embryogenesis (SE) process, ET is essential for embryogenic competence acquisition but impairs somatic embryo development. Hormonal and metabolic analysis revealed that, during the SE process, cell dedifferentiation requires an increase of ACC and CK. Simultaneously, somatic embryo development involves a decrease in ACC and maintenance of CK. This profile agrees with our observations, where ethylene is required for embryogenic callus induction, while its inhibition is essential for the differentiation of somatic embryos. Our study provides new insights into how ET influences tamarillo micropropagation, with possible applications in the overcoming of regeneration bottlenecks of other woody species.

Improvement of *in vitro* regeneration in *Passiflora quadrangularis* - a recalcitrant species

Paula Oros, Corina Catana

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

corina_catana@yahoo.com

Keywords: contamination, recalcitrance, organogenesis

The *Passiflora* genus includes species of great ornamental and therapeutic value. Contamination and recalcitrance have also been associated with *in vitro* culture of *Passifloraceae*. The present research aims to provide solutions to these difficulties. From nodal segments as explants, this study aimed to perform the first extensive *in vitro* regeneration protocol in *P. quadrangularis*. Out of a total of 15 treatments tested for explants asepsis, the most effective (61.67%) was T_{14}: the pretreatment of EtOH (70%, 1 min) + NaClO (50%, 10 min) followed by the treatment with the mixture Rifampicin (15 µg/ml) + Benomyl (2 g/l). In the preliminary initiation stage using Murashige & Skoog (MS) medium in 13 treatments, the most favorable morphogenic responses (33.33%) was on MS + 2 mg/l BAP. Adding $AgNO_3$ and Pluronic F-68 (PF-68) to the culture media reduced the impact of leached phenols and explant browning. Using 0.2% PF-68 on MS medium with 2 mg/l BAP, the regeneration rate is increased to 84.44%. From one shoot, a maximum of 7.17 shoots were obtained on the MS medium supplemented with 2 mg/l BAP and 1 mg/l TDZ in the sixth subculture. Indirect organogenesis is a crucial method for *in vitro* clonal propagation of *P. quadrangularis* because it avoids recalcitrance, ensuring a high yield (32.67%) of adventitious bud formation. At acclimatization, two substrates were tested. On the peat + perlite 1: 1 substrate, 73.33% of the plantlets survived.

ORAL PRESENTATIONS

WG1

Topolin cytokinins enhanced shoot proliferation, reduced hyperhydricity and altered cytokinin metabolism in *Pistacia vera* L. seedling explants

[1,2]Dhekra Abdouli, [3]Lenka Plačková, [3,4]Karel Doležal, [2]Taoufik Bettaieb, [1]Stefaan P.O. Werbrouck

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

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

[3]Laboratory of Growth Regulators, Palackỳ University and Institute of Experimental Botany, AS CR, Šlechtitelů 11, 783 71 Olomouc, Czech Republic

[4]Department of Chemical Biology, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic

abdouli.dhekra@gmail.com

Keywords: Pistachio, meta-topolin, Endogenous cytokinin, hyperhydricity

Pistacia vera is an important economic crop throughout the Mediterranean region. However, climate change poses a serious threat to the sustainability of pistachio cultivation. *In vitro* culture provides an important tool for clonal production of rootstocks, allowing the selection of those best adapted to the challenges posed by climate change. However, the success of *in vitro Pistacia vera* culture is limited by factors such as low proliferation rates and physiological disorders, including hyperhydricity and tissue necrosis. The effects of 10 µM *meta*-topolin (mT) and *meta*-topolin riboside (mTR) on the *in vitro* proliferation and abnormalities of *Pistacia vera* L. were evaluated and compared with those of 6-benzylaminopurine (BA). The highest proliferation rate (15.6) was recorded in the mT medium, with a value 6 times higher than in the BA medium. Moreover, the lowest percentage of hyperhydric usable shoots (58.9%) was found in mTR-treated shoots. Shoot tip and leaf necrosis were not influenced by cytokinin (CK) type. Endogenous CKs and their metabolites were determined in seedlings and, for the first time, the metabolism of exogenous BA, mT and mTR was studied in pistachio. Quantitative and qualitative analyses of CK metabolites provided some initial clues as to why topolin would be superior to BA in terms of proliferation rate and allowed a better understanding of the effect of exogenous administration of CK.

ORAL
PRESENTATIONS
WG1

Plant Growth regulators in somatic embryogenesis of Norway spruce and Scots pine- testing the effect of cytokinin oxidase inhibitors

[1]Saila Varis, [2]Stijn Maris, [1]Sakari Valimaki, [2]Stefaan P.O. Werbrouck, [1]Tuija Aronen

[1]Production Systems Unit, Natural Resource Institute Finland, Vipusenkuja 5, 57200 Savonlinna, Finland

[2]Department of Applied Bioscience, Faculty of Bioscience Engineering, Ghent University, 9000 Gent, Belgium

saila.varis@luke.fi

Cytokinin oxidase/dehydrogenase degradation is a key mechanism that regulates cytokinin homeostasis in plants. Recently, a new class of potent inhibitors targeting these enzymes has been developed. While cytokinin oxidase inhibitors have shown to be functional in somatic embryogenesis (SE) of coffee, their efficacy in conifer SE remains unexplored. Initiation of SE in Norway spruce using zygotic embryo (ZE) explants generally yields a high success rate, however, the genetic background significantly affects the outcome. In contrast, Scots pine faces greater challenges in SE initiation from ZE, with success rates generally falling below 10%. The tested cytokinin oxidase inhibitor, 2 micromolar JN4, was added to semi-solid mLM media. Traditionally, coniferous SE initiation rely on the cytokinin BA. In this study, we also investigated isoprenoid cytokinin 2iP, as it serves as a substrate for cytokinin oxidase/dehydrogenases. Open pollinated immature ZEs from three Scots pine and Norway spruce trees were used, as well as a controlled Norway spruce crossing. Additionally, mature ZEs from two families were used. In both species, no significant differences were found in SE initiation rates or the number of somatic embryos produced from established embryogenic cultures when BA or 2iP was used with or without JN4. However, in Scots pine, 2iP led to an elevated SE initiation success rate, increasing from 4.9% to 7.3%, while JN4 enhanced embryo production with both cytokinins. Conversely, in Norway spruce, substituting 2iP for BA resulted in a decreased initiation rate, dropping from 48% to 27%.

ORAL PRESENTATIONS

WG2

Cryotherapy, breeding instrument, commercial stock deposit; Cryopreservation is more than a long term conservation tool for of plant genetic resources

[1,2]Bart Panis

[1]Alliance of Bioversity International and CIAT, Willem de Croylaan 42 bus 2455, 3001 Leuven, Belgium

[2]Dept. Biosystems, KU Leuven, Willem de Croylaan 42 bus 2455, 3001 Leuven, Belgium

b.panis@cgiar.org

Cryopreservation is considered as safest long term conservation methodology for vegetatively propagated, sterile or recalcitrant seed producing crops and trees. Well known examples of cryopreserved collections are apple, bananas, potato, mulberry and garlic that have each more than 1000 accessions stored in liquid nitrogen. Currently, between 20.000 and 25.000 accessions of wide variety of species are safely preserved in liquid nitrogen and more initiatives to increase these numbers are in the pipeline.

Cryopreservation can, however, also be applied as a tool in classical as well as modern breeding initiatives. For example, for many adult trees (such as conifers) clonal propagation is only possible through somatic embryogenesis (starting from seeds). After breeding, it takes, however, decades before the value of a selection/cross of a tree can be determined. The storage of embryogenic cultures could meanwhile happen through cryopreservation. As an example as modern breeding tool, cryopreservation is applied to store thousand cryotubes containing transformation competent cell lines of banana.

For many crops such as banana, sweet potato, grapevine and potato cryopreservation is also used for pathogen eradication. This effect of cryotherapy is not based on killing pathogen such as viruses, phytoplasmas and bacteria by freezing but on the selection of the most meristematic part of the plant that has also the highest chance to be free of those pathogens.

Finally, cryopreservation can also be applied to store large amounts of independent in vitro cultures that are being produced and conserved by *in vitro* production companies.

3D plant imaging in woody tissue culture practice

Corina Catana, Paula Oros

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

Keywords: 3D plant imaging, woody plant tissue culture, computer tomography

Integrating 3D imaging into woody plant tissue culture studies can provide valuable insights into the dynamics and health of the tissues, contributing to the optimization and success of tissue culture processes. Using computer tomography (CT) for plant imaging is a viable and increasingly utilized technique. X-ray CT, also known as micro-CT when applied to small specimens like plants, enables non-destructive imaging of the internal structures in 3D. CT is applied to woody plant studies due to its non-destructive nature, high-resolution 3D images, allowing to explore the internal architecture of plants in great detail. It can be used for quantitative analysis, such as measuring volumes, porosity, water content. 3D plant imaging is capable of capturing dynamic processes in study changes in plant structures over time, as well. In the context of initiating *in vitro* cultures helps to understanding the internal structures allows researchers to pinpoint regions with high concentrations of meristematic cells. 3D imaging can help identify and select optimal explants and the precise isolation of explants, ensuring that the selected tissue is healthy, undamaged, and has the desired characteristics. Understanding the physiological state of internal tissues allows for the development of protocols that enhance the success rate of tissue culture initiation. Our group propose integrating CT imaging as new tool in woody plant tissue culture. Optimizing CT parameters for plant imaging require further experimentation and adaptation.

ORAL PRESENTATIONS

WG2

Chestnut gene soldiers against *Phytophthora cinnamomi* infection

[1]Saleta Rico, [1]Jesús Vielba, [2]Beatriz Cuenca, [1]Nieves Vidal, [1]Conchi Sánchez

[1]Department de of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, 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

conchi@mbg.csic.es

Keywords: ink dissease, in vitro inoculation, signalling pathways, transcriptomics

European chestnut (*Castanea sativa* Mill.) is a long-lived multipurpose tree cultivated worldwide for its nuts and timber. Among other threats, chestnut populations are affected by "ink disease" caused by *Phytophthora cinnamomi*, a fungus-like eukaryotic microorganism belonging to the oomycota class. This disease has significantly contributed to the drastic decline of chestnut distribution in Europe. Trees exhibit different levels of susceptibility to *P. cinnamomi* infection, indicating potential genetic control over tolerance. Although the mechanisms involved in this process remain unclear, the response to infection trigger a series of modulations of key genes to fight against the oomycete.

Next Generation Sequencing (NGS) allow us to investigate the plant response to biotic stresses through transcriptomic analysis. In this study we identified differential expressed genes in response to *P. cinnamomi* inoculation by comparing transcriptomic profiles of two *in vitro* chestnut clones with different resistance levels to this pathogen. CS12 is a pure *C.sativa* clone highly sensitive to infection while PO11 is a *Castanea* hybrid resistant clone. Rooted plantlets were inoculated *in vitro* with mycelial fragments of *P. cinnamomic*, and leaf samples were collected 48 hours after infection for RNA-seq analysis. Non-inoculated plants were used as control.

Signalling pathways and differentially expressed genes involved in the regulation of plant immune response and stress adaptation and recovery were identified. Understanding the genetic basis underlying major events in the plant response to *P. cinnamomi* may contribute to the development of novel strategies to control ink disease.

ORAL PRESENTATIONS

WG2

Detection of plant viruses with high-throughput sequencing and *in vitro* elimination of detected viruses

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

[1]Flanders Research Institute for Agricultural Fisheries and Food (ILVO), Plant Sciences Unit, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium

[2]Laboratory of Applied In Vitro Plant Biotechnology, Dept. Plant & Crops, Faculty of Bioscience Engineering, University Ghent, Belgium

ine.dewitte@ilvo.vlaanderen.be

Keywords: High-throughput sequencing, thermotherapy, yam

Starting with virus-free plant material is crucial to prevent yield losses and limit the spread of viruses, especially for companies involved in vegetative propagation. When a mother plant is infected, all clones produced via vegetative propagation will carry the virus as well. Currently, companies mainly rely on conventional virus diagnostic tools such as PCR or ELISA, or visual symptom assessment (e.g. by indexing on indicator plants). However, these techniques only detect a limited set of specific viruses and require prior knowledge. Comprehensive testing for an entire list of viruses is time-consuming and does not guarantee the absence of other viruses. These limitations underscore the need for more advanced approaches. In recent years, High-throughput sequencing (HTS), specifically total RNA sequencing, has shown its potential in virus discovery and diagnostics. HTS allows to assess the entire virome of plants in one run without prior information. Replacing targeted PCR or ELISA tests with RNAseq can significantly cut down both costs and time in specific cases. In practice, the HTS strategy involves extracting total RNA from samples, whether or not followed by ribosomal RNA depletion to enrich the proportion viral RNA in the sample. Subsequently, the RNA is pooled for sequencing, either by Illumina (short read) or nanopore (long read) sequencing. The results then undergo analysis using a bioinformatics pipeline, followed by virus detection and discovery using the VirusDetect pipeline. Post HTS, viruses can be eradicated using advanced *in vitro* techniques that combine thermotherapy, cryotherapy and chemotherapy with meristem culture. This HTS-based strategy has successfully been applied to several crops, including Yam (*Dioscorea* spp.), a vital staple food in many developing countries. Notably, nine distinct viruses were detected using HTS in the yam cultivars pool, including important yam viruses such as Yam virus y (YVY), Dioscorea mosaic associated virus (DMaV), Dioscorea bacilliform virus (DBV), Yam yellow spot mosaic virus (YYSMV) as well as sweet potato feathery mottle virus (SPFMV). To eliminate these viruses, the plants underwent a four-week treatment at 36±1°C, followed by the isolation of meristem tips. Three viruses, YVY, YMMV, and YCNV, were successfully eliminated in one yam cultivar, while SPFMV was successfully eliminated in the other cultivar.

ORAL PRESENTATIONS

WG2

Susceptibility genes: the new frontier of improving plant tolerance to pathogen

[1]Vera Pavese, [1]Andrea Moglia, [1]Lorenzo Antonio Marino, [1]Anna Maria Milani, [2]Elena Corredoira, [2]Mª Teresa Martínez, [1]Daniela Torello Marinoni, [1]Roberto Botta

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari—DISAFA, Università degli Studi di Torino, Largo Paolo Braccini 2, Grugliasco, 10095 Torino, Italia

[2]Misión Biológica de Galicia, Sede en Santiago, Consejo Superior de Investigaciones Científicas, Avd. Vigo, s/n, 15705 Santiago de Compostela, La Coruña, Spain

vera.pavese@unito.it

Castanea sativa, a multipurpose tree species, is affected by ink disease caused mainly by the oomycete *Phytophthora cinnamomi*. The loss-of-function of the susceptibility genes (S genes), required for plant/pathogen interaction can potentially increase plant tolerance to diseases. For this reason, in the present work, we evaluate the expression dynamics of four S genes in the Castanea genus, after pathogen infection. Results highlighted the upregulation of *pmr4* gene, during the infection. We tested the hypothesis if the knock-out of this gene might increase chestnut tolerance.

The CRISPR/Cas9 technology was applied, for the first time, in chestnut targeting the *phytoene desaturase* gene, delivered using *Agrobacterium tumefaciens* or as a ribonucleoprotein (RNP) form. The established CRISPR/Cas9 system was applied to target knock-out the *pmr4* gene. Somatic embryos from two lines (CI-3 and CI-9) were transformed with the EHA105 nptII_Cas9_pmr4_gRNA1_gRNA2 strain. After eight weeks, kan-resistant explants were observed only in the CI-9 line, and the best results were observed with 5 days of coculture (6.7%). Editing efficiency was evaluated through DNA extraction and Sanger target sequencing. All the lines showed an editing efficiency higher than 93% with an accuracy of R2 > 0.94. The tolerance assay was performed and after 5w from infection over 80% of the edited lines showed tolerance to *P. cinnamomi*, while 100% of the wild-type lines were necrotic. After two months, an average of 65% of the edited lines were still tolerant, without significant signs of necrosis. Trypan blue assay was performed highlighting the reduction of the pathogen penetration in the edited lines.

ORAL PRESENTATIONS
WG2

Enhancement of medicinal bioactive compounds of *Gynura procumbens* by silver nitrate and phytohormones: perspective in phytotherapy for diabetes and cancer

[1]Viktor V. Husak, [1]Olha A. Bulii, [1]Yurii O. Vakiv, [1,2]Volodymyr I. Lushchak

[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

Keywords: Gynura procumbens, polyphenols, flavonoids, diabetes, cancer, oxidative stress

Gynura procumbens is recognized for its traditional medicinal properties, particularly in the treatment of diabetes and cancer. Our research has focused on the modification of levels of bioactive compounds in this plant. The study aimed to increase the levels of polyphenols and flavonoids through the application of silver nitrate and phytohormones during their micropropagation. We observed a significant increase in flavonoid content at a concentration of 1 mg/L of silver nitrate. In addition, administration of 0.5 mg/L naphthalene acetic acid (NAA) increased not only flavonoid levels, but also polyphenolic compounds known for their antioxidant activities, which are critical in combating oxidative stress associated with diabetes and cancer. Remarkably, the callogenesis with 10 mg/L NAA further enhanced the levels of these bioactive compounds, suggesting a dose-dependent response that could be exploited for medicinal purposes. Our results suggest that the application of silver nitrate and NAA not only stimulates the growth and reproduction of *G. procumbens* but also significantly enriches its content of bioactive compounds. This enhancement in polyphenolic and flavonoid compounds could potentially amplify the plant's therapeutic efficacy. This research offers promising insights into the cultivation and enhancement of *G. procumbens* as a potent medicinal resource, highlighting its role in the future of phytotherapy for chronic diseases.

ORAL PRESENTATIONS

WG2

Dormant bud cryopreservation to supplement elm genetic resources conservation in Finland

[1]Sakari Välimäki, [2]Leena Yrjänä, [2]Mari Rusanen, [1]Tuija Aronen

[1]Natural Resource Institute Finland, Vipusenkuja 5, 57200 Savonlinna
[2]Natural Resources Institute Finland
tuija.aronen@luke.fi

Keywords: cryo-bank, ex situ conservation, slow cooling, tissue culture, organogenesis

Ulmus laevis (Pall.) and *Ulmus glabra* (Huds.) are endangered and protected species in Finland. Elms are globally threatened by the Dutch elm disease which is likely to spread also to Finland. The genetic diversity of elms in Finland is conserved in dynamic *ex situ* collections which have grafted material originating from the small and scattered Finnish elm populations. To prevent the loss of trees in conservation collections to Dutch elm disease or other hazards, cryopreservation and tissue culture methods were developed and tested for *U. laevis* and *U. glabra* dormant buds. The buds were collected in winter, frozen using a programmable freezer at the rate of –0.17 °C/min to –38 °C and plunged into liquid nitrogen. For *U. laevis* 50% and 63% of the cryopreserved dormant buds were recovered in two test thawings through tissue culture. However, for *U. glabra*, the proportion of successful tissue culture initiations was considerably lower, at 6% and 13%. This was both due to decreased bud viability after cryopreservation and higher contamination rate. However, dehydrating *U. glabra* buds from initial average 52% moisture to 32% improved the post-thaw viability and reduced the contamination rate so that 43% of the dehydrated buds were recovered instead of 11% of buds frozen without dehydration. Even though cryopreservation itself does not solve the of susceptibility of the material to Dutch elm disease, most genotypes can now be recovered from cryostorage if lost from the collections.

CopyAlderTrees - propagation of selected genotypes tolerant to *Phytophthora*

**[1]Daniela Cordeiro*, [1]Alberto Pizarro*,
[2]María Teresa Cervera, [1]Carmen Díaz-Sala**

[1]Department of Life Sciences, Faculty of Science, University of Alcalá, 28805 Alcalá de Henares, Madrid, Spain

[2]Department of Forest Ecology and Genetics, Institute of Forest Science (ICIFOR-INIA-CSIC), 28040 Madrid, Spain

*Both authors contributed equally to this work.

carmen.diazsala@uah.es

Keywords: conservation, forest disease, in vitro culture, natural resources, riparian forest ecosystems

Deforestation and increasing invasions of pests and pathogens, resulting from globalization of trade and free market policies, coupled with rapid climate change, represent the greatest challenges to sustainable forestry and the continued functioning of forest ecosystems. The preservation and conservation of riparian forest ecosystems depend largely on alders. However, in recent decades, Europe has witnessed a severe decline of alders, mainly caused by fungal attacks of *Phytophthora*. Therefore, this work focuses on the urgent demand to find methods for the conservation and sustainable use of natural genetic resources of alders. For this purpose, seven alder genotypes were selected from Spanish riparian forest ecosystems for their absence of symptoms to *Phytophthora*. A protocol for *in vitro* propagation of alder has been successfully used and has allowed the propagation of five of these seven genotypes in our laboratory. Improvement of the procedure using a double-phase culture system is being tested. The other two genotypes remain recalcitrant to *in vitro* culture, due to the difficulty in obtaining explants and the high contamination rate obtained when introduced directly from the field. Although with varying rates, all genotypes are also being propagated *ex vitro*, as well as their progeny, for further characterization. This work may support the monitoring and management of one of the most aggressive diseases of riparian ecosystems, focusing on ecological restoration.

ORAL PRESENTATIONS

WG2

Micropropagation tools for the characterization and conservation of valuable agri-food germplasm: case studies from the CULTIVAR Project in Portugal

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

[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 CoLab, Estrada de Gil Vaz, Elvas, Portugal

sandra.correia@iplantprotect.pt

The Centro Region in Portugal is particularly rich in agro-biodiversity, combining the culture of local varieties in interaction with modern horticulture and fruticulture. Unfortunately, the genetic diversity of cultivated crops in this region faces high risks of erosion and significant constraints due to extreme climatic events and the emergence of new diseases. In this context, one of the main objectives of the CULTIVAR Integrated Network (https://icultivar.pt) was the conservation of several endogenous genetic resources with agri-food relevance. For that, several traditional varieties were identified and selected, including several woody fruit crops (e.g. *Prunus* spp., *Cydonia oblonga*, *Citrus* spp.). For these resources, plant tissue culture approaches were optimized for the in vitro establishment and multiplication of the selected varieties. In parallel, phenotypic characterization was conducted, and, for some of the resources, gene expression analysis of the *in vitro* regeneration responses, or genetic variation evaluation using molecular markers (ISSRs) was carried out. The development of biotechnological tools for the *ex-situ* conservation of traditional varieties represents a critical tool for their protection. Also, the maintenance of such germplasm in controlled laboratory and/or nursery conditions allows for the evaluation of stress resilience and further selection of valuable germplasm for future breeding and propagation programs.

Micropropagation and *in vitro* conservation of *Zizyphus spina-christi* L. germplasm by using abscisic acid

[1,2]Doaa Elazab, [3]Claudia Ruta and [1]Maurizio Lambardi

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

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

[3]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A.), University of Bari Aldo Moro, 70125 Bari, Italy

doaa.elkassas@agr.au.edu.eg

Keywords: Zizyphus spina-christi, jujube, micropropagation, plant growth regulators, slow growth storage, abscisic acid

Jujube (*Zizyphus spina-christi* L., in Arabic "Sidr-indian") belongs to the buckthorn family (Rhamnaceae). *Zizyphus* species are important fruit trees for several countries, such as Egypt, India and Golf area, for the fleshy drupes which are rich in sugars and vitamins. Traditional tree improvement programs are time-consuming and difficult, and *in vitro* propagation methods provide an effective alternative. A micropropagation protocol for *Z. spina-christi* was optimized in this study by investigating the composition of the culture medium MS or Nitsch&Nitsch (NN), particularly as for the plant growth regulator component. 6-benzyladenine (BA) at 5.0 mg/l on MS medium was selected to induce a high proliferation rate for mass production. Culture conditions were: temperature 23+2°C, light intensity 40 µmol m-2 sec-1, photoperiod 16h. Subcultures were done every 6 weeks. As for slow growth storage, this is a technique that allows in vitro storage of shoots for period ranging from 6 months to 2 years depending on the species, after which shoots can be returned to standard culture conditions and micropropagated when desired. In this study, *in vitro* germplasm conservation of *Z. spina-christi* was conducted using abscissic acid (ABA) as Osmotically Active Compound (OAC) in different media (MS, ½MS and NN), and maintaining the above mentioned standard culture conditions. Different concentrations of ABA (0, 7 and 9 mg/l) were evaluated for 12 months, with observations on the quality of recovered shoots performed every 3 months. After 12 months of storage, ABA treatment on full-strength MS medium could maintain high survival percentages of shoots after the return to the standard culture medium. In particular, ABA induced 94% survival in recovery medium when was used at 7 mg/l in the MS storage medium, while it induced 53% survival when used at 9 mg/l, in comparison with the control (ABA-free medium) where recovery was only 45% in post-conservation. With reference to the quality of recovered plantlets, at 7 mg/l of ABA, height (cm), proliferation rate and fresh and dry weight (g) were the highest, with a significant difference with 9 mg/l of ABA and the control medium. The study showed the possibility of an effective long-term conservation of jujube shoots by just using ABA as OAC in the storage medium.

ORAL PRESENTATIONS

WG3

Somatic embryogenesis in pines: from mass propagation to stress response and adaptation

[1]Ander Castander-Olarieta, [1,2]Catia Pereira, [1]Mikel Hurtado, [1]Itziar A. Montalbán, [1]Paloma Moncaleán

[1]Department of Forestry Science, NEIKER-BRTA, 01192 Arkaute, Spain

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

pmoncalean@neiker.eus

Keywords: epigenetic memory, in vitro culture, multivarietal forestry, priming

Somatic embryogenesis offers a bunch of opportunities for pine species: micropropagation of elite individuals, cryopreservation of plant material, and implementation of multivarietal forestry, among others. In this sense, during the last decade our group has overcome several bottlenecks of the somatic embryogenesis process and has developed different strategies for its optimization and scale up. Nonetheless, somatic embryogenesis goes beyond mass propagation: this technique can be used as a powerful research tool, not only to study embryo development, but also to uncover the molecular mechanisms underlaying plant stress response and epigenetic memory acquisition. Work carried out in our laboratory has demonstrated that the modulation of the culture conditions (water availability and temperature) during the different stages of somatic embryogenesis provokes mid- and long-term effects on the success and efficiency of the process, together with alterations on the characteristics and performance of the resulting somatic embryos and plants. Research is being conducted using transcriptomic, proteomic, metabolic and physiological approaches to elucidate if these modifications can result in plants better adapted to stress conditions. Field monitoring of this work will be performed thanks to the deployment of the generated plants in plantations with different edaphoclimatic conditions along the Basque Country.

Funding: These results are part of the Project of I+D+i PID2020-112627RB-C32 funded by MCIN/ AEI/10.13039/501100011033/

Production of high-value molecules using apple cell suspension cultures

Xuan Xu, Salma Halime, Kjell Sergeant, Diego Rios Salgado, Samuel Jourdan, Jean-Francois Hausman, Jenny Renaut, Gea Guerriero, Sylvain Legay

Environmental Research and Innovation Department, Luxembourg Institute of Science and Technology, 5, rue Bommel, L-4940 Hautcharage, Luxembourg
xuan.xu@list.lu

Keywords: plant cell suspension cultures, bioreactor, metabolomics, transcriptomics

Higher plants synthesize a diverse range of bioactive molecules, offering enormous industrial potential. However, the commercialization of these molecules is largely hampered by the low production levels in plants and the infeasibility of chemical synthesis due to the complex chemical composition and structure. To this end, plant cell suspension culture serves as a viable alternative for the sustainable production of desired molecules. We hereby showcase a study in which cell suspension cultures of apple cultivar "Belle de Boskoop" were developed to produce high-value molecules, such as phloretin and phloridzin. The optimization of biomass production using bioreactor technology will be presented and discussed. The optimized process parameters were applied to cultivate the cell line in the pilot scale (300L bioreactor). Metabolomic profiling was performed on cell suspension cultures growing under different growth conditions. Transcriptomic analysis was conducted to understand why pH regulation is indispensable for the growth of Boskoop cells.

ORAL PRESENTATIONS

WG3

Temporary Immersion System and addition of silver nanoparticles to eliminate pathogens in apricot (*Prunus armeniaca* L.)

Cristian Pérez-Caselles, Marina Martín-Valmaseda, Javier Alfosea, Nuria Alburquerque, Lorenzo Burgos

Fruit Biotechnology Group. Department of Plant Breeding, CEBAS-CSIC, Campus Universitario de Espinardo, Edificio Nº25, 30100 Murcia, Spain

burgos@cebas.csic.es

Temporary Immersion Systems (TIS) are necessary to micropropagate apricot in liquid medium and paramount to apply silver nanoparticles (AgNPs) since their availability is restricted in semisolid medium. AgNPs have been described as antimicrobial agents and, in our laboratory, we have tested their effect on micropropaged shoots of sharka-infected 'Canino' and Hop Stunt Viroid-infected 'Mirlo Rojo'. The aim of this work is to produce PPV- and/or HSVd-free apricot plants under in vitro conditions.

AgNPs have been added to the liquid shoot multiplication medium in TIS at different concentrations (0, 25, 50, 75, and 100 mg/L). After eight weeks of culture with the AgNPs, meristems were isolated and cultured on appropriate media for meristem multiplication. The viability of the meristems was evaluated and the presence/absence of the pathogens was analyzed by RT-PCR in the established shoots.

In both cultivars the highest viability of isolated meristems was observed at 0 mg/L AgNPs (83% approximately) and this percentage significantly decreased when meristems were isolated from shoots treated with AgNPs, although not significant differences were found between AgNPs treatments. Regarding the elimination of PPV, free-virus 'Canino' shoots were produced in all treatments. Maximum virus elimination rates (75%, percentage of clean plants regarding the number of shoots established from surviving meristems) were achieved with the application of 75 mg/L of AgNPs in TIS. However, HSVd-free 'Mirlo Rojo' plants were not obtained from any of the treatments.

Overcoming challenges for micropropagation of *Prunus domestica* cv. Tropojane in various TIS bioreactor systems

[1]Brunilda Çuko, [1]Ledina Shkëmbi, [1,2]Valbona Sota, [2]Efigjeni Kongjika

[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

cuko.brunilda@gmail.com

Keywords: plum, immersion frequency, hyperhydricity, shoot tips necrosis

Prunus domestica cv. Tropojane is an important autochthonous fruit crop in Albania, and it is widely used for its well-known values in the food industry. Beyond the traditional propagation methods, efficient methodologies and protocols have recently been established for the in vitro micropropagation of this fruit species using conventional micropropagation methods. However, the new approaches in the field, such as temporary immersion systems, bring new perspectives to the large-scale micropropagation of *P. domestica*. This study presents some strategies and methodologies for overcoming the challenges faced during the micropropagation of *P. domestica* cv. Tropojane in the Plantform and SETIS bioreactors. Besides testing different parameters related to physical - chemical growth conditions and immersion and ventilation frequency, some cultural practices to decrease contamination rate were optimized. Plum shoots grown in a jellified nutrient medium were isolated at 1-1.5 cm in length, and placed in the specific containers of bioreactors. Previously, the liquid nutrient medium was placed in the respective containers and sterilized inside the bioreactor to avoid culture contamination by reducing the manipulation time in the laminar flow. Different basal media such as MS, WPM, and DKW were tested in each bioreactor. Optimizing the Ca^{2+} concentration in the liquid nutrient medium, and the immersion and ventilation frequencies are strategies used to decrease the hyperhydricity and shoot tip necrosis. Optimization needs to be done regarding the type and concentrations of cytokinines in the nutrient media to achieve a high regeneration rate of lateral shoots, with reduced symptoms of oxidative stress and plantlets necrosis.

ORAL PRESENTATIONS
WG3

Recent advances in somatic embryogenesis induction in *Aesculus* species

Snezana Zdravkovic-Korac, Dusica Calic, Jelena Milojevic, Maja Belic, Sladjana Jevremovic

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

Keywords: aescin, embryogenic tissue, liquid culture, somatic embryos, tissue culture

Aesculus species are valuable ornamental plants, while their seeds are used as a source of health-promoting aescin. The somatic embryos (SE) of *A. hippocastanum* (horse chestnut) contain aescin in similar amounts as the seeds. Different pathways of propagation by tissue culture for several *Aesculus* species (*A.hippocastanum, A. flava* and *A. carnea*) have been investigated for few decades in our laboratory. Recently, an efficient protocol for somatic embryogenesis induction in stamen filaments culture has been developed. However, the frequency of embryogenic tissue (ET) induction varied markedly (0–50%) for the same genotypes from year to year. In *A. flava*, a significant increase (80–100%) in the frequency of ET induction was achieved by using liquid culture of friable calli. After the establishment of stable liquid cultures, lines with high ET proliferation and SE development were selected. Significant fresh weight increase (76–167-fold), with 256–669 newly developed SEs per 100 mg of initial inoculum, were achieved four weeks after plating on solid medium. In addition, most SEs (19–39%) reached the late torpedo and cotyledonary stage of development. SEs have high germination rate (80%), but conversion rates still need to be improved to enable sustained clonal propagation of elite specimens. The established protocols for ET induction and SE production represent a clean and safe technology for the *in vitro* production of *Aesculus* species, including alternative sources of aescin.

Morpho-physiological evaluation of *Solanum betaceum* Cav. *in vitro* cloned plants: a comparison of different micropropagation methods

[1]Mariana Correia, [1]Tércia Lopes, [1]Ana Patrícia Puga, [2]Glória Pinto, [1]Jorge Canhoto, [1,3]Sandra Correia

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

[2]Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, Portugal

[3]InnovPlantProtect CoLab, Estrada de Gil Vaz, Elvas, Portugal

mjcorreia324@gmail.com

Keywords: axillary shoot proliferation, organogenesis, plant physiology, somatic embryogenesis, tamarillo

Tamarillo (*Solanum betaceum* Cav.) is a subtropical solanaceous tree, with increasing agronomic interest due to its nutritious edible fruits. Growing demand for tamarillo plants and fruits requires an optimization of existing propagation methods and scaled-up systems that allow large-scale cloning of selected germplasm. This report offers insight in the three most prominent propagation *in vitro* techniques and optimizations of the protocols already established to bypass some of the challenges these techniques display. Axillary shoot proliferation in semisolid medium organogenesis and somatic embryogenesis procedures were evaluated, and culture media optimized. Variables such as the age of the established shoot cultures, and rooting treatments were also analyzed. Morphological and physiological quality of acclimatized plants derived from all the methodologies were compared, having seed-derived plants as a control group. Overall, the results obtained show that *in vitro* derived plants have a similar development when compared with seed-derived plants. Micropropagation by axillary shoot proliferation was highly efficient, with rooting rates above 80% in most treatments. Organogenesis and somatic embryogenesis-derived plants were also morphologically and physiologically equivalent to seed- and axillary shoot-derived plants. The use of each specific micropropagation method is further discussed.

ORAL PRESENTATIONS
WG3

Somaclonal variation in *in vitro* culture

Yildiz Aka Kacar

Department of Horticulture, Faculty of Agriculture, Cukurova University, 01330 Adana, Türkiye
ykacar@cu.edu.tr

Keywords: genetic stability, somaclonal variations, DNA markers

Somaclonal variation is defined as genetic or epigenetic changes that occur between plants obtained from *in vitro* conditions and the donor plant. These changes are represent a combination of morphological, cytological, biochemical, and genetic/epigenetic changes or abnormalities in DNA. Somaclonal variation in plant tissue culture has a negative impact, especially on the mass production of clonal plants coming by classical tissue culture technique and/or temporary immersion systems can cause major problems.

Many factors such as genotype and ploidy level, plant growth regulator balance, source of explants, culture duration, macro and micro elements used in *in vitro* culture, and physiological stress induce somaclonal variation under *in vitro* conditions.

Somaclonal variation can be assessed by analysis of phenotype, chromosome number and structure, proteins, or direct DNA evaluation of plants. Molecular markers are one of the most effective methods to reveal somaclonal variations occurring in tissue culture. Molecular marker systems have investigated genetic stability or somaclonal variation of plants after micropropagation, long term preservation or cryopreservation under *in vitro* conditions, and also of plants coming from long-term culture conditions or following a large number of subcultures.

This review addresses somaclonal variation encountered in the *in vitro* propagation of woody plants. Possible reasons for formation of the somaclones, their disadvantages, methods for their identification and solutions to the problem by new approaches are presented comparatively for different woody plant species by presenting examples from the studies we have done for many years.

Vineyard evolution: advancing with embryogenic callus technology

[1]Pierre Videau, [1]Katerina Labonova, [1]Emma Coulonnier, [1]Camille Mietton, [2]Olivier Zekri

[1]Research & Development department, Novatech lab, Le Gué de Velluire, France
[2]Selection department, Mercier Frères S.A.R.L, Vix, France
olivier.zekri@mercier-groupe.com; pierre.videau@mercier-groupe.com

Keywords: resistance, somatic embryogenesis, biotechnology, vine

Since some years, the French wine sector has encountered significant strategic challenges primarily attributable to climate change. Increasing disease, early frosts, hailstorms, drought, and shifting grape ripening patterns have led to reduced productivity and yields. Mercier, a prominent viticultural nursery located in Vendée, France, specializes in vine plant production, in collaboration with his laboratory, Novatech, want to answer the growing demand about resistant varieties. The "NEXT" program consists of introducing new resistance traits via genetic engineering to decrease the impacts of biotic and abiotic stresses. The aim of the project is to identify target genes associated with many stresses and integrate them into elite cultivated varieties. Novatech has initiated large-scale production of transformable material from wine grape varieties, specifically embryogenic calluses. From these calluses to the final transformed plants, Novatech is dedicated to overcoming technical barriers and refining protocols to obtain rapidly edited plants. Collaborations with research teams worldwide facilitate the incorporation of known genes, enhancing the efficiency of incorporating multiple resistance traits. Through these concerted efforts, Mercier and Novatech are poised to revolutionize the wine industry by introducing resilient grapevine varieties adapted to the challenges posed by a changing climate.

ORAL PRESENTATIONS

WG3

Genetics, genomics, and machine learning applications in the propagation of woody plants: case studies on laurel and Peruvian Rosewood

[1]Faheem Shehzad Baloch, [2]Durmuş Alpaslan Kaya, [3]Gülşah Karataş, [2]Nafiz Çeliktaş, [4]Muhammad Asim, [4]Muhmmad Azhar Nadee

[1]Department of Biotechnology, Faculty of Science, Mersin University, Mersin, Türkiye

[2]Department of field crops, Faculty of Agriculture, Hatay Mustafa Kemal University, Hatay, Türkiye

[3]Department of Food engineering, Faculty of Architecture and Engineering, Tokat Gaziosmanpaşa University, Tokat, Türkiye

[4]Faculty of Agricultural sciences and Technology, Sivas University of science and Technology, Sivas, Türkiye

balochfaheem13@gmail.com

Keywords: Artificial Neural Network, genetic algorithm, laurel, Peruvian Rosewood, micropropagation, genomics

Turkey is the world's largest producer of laurel, contributing to over 90% of the global export market with a production of 21.788 tons of leaves, while Rosewood (*Aniba rosaeodora*), is an endangered species in the Amazon forests, facing depletion due to over-exploitation for the cosmetic industry. The main objective of this study is to explore genomic regions affecting agronomic, antioxidant, and phenolic properties of the laurel gene pool from Turkey and rosewood from Peru. It involves DArTseq, ISSR markers, and retrotransposon analysis to identify optimal genotypes for commercial propagation. More than 50.000 SNPs were identified to estimate the genetic diversity and population structure of laurel and Peruvian germplasm. Structure, PCoA, and UPGMA clustered the germplasm based on their collection provinces and regions. GWAS analysis led to the identification of molecular markers linked to leaf traits and essential oil amount in laurel. These findings will facilitate marker-assisted selection in laurel breeding and contribute to genomic studies. Based on GWAS and molecular characterization two genotypes of both species will be selected to develop an effective protocol for *in vitro* propagation. Optimization of input factors will be done by response surface regression analysis. Results will be validated and predicted using artificial neural network and machine learning models. *In vitro* propagated plantlets will be employed for commercial propagation, and a laurel orchard will be established in Hatay (Turkey). This comprehensive genomic study is the first of its kind for these medicinal woody plants and will serve as a reference for other plants of interest.

The impact of regulatory oversight on the development and adoption of plant biotechnology

Selim Cetiner

Sabancı University, 34956 Istanbul, Turkey
cetiner@sabanciuniv.edu

Keywords: genome editing, New Breeding Techniques, biosfety, public perception

It is a fact that molecular techniques are providing important possibilities in boosting the agricultural production. However, the perceived negative effects on human health and environment of insect resistant and herbicide tolerant plant varieties, developed during the last 30 years through modern biotechnology methods or genetic engineering techniques, are the subject of heated debates. Recently, genome editing or New Breeding Techniques are facing the same problem, and possibilities offered by these new technologies are being questioned from many different standpoints.

Especially in the EU and in many of the developing countries, possible adverse affects of transgenic crops on human health and environment are quite controversial issues. It cannot be denied that these arguments are based on ideological, sentimental, personal and economic choices rather than hard scientific facts.

Despite these negative public perceptions, several national, regional and international regulations are in place to address these issues. However, it is still not possible to say that a complete consensus is reached in the international community. For instance, the biosafety legislation of the USA is quite different from that of the EU, where even the existing regulations are still not interpreted and implemented in a harmonious way by the member states.

The presentation will address the prospects and constraints of plant biotechnology from the perspective of regulatory oversight developed by national, regional and international institutions.

ORAL PRESENTATIONS

WG4

Somatic embryogenesis in conifers: present state and application in Germany

Madlen Walther, Juliane Raschke, Jana Seifert, Andrea Rupps

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

madlen_walther@web.de

Keywords: somatic embryogenesis in conifers, genotype collection, transfer into economy, approval of somatic plants

In Germany, we are currently aiming to transfer our expertise and solely academic knowledge on somatic embryogenesis in conifers to colleagues from nurseries and forestry. We approach this task with two different projects:

1. "OPAL"
This project is based on our genetically diverse clone bank of cryopreserved hybrid larch and fir genotypes and aims to turn critical points of the biotechnological process chain into applicable methods, like multiplication of the somatic embryos in bioreactors, a stable embryo maturation and a cost-benefit efficient conversion. Based on this, a highly productive and partially automated process for the cultivation of in vitro propagated plant material is to be established and integrated to form routine processes in tree nurseries thus completing the transition from research to applied mass production.

2. "LarchForFlexibility"
This project aims to expand our hybrid larch clone collection with genotypes of above-average economic characteristics, increased vitality and drought tolerance. In addition, specific, but lengthy clone tests on the field are necessary for the approval under forest plantation laws. In this context, we are defining molecular markers in order to provide a reliable tool for the required genotype identification. At this point, it is of economic interest to prepare the market for the introduction of the adapted strategy and the advantageous propagation material and to make it accessible through public relations work.

Micropropagation of *Phalaenopsis* using temporary immersion system

[1]Bora Onur Hallaç, [2]Yeşim Yalçın Mendi, [3]Soner Yağ

[1]FSB Biotech Company, Adana, Türkiye

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

[3]FSB Biotech Company, Adana, Türkiye

bora@fsbbiotech.com

Keywords: Phalaenopsis, micropropagation, ornamental

Phalaenopsis orchids are the most popular among indoor potted and flowering ornamental plants. While the annual consumption amount in Turkey is 6,000,000 units, this figure reaches hundreds of millions of units in the world. Although there are serious processes at every stage of orchid production, the healthy supply of seedling material is of great importance. *Phalaenopsis* seeds, although quite small, require artificial medium for germination since they do not contain endosperm. The production of this plant, whose vegetative propagation in nature is not commercially efficient, is done using tissue culture techniques. In generative production methods, since the plants do not have clone characteristics, production is achieved vegetatively, not by seeds, but by using various growth regulators in artificial media of structures taken from various organs of the plant. Direct organogenesis and somatic embryogenesis methods are used in commercial production. In the project, somatic embryogenesis was used as the method. The flower stems of the plants were sterilized with chemicals such as sodium hypochlorite (0.5% - 1.5%) and mercuric chloride (0.05% - 0.1%) and then transferred to the *in-vitro* environment. After the first shoots were obtained, PLB was obtained by applying thidiazuron at concentrations between (1mg - 4mg). Media such as Vacin&Went, Hyponex, ½ MS were used in the preparation of these media. The resulting PLBs were replicated in the next step. Replicated PLB structures; They were transferred to media containing activated carbon and grown in clusters. Plants that reached a certain maturity were sorted and transferred to rooting media. Rooted plants were removed from the medium and transferred to quick-plug growing media in order to adapt to external conditions. Here, the temperature was gradually increased to 28 °C and the light intensity to 5000 lux. Humidity was gradually reduced to 85%. Protective practices have been carried out against bacteria, fungi, insects and mites. The resulting plants were defined as seedlings ready to be potted.

Semi-solid culture methods are generally used throughout the world in the commercial production of *Phalaenopsis* orchids. Both semi-solid culture and bioreactors with temporary immersion systems were used in the project. The bioreactors used were designed by FSB Biotechnology. Comparisons made at all stages showed that bioreactors have superior qualities compared to semi-solid culture media in parameters such as tillering rate, growth rate and plant quality.

ORAL PRESENTATIONS

WG4

Strategic innovations in *in vitro* woody crop production: balancing technical excellence with market and stakeholder dynamics

[1,2]Buhara Yücesan, [2]Ahmet Tigrel

[1]Dept. Of Seed Science and Technology, Faculty of Agriculture, Bolu Abant Izzet Baysal University, Bolu, Türkiye

[2]Xplant R&D Co., Dudullu OSB, DES:113-27, Ümraniye, Istanbul, Türkiye

Keywords: in vitro regeneration, stakeholder engament, market dynamics, sustainable agriculture

Plant factories, leveraging vertical farming and plant tissue culture, offer significant advantages over traditional and semi-open greenhouse systems for large-scale crop production. This study examines the critical factors for successful *in vitro* crop production, emphasizing the balance between internal technical requirements and external market and stakeholder influences. Internally, the focus is on academic research, protocol development, and the application of innovative methodologies to optimize culture media and enhance plant regeneration processes. Externally, we address the realities faced by stakeholders and investors, including labor costs, energy consumption, market volatility, order cancellations, and logistics. Particularly, we explore the strategy for *in vitro* regeneration of woody crops, considering short-term production of rootstocks and long-term strategies for green foliage crops. The selection of starting materials is guided by market demand, highlighting the importance of strategic planning in tissue culture systems. This research presents a comprehensive risk assessment of plant cloning, aiming to stimulate technological awareness and acceptance among stakeholders. By analyzing labor efficiency and its correlation with regeneration rates, we propose a model for sustainable, cost-effective crop production. With the global horticulture market becoming increasingly competitive, particularly in Asia where costs are driven down, this study underscores the need for innovative approaches to maintain profitability in woody crop production. By aligning tissue culture practices with market demands and stakeholder expectations, we aim to contribute to the advancement of commercial *in vitro* crop production.

Addressing forest seed challenges: exploring somatic embryogenesis and tissue culture for tree propagation

ORAL PRESENTATIONS

WG4

Branislav Cvjetković

Faculty of Forestry, University of Banja Luka, Blvd Petra Bojovića 1A 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina

branislav.cvjetkovic@sf.unibl.org

Keywords: new forests, tissue culture, somatic embryogenesis

Forest ecosystems face numerous challenges, including habitat degradation, climate change, and invasive species, which pose significant threats to tree populations worldwide. One critical aspect of forest conservation, new forest establishment and restoration efforts lies in effective tree propagation methods to gain high productivity and maintain genetic diversity and enhance population resilience. Traditional seed-based propagation methods encounter various limitations, which is more pronounced in last decade and it including omitting of mast years, low germination rates, seed dormancy, susceptibility to predation and diseases, and the potential for genetic drift.

Somatic embryogenesis (SE) and tissue culture techniques offer promising alternatives for forest tree propagation. It makes possible to bypass many of the challenges associated with conventional seed-based methods propagation. SE involves the induction of embryogenic tissues from somatic cells, leading to the development of somatic embryos capable of germination and plantlet regeneration. Tissue culture, including techniques such as micropropagation, allows for the rapid multiplication of elite genotypes and the production of disease-free planting material.

This presentation explores the potential of SE and tissue culture as methods for forest tree propagation, highlighting their advantages, challenges, and applications in the context of new forests and forests planation establishing, conservation and restoration. It is stressed that the importance of using superior genetic material through clonal multiplication in tissue-cultured plants, as well as strategies for scaling up production and integrating these techniques into forest seed and nursery practices. Using the capabilities of SE and tissue culture, nurseries, forest managers and conservationists can enhance the efficiency and effectiveness of tree propagation efforts, contributing to the sustainable establishment, management and restoration of forest ecosystems and forest plantations in the face of global environmental challenges.

ORAL PRESENTATIONS

WG5

Using micropropagated trees in a community-owned revegetation project on neglected land

[1]Carlos Sobrino, [2]Lucía Saborido, [3]David García, [1]Conchi Sánchez, [1]Anxela Aldrey, [1]Puri Covelo, [1]Mª José Cernadas, [4]Bruce Christie, [1]Nieves Vidal

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

[2]Community forest owners of Araño, Rianxo, A Coruña, Spain

[3]Dept. Pedagogy and Didactics, Facultade de Ciencias da Educación de la Universidad de Santiago de Compostela, Rúa Prof. Vicente Fráiz Andón, s/n, Santiago de Compostela, A Coruña, Spain

[4]The Greenplant Company, Palmerston North 4410, New Zealand

brucechristie101@gmail.com

nieves@mbg.csic.es

Keywords: biodiversity, community building, forest restoration, networking, plant survival

In vitro culture is a tool for *ex situ* biodiversity conservation enabling restoration of degraded land. This study used native trees cultured *in vitro* for replanting fire damaged riparian forest in Galicia, where invasive species like *Acacia melanoxylon* and *Eucalyptus globulus* were establishing on neglected land. Plant material was grown from the germplasm collection at the Institute or seeds collected from the proposed revegetation area.

All activities for the project were carried out with the support of local community, local government agencies, NGOs and researchers collaborating on the project. Tree planting occurred in April 2022 and March 2023 using micropropagated plants of *Quercus robur, Q. suber, Betula pubescens, Salix viminalis, Pyrus cordata, Corylus avellana, Prunus avium* and *P. domestica* plus some *Quercus robur* seedlings. All propagation material was collected from the area for replanting.

Results indicate that with adequate instruction and supervision previously inexperienced school children and adults could successfully plant micropropagated material in an area that did not receive the post planting care expected in experimental or plant production plots. Technical considerations arising from the project included at a bureaucratic level factoring in time to get the site cleared of invasive plants and project approval. Technical lessons learnt included the need for quality plant labels, plus for weeding and irrigation in the first summer after planting.

PInK-net: Resuming the network idea of *in vitro* tree labs in Germany

[1]Andrea Rupps, [1]Juliane Raschke, [1]Madlen Walther, [1]Emma Ehmke, [2]Lena Safranek, [2]Julia Eckard, [2]Antje Schmidt, [2]Winston Beck, [3]Ben Bubner, [3]Anne-Mareen Eisold, [3]Franka Thiessen

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

[2]Dept. Urban Plant Ecophysiology, Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt-Universität zu Berlin, Lentzeallee 55/57, 14195 Berlin, Germany

[3]Institute of Forest Genetics, Johann Heinrich von Thünen Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, Eberswalder Chaussee 3a, 15377 Waldsieversdorf, Germany

andrea.rupps@hu-berlin.de

Keywords: PInK-net, national scientific association, vegetative propagation of conifers and deciduous trees, in vitro techniques

Research groups with focus on *in vitro* (iv) culture and clonal tree propagation are scarce in Germany. The only research/economy association ADIVK (Association for German iv cultures) dissolved in 2023. That's why a group of at first three institutes from Berlin/Brandenburg (Institute of Biology and Thaer Institute, Humboldt-Universität zu Berlin; Institute of Forest Genetics, Johann Heinrich von Thünen Institute, Waldsieversdorf) saw the need to set up a new national cooperation combining knowledge, expertise and researchers focused on iv culture of woody plants: the PInK-net (plant iv culture network). Beyond continuous material and methodological exchange, our aims are mutual support in scientific and administrative procedures, submission of common project proposals and troubleshooting at specific iv culture steps. These comprise different multiplication techniques, like somatic embryogenesis as well as micro cuttings for various conifers and deciduous trees, the establishment of iv cultures using different explants, handling of subsequent culture and storage. We are sharing details about the application of stress tests, analysis of pathogen infestation and molecular processes. Another common objective is the involvement and information of the public and further stakeholder groups at an early entry point. By this, we hope that clonal reproduction will become socially accepted and economically affordable for nurseries and forestry in our country in the foreseeable future.

ORAL PRESENTATIONS

WG5

Epigenetics as a regulator of tree specialized metabolites *in vitro* production

Stéphane Maury

Physiology, Ecology and Environment (P2e), INRAE, Université d'Orléans, EA 1207 USC 1328, 45067 Orléans, France

stephane.maury@univ-orleans.fr

Keywords: Forest trees, specialized metabolites, epigenetics, in vitro

Specialized metabolites correspond to millions of natural molecules from different chemical families depending on plant taxa that play a key role in ecological interactions during their life cycle. Due to their chemical properties, plants' specialized metabolites have been exploited for a long time for various industrial applications. However, the limitations in natural population resources as well as the difficulties of their cultivation in terms of production quality or product safety have not always been satisfactory, notably for perennials such as forest trees. Reliable and eco-adapted practices to produce specialized metabolites such as *in vitro* cultures provide a useful and powerful alternative to agronomic cultures. Modern omics have allowed the identification of metabolite pathways but have also raised the question of their complex regulation to improve their production. Among the major regulatory players, epigenetics have been shown in recent years to be involved in plant development and the response to environmental variations. Here, the state of the art concerning the epigenetic control of plant specialized metabolite *in vitro* production as well as the challenges in forest trees are presented (Maury, 2024, https://doi.org/10.3390/f15010141).

ORAL PRESENTATIONS

WG5

Present and future perspective on knowledge-sharing and stakeholder engagement strategies on CopyTree

[1]Valbona Sota, [2]Lucie Fischerová, [3]Maurizio Lambardi, [4]Stefaan P.O. Werbrouck

[1]CopyTree Science Communication Coordinator; University of Tirana, Albania

[2]CopyTree Scientific Representative; Institute of Experimental Botany of CAS, Czech Republic

[3]CopyTree Vice-Chair; Institute of BioEconomy (CNR-IBE), Florence, Italy

[4]CopyTree Chair, Ghent University, Brussels, Belgium

valbona.sota@fshn.edu.al

Keywords: international network, micropropagation, in vitro technology transfer, awareness-raising

Technology transfer and stakeholder engagement within the CopyTree community are mutually beneficial and form a network outcome. The success of this process depends on the quality of the strategies used to achieve specific goals. In the context of this COST Action Network, the focus is on optimising the interaction between Knowledge Producers (KP) and Knowledge Users (KU). Stakeholder engagement, an ongoing process of information sharing and feedback gathering, drives this ambition. The CopyTree community currently comprises 257 members from 43 countries, demonstrating strong interest and effective global distribution. Of the 165 unique affiliations, a) 52% are Universities or other educational institutions involved in micropropagation and *in vitro* technologies, b) 37% are research Institutes and centers (both public and private), some with national or governmental support, and c) 11% represents diverse stakeholders such as commercial micropropagation companies, private nurseries, scientific societies, consultants, and agribusinesses. In order to increase the involvement of KU, and to improve the bridge between research and development (R&D) for profitable knowledge acquisition, considerations should be given to (i) directly inviting companies benefiting from CopyTree results, (ii) identifying and prioritising user interests, (iii) conducting listening sessions with targeted stakeholders to understand challenges and explore solutions, (iv) exploring additional effective mechanisms to influence stakeholders, and (v) launching awareness-raising campaigns on social media channels presenting how research and innovation on *in vitro* woody plant production can bring benefits to the society. All of the above should be managed and implemented by each Working Group independently and interdependently, while thinking and acting on the overall CopyTree mission and goals.

Posters

WG 1 - RECALCITRANCE

WG 2 - SANITATION and CONSERVATION

WG 5 - COMMUNICATION

WG 3 - AUTOMATION

WG 4 - RISK ASSESSMENT

POSTERS
WG1

Biotechnological advances for *Melia volkensii*, a climate-resilient tree for reforestation in East Africa

Stefaan Werbrouck

University Ghent, Belgium, Fac. Bioscience Engineering, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium

Stefaan.werbrouck@ugent.be

Keywords: Melia, reforestation, Mahogany, vitro

Melia volkensii, a Kenyan savanna tree, possesses valuable traits for reforestation due to its climate resilience, high-quality timber, and resistance to locusts. However, overexploitation led to population decline. Fortunately, successful reforestation efforts have restored millions of trees within 15-20 years, generating valuable genetic diversity for future breeding programs. *In vitro* cloning offers significant potential to accelerate reforestation beyond seedling-based approaches. Our research addressed challenges associated with *M. volkensii in vitro* culture, including recalcitrant seed germination, stunted shoot growth without rooting, and callus formation. Acclimatization also posed a significant hurdle.

This overview presents solutions developed in collaboration with local teams to streamline the *in vitro* biotechnology of *M.volkensii*. We implemented strategies including: (1) enhanced micropropagation using meta-topolin derivatives, (2) modified rooting medium, (3) applying ABA homologues for acclimatization regulation, (4) field trials involving beneficial microbes and mycorrhiza, (5) techniques for adventitious shoot and somatic embryo induction and (6) application of these techniques for genetic transformation, polyploidy induction, and early embryo rescue.

Transgene-free genome editing in *Quercus ilex* L.: a way to improve traditional breeding

[1]Vera Pavese, [1]Andrea Moglia, [1]Lorenzo Antonio Marino, [1]Anna Maria Milani, [2]Elena Corredoira, [2]Mª Teresa Martínez, [1]Daniela Torello Marinoni, [1]Roberto Botta

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari—DISAFA, Università degli Studi di Torino, Largo Paolo Braccini 2, Grugliasco, 10095 Torino, Italia

[2]Misión Biológica de Galicia, Sede en Santiago, Consejo Superior de Investigaciones Científicas, Avd. Vigo, s/n, 15705 Santiago de Compostela, La Coruña, Spain

vera.pavese@unito.it

Keywords: axillary shoot proliferation, organogenesis, plant physiology, somatic embryogenesis, tamarillo

The CRISPR/Cas9 technology represents a revolution in the biotechnology area, facilitating the production of cultivars deprived of negative traits. The CRISPR/Cas9 system can be delivered through the *Agrobacterium tumefaciens* strain; this method may lead to the Cas9 integration into the host genome and remain active, causing off-target events. To circumvent these issues, the CRISPR/Cas9 machinery can be introduced as a ribonucleoprotein (RNP) complex, targeting the specified gene before being rapidly degraded. This approach represents a new era of gene editing, paving the way for the production of plants without transgenes.

In woody species, especially *Quercus ilex* L. traditional breeding poses significant challenges due to prolonged juvenile phases, recalcitrance to clonal propagation and high heterozygosity level. Consequently, the adoption of new genomic technology can support and improve breeding.

In the present work, we present the first protoplast isolation protocol in holm oak, highly valued in the European Mediterranean zones. Protoplasts were successfully extracted from both *in vitro* leaves and proembriogenic masses. Proembryogenic masses represented the best source for high protoplast yield (11 x 106 ± 2 x 106 protoplasts/ml) and viability (92% ± 0.5).

CRISPR/Cas9 RNPs targeting the *phytoene desaturase* gene were successfully delivered into protoplasts, demonstrating an editing efficiency of 5.6% ± 0.5. Protoplasts were then cultured in semi-solid media and after a 45-day embryogenic calli development were observed.

POSTERS
WG1

How does secretome composition affect the embryogenic capacity in *Pinus nigra* cell lines?

[1]Miroslav Pernis, [1]Terezia Salaj, [2]Jana Bellova, [1]Maksym Danchenko, [2]Peter Barath and [1]Katarina Klubicova

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

[2]Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 84538 Bratislava, Slovakia

miroslav.pernis@savba.sk

Somatic embryogenesis (SE) offers an effective tool for large-scale vegetative propagation, conserving valuable or endangered genotypes and studying the regulation of embryo development. The relation between the secretome composition and the embryogenic capacity in conifers is not yet well understood. Here, we used suspension cell cultures of *Pinus nigra* to study extracellular proteins secreted into liquid growth media. We aimed to obtain overall secretome profiles of cell lines with contrasting embryogenic capacity and compared them to identify possible players in SE. The majority of differentially accumulated proteins were cell wall-related and carbohydrate-acting proteins. Proteins involved in the cell wall stiffening or cell wall plasticity and cell were more abundant in the medium of high embryogenic capacity cell lines. On the other hand, the medium of low embryogenic capacity cell lines was more abundant in proteins associated with cell wall loosening or softening. In accordance, microscopic findings in the latter showed long suspensor cells without proper assembly and loosely organised meristematic cells.

Furthermore, peroxidase and α-amylase activity assays showed significantly higher activity in high embryogenic cell lines, validating the proteomic data and suggesting given enzymes as potential markers of embryogenic capacity.

Acknowledgements: This work was supported by the Slovak Grant Agency VEGA, Proj. No. 2/0032/22.

POSTERS
WG1

In vitro propagation of alternative species to the radiata pine in the Basque Country

[1,2]Alejandra Rojas-Vargas, [2]Ander Castander-Olarieta, [2]Paloma Moncaleán, [2]Itziar A. Montalbán

[1]Instituto de Investigación y Servicios Forestales, Universidad Nacional, Heredia 86-3000, Costa Rica

[2]Forestry Science Dept., Neiker-BRTA, Centro de Arkaute, N-104 km. 355; 01192 Arkaute (Álava)

imontalban@neiker.eus

Keywords: Cryptomeria japonica, forestry, organogenesis, Pinus ponderosa, Sequoia sempervirens

In the Basque Country, a region in the northeast of Spain, *Pinus radiata* is the most important forest species. Unfortunately, in recent years this species has been affected by several diseases caused mainly by fungi: pine pitch canker, caused by *Fusarium circinatum*; red bands caused by *Dothistroma septosporum* and *D. pini* and brown bands caused by *Lecanosticta acicula*. In this sense, radiata pine plantations in the Basque country have decreased from 123.921 ha (2016) to 102.488 ha (2022); decrease that coincides with a historical outbreak of the bands disease during the years 2018-2019. Therefore, among other actions, it is necessary to look for alternative species that can adapt to our edaphoclimatic conditions and that can be profitable for the stakeholders.

The objective of our work was the development or optimization of organogenesis protocols for elite individuals of alternative forest species such as *Sequoia sempervirens, Cryptomeria japonica* and *Pinus ponderosa*. For this purpose, apart from the traditional benzyladenine and fluorescent illumination, other cytokinins (meta-Topolin, thidiazuron or kinetin) and other light sources were assayed (different LED colours).

Acknowledgements: Grants PID2020-112627RB-C32 and AGL2016-76143-C4-3R funded by MCIN/AEI/10.13039/501100011033 and by ERDF A way of making Europe by the European Union. Also funded by DECO (Basque government), Universidad Nacional de Costa Rica and Instituto de Investigación y Servicios Forestales (INISEFOR).

POSTERS
WG1

Exploring the role of an rRNA methyltransferases gene family during somatic embryogenesis in dicot species

[1]Ricardo Ferraz, [2]Cláudia Marinho, [1]Patrícia Fernandes, [1,3]Sandra Correia, [4,5]Sílvia Coimbra, [1]Jorge Canhoto

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

[2]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL), Rua Pedro Soares, 7801-908 Beja, Portugal

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

[4]Faculdade de Ciências da Universidade do Porto, Departamento de Biologia, Universidade do Porto, rua do Campo Alegre, 4169-007 Porto, Portugal

[5]LAQV Requimte, Sustainable Chemistry, Universidade do Porto, 4169-007 Porto, Portugal

rikayferr@hotmail.com

Keywords: endodermis, lateral root, NEP-TC, somatic embryo, woody species

Somatic embryogenesis (SE) is a valuable tool for plant breeding. *Solanum betaceum* Cav. (tamarillo) is a tree for which well-established SE protocols have been developed. In previous work, an rRNA methyltransferase (MTase) expressed in non-embryogenic calli (NEC) of tamarillo (NEP-TC) was identified as being putatively involved in SE induction. Despite being associated with plant stress and development, the mechanism of action of plant RNA MTases remains unknown. Given the lack of genetic resources for tamarillo, *Arabidopsis thaliana* was chosen in this study. Previous analyses have revealed the existence of a NETP-TC's orthologue in *Arabidopsis*, together with other five proteins of the same family. Here we characterise *Arabidopsis* mutant lines for genes coding three of these MTases: *AT4G15520*, *AT4G17610* and *AT5G15390* (*nep, mt2* and *mt5*, respectively). The response to SE induction, as well as several developmental traits, were analysed. Moreover, *mt2mt5* double mutant was obtained and characterised. It was found that while *nep* mutants exhibited precocious development, longer roots, higher number of lateral roots and higher rates of NEC formation and lower rates of germination during SE, *mt2mt5* mutants showed opposite responses. The other two single mutants did not exhibit significant phenotypes. No differences were found between mutants and wild-type roots anatomy. This suggests that MTases play a role in development and that *MT2* and *MT5* may have redundant roles.

Isolation and characterization of extracellular vesicles in *Solanum betaceum* Cav. somatic embryogenesis

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

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

[2]InnovPlantProtect CoLab, 7350-478 Elvas, Portugal

*These authors contributed equally to this work

miguel9_rito@hotmail.com

Keywords: calli, cell signaling, embryogenic competence, extracellular vesicles, somatic embryogenesis

Solanum betaceum Cav. (tamarillo) has shown to be an interesting model for somatic embryogenesis (SE) analysis in dicot woody species. Using exogenous auxins and high sucrose levels, embryogenic (EC) and non-embryogenic (NEC) *calli* can be induced and subcultured in the same conditions, allowing plant cloning and molecular studies to analyse the acquisition of totipotency. Although various regulatory pathways for the expression of embryogenic competence are known, there is still little information on cell signalling mechanisms involved. In this work, we aimed to study extracellular vesicles (EVs) and characterize them in *S. betaceum* EC and NEC. EVs are nano-sized structures with a key role in cell signalling and stress responses by transporting biomolecules. This is currently a hot topic in plant research but only a few studies were made during SE. To isolate EVs, both *calli* were infiltrated with buffer solution and centrifuged in low-speed centrifugation to collect the EVs, followed by differential centrifugation to pellet them. EC and NEC-derived EVs were compared in size, concentration, and morphology, using transmission electron microscopy and nanoparticle track analysis. We observed that NEC produced a heterogenic population of EVs with peaks at 120, 300 and 400 nm and EC a homogeneous one (peak at 120 nm). Molecular analysis of EVs content is ongoing. This work contributes to EVs characterization in SE and provides results that can be important to set up future research.

POSTERS
WG1

Nanoparticle controlled gradual release of plant growth regulators in tissue culture

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

[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

Traditionally, plant growth regulators (PGRs) are dissolved in the culture medium, leading to uniform distribution within plant tissues in contact with the medium. However, during various stages of plant development, spatial and temporal gradients of hormones and signaling molecules play a crucial role. To address this, a controlled and gradual release system for PGRs has been implemented. Mesoporous $CaCO_3$ microparticles were designed to load and release PGRs gradually, creating gradients within plant tissues. These particles were loaded with different types of auxins and cytokinins, and their effectiveness was tested in *Arabidopsis* and tobacco for root and shoot induction. The results showed promising outcomes compared to traditional phytohormone use. Additionally, this technology facilitated in vitro rooting of recalcitrant olive shoots, suggesting its potential as a unique and powerful tool in plant biotechnology for precise manipulation and enhancement of plant growth.

Combining cytokinines with an cytokinin oxidase inhibitor for improving adventitious regeneration in olive

[1]Saba Taheri, [2]Isaac Kofi Bimpong, [1]Stefaan P.O. Werbrouck

[1]Laboratory for Applied In Vitro Plant Biotechnology, Faculty of Bioscience Engineering, Ghent University, Belgium

[2]Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna International Centre, Vienna, Austria

seyehehsaba.taheri@ugent.be

Keywords: Cryptomeria japonica, forestry, organogenesis, Pinus ponderosa, Sequoia sempervirens

Olive trees (*Olea europaea*) hold substantial economic value, but their *in vitro* culture challenges due to a low success rate influenced by genotype. Addressing this issue is crucial for both established and newly released cultivars. Moreover, the induction of adventitious shoots, essential for random mutagenesis or gene editing, presents an even greater challenge. In leaf explants, shoot induction is predominantly restricted to the petiole region and successful only in a limited number of genotypes. To develop an improved protocol for olive micropropagation, we investigated a novel approach involving a promising cytokinin oxidase inhibitor (Br,OCF3-2HE). Specifically, we applied combinations of 5 µM and 10 µM zeatin, thidiazuron (TDZ), 6-(dimethylallylamino) purine (2ip), and 6-(dimethylallylamino) purine riboside (2ipR) with x µM Br,OCF3-2HE in Rugini's Olive Medium under both shade and dark conditions. Our results indicate that the combination of 2ipR with Br,OCF3-2HE was as effective as the commonly used TDZ. Notably, a better response was achieved under dark conditions. Reasonable shoot induction occurred when 10 µM TDZ was used with or without CKXI, as well as when 2ipR at 5 and 10µM was supplemented with Br,OCF3-2HE. Furthermore, a high concentration of cytokinin significantly impacted callus formation. For future studies, we recommend exploring the use of 2ipR supplemented with cytokinin oxidation inhibitors in a dark condition.

POSTERS
WG1

Regeneration capacity - key feature for application of modern biotechnological techniques in fruit trees

[1]Susan Schröpfer, [1]Ofere Emeriewen, [1]Andreas Peil, [1]Henryk Flachowsky

[1]Julius Kühn-Institut (JKI) - Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Fruit Crops, Dresden, Germany

susan.schroepfer@julius-kuehn.de

Keywords: fruit tree, regeneration, genotype, genome editing, genetic stability

The application of modern biotechnological methods holds great promise for breeding and research in fruit crops. Low *in vitro* regeneration rates and recalcitrance in some fruit tree species or genotypes within a species hinder the application of biotechnical approaches. In this contribution, we present our experience with genetic transformation attempts on the recalcitrant wild apple species *Malus fusca* and hybrid genotypes resulting from crosses between *M. fusca* and cultivated apple (*Malus domestica*). These experiments underscore the impact of the genotype on the regeneration capacity. Further, this talk aims to shed light on the challenges posed by recalcitrance, in particular to regeneration, in different fruit tree species when applying modern biotechnological techniques. Particularly for genome editing approaches, efficient *in vitro* regeneration from individual genome-edited cells is crucial for the production of genetically uniform plants. In this context, we present data from various genome-editing experiments conducted on apple using advanced techniques like high-resolution fluorescent PCR capillary electrophoresis, DNA sequencing, and phenotypic evaluations to monitor induced genetic variations. Our findings offer insights into the distribution, transmission, and stability of genetic changes during *in vitro* multiplication and regeneration processes, highlighting the importance of successful regeneration for generating genome-edited plants.

Transcriptomic analysis of auxin and gibberellin-inhibitor treatments during adventitious rooting in chestnut

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

Department of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela, CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
conchi@mbg.csic.es

Keywords: auxin, gene expression, gibberellin, microshoots

Adventitious rooting is a complex post-embryogenic process necessary for the mass propagation of sweet chestnut (*Castanea sativa* Mill.), a tree with key ecological and socio-economic roles in Europe. Auxin is the main plant growth regulator involved in adventitious rooting. However, other hormones, such as gibberellins, also influence the adventitious rooting process.

Experiments were carried out to determine the effects of auxin and gibberellins on the initiation of adventitious roots. The analysis was performed in a juvenile-like chestnut clone derived from basal shoots. Auxin and the gibberellin biosynthesis inhibitor paclobutrazol (PBZ) were applied to microshoots and the results suggest that PBZ stimulates root formation in chestnut, even in the absence of exogenous auxin.

A transcriptomics analysis was developed to elucidate the genetic basis of the responses to auxin and PBZ. Around 600 Differentially Expressed Genes (DEGs) were detected in IBA-treated microshoots, while 900 DEGs with significant differential expression were found in PBZ-treated microshoots. IBA-induced genes were mainly related to the auxin signalling pathway. In PBZ-treated tissues, significant expression was found for genes linked to the biosynthesis of secondary metabolites, the ethylene signalling pathway and gibberellin receptors. These findings will be confirmed with other experiments but allow a deepening in our understanding of the genetic basis of adventitious rooting regulation in chestnut.

POSTERS
WG1

Challenging seabuckthorn (*Hippophae rhamnoides* L.) propagation by *in vitro* culture

Līga Lepse

Institute of Horticulture, LatHort, Graudu iela 1, Ceriņi, Krimūnu pag., Dobeles nov., LV-3701

liga.lepse@lbtu.lv

Keywords: recalcitrance, growing media, initiation, explant

One of the theoretically possible ways to obtain healthy plant material for seabuckthorn is through micropropagation *in vitro*. Research has been conducted worldwide with various success. Currently, the technological solutions for seabuckthorn *in vitro* propagation are practically non-existent. This is also evidenced by the fact that productive technological solutions for the proliferation and rhizogenesis stages are not available. One of the challenges of the project "Development of biotechnology competencies for the production of high-value horticultural products" financed by the Ministry of Agriculture of Latvia, was to find an effective technological solution for seabuckthorn micropropagation. During the project, 13 media compositions for seabuckthorn initiation *in vitro* were tested for five culture initiation periods of the explants for the varieties: `Mary`, `Prozracnaja`, and `Tatyana`. Seabuckthorn varieties were introduced into culture at different stages of the vegetation period to test the viability of explants at different levels of endogenous plant hormone balance in the plant: in February - March (end of dormancy stage), April - May (just beginning of vegetation), June (developed shoots of 3-5 and 10-15 cm respectively), July (shoots already mature). Unfortunately, all tested initiation media did not yield the expected results - the percentage of successfully introduced plants in culture was below 10%. These plants grew and developed slowly, forming poorly developed microplants. In the next proliferation passage, they also perished becoming necrotic. It can be concluded that a suitable technological solution for seabuckthorn micropropagation was not found.

Somatic embryogenesis of selected conifer species

Miroslav Pernis, Terezia Salaj, Maksym Danchenko, Jozef Mravec and Katarina Klubicova

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

katarina.klubicova@savba.sk

The study of various aspects of somatic embryogenesis of selected conifer species has a long history/tradition at our Institute. We established protocols for induction of embryogenic tissues from immature zygotic embryos enclosed in megagametophytes (*Pinus nigra*) and juvenile explants such as immature or mature zygotic embryos (*Abies alba* and *A. alba* × *A. numidica*) more than 30 years ago. The established protocols resulted in successful development of somatic seedlings. Later, we focused on discovery of molecular factors, especially proteins related to embryogenic competence. Pinus nigra embryogenic tissue is known to loose embryogenic capacity after long term cultivation. We studied this phenomenon at proteome level as well. In recent years, we investigate the role of subproteomes, such as secretome and cell wall proteome in somatic embryogenesis and look for potential markers.

Our group uses proteomics and biochemical methods, as well as novel visualisation methods laser-scanning confocal microscopy.

Acknowledgements: Our research is supported by grant from the Slovak Grant Agency VEGA 2/0032/22.

POSTERS
WG1

Strategies for *in vitro* micropropagation of the woody plant *Vitellaria paradoxa* (C.F. Gaertn.), the shea tree

[1,2,4]Saraka D.M. YAO, [1,2]Nafan Diarrassouba, [3]Christophe Kouame, [4]Stefaan P.O. Werbrouck

[1]Genetics educational and research unit, Biochemistry-Genetics Department, Biological Sciences Training and Research Unit, University of Peleforo GON COULIBALY (UPGC), BP 1328 Korhogo, Ivory Coast

[2]Centre Africain de Recherches et d'Applications sur le Karité (CRAK), Korhogo, Ivory Coast

[3]World Agroforestry Centre (ICRAF), Ivory Coast

[4]Laboratory of Applied In Vitro Plant Biotechnology, Applied Biosciences Department, Faculty of Bioscience Engineering, Gent University, Valentin Vaerwyckweg 1, 9000 Gent, Belgium

didierys@yahoo.fr

Keywords: shea tree, micropropagation, somatic embryogenesis, callus, secondary metabolites

In the northern region of Ivory Coast, *Vitellaria paradoxa*, or shea tree, is growing in the wild. Local communities harvest its nuts to produce shea butter, a sought-after cosmetic cream. The tree's lengthy juvenile phase of 15-25 years restricts vegetative propagation to grafting of mature buds of elite trees grafting on non-specific seedlings, introducing variability. To maintain genetic consistency in disseminated shea plant material, *in vitro* micropropagation of rootstocks that induce early flowering presents a viable alternative to seedling rootstocks. The *in vitro* production of complete clones from mature elite trees is also worth exploring, though potential delays in flowering due to rejuvenation must be considered. The objective of the study was *in vitro* initiation and axillary or adventitious shoot or somatic embryo induction, to start micropropagation. Another intriguing possibility with the produced callus is the production of cell suspension cultures to identify and quantify secondary metabolites that are beneficial for the pharmaceutical and cosmetic industries. The initial findings will be presented. Various types of callus were generated from leaf explants. Additionally, the response of flower and immature seed explants to combinations of auxin (2, 4-D), cytokinin (2iPR), and cytokinin oxidation inhibitors after being transferred to Murashige and Skoog (MS) basal medium will be demonstrated and discussed.

Possible applications of new approaches to minimize recalcitrance in woody plant species

POSTERS
WG1

[1]Vladislava Galovic, [2]Tatjana Vujović, [1]Saša Orlović

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

[2]Fruit Research Institute, Kralja Petra I 9, 32102 Čačak, Serbia

Keywords: woody species, recalcitrance, new in vitro approaches

In the laboratory of *in vitro* culture of the Institute of Lowland Forestry and Environment (ILFE) various woody plant species were successfully regenerated *in vitro*. That were various poplar species and their clones and cultivars like *Populus deltoides* (cl. B229, cl. 182/81, PE19/66), *Populus alba* (cl. L12, L50, L80), *P. alba* cult. Villa Franka, hybrid Pannonia-M1 (*P.* x *euramericana* - *P. nigra* x *P. deltoides*) and recently Wild cherry (cl. 6A and 8A). Those species were the starting material for *in vitro* testing their physiological, biochemical, transcriptomic response to various abiotic and biotic stresses like drought, salinity, heavy metal pollution, pests. The usual techniques that were applied were different kind of tissue introduction, micropropagation and rhizogenesis, somatic embryogenesis by varying hormonal and nutrient status of the corresponding nutrient medium. The response in the regeneration rates and micropropagation were the best in poplar M1 clone but the best induction in the medium, shoot regeneration rates and elongation and its micropropagation were found in *Populus alba* clones. Using expression platforms of various abiotic responsive genes including transcription factor genes (GRAS family TFs like PtGRAS17 and PtGRAS16, PtDREB2 of DREB family TFs and abiotic stress-inducible genes PtP5SC1, PtSOS1, Rd29A, Rd29B genes and genome editing technology using CRISPR/Cas9 design of WRKY TF genes, bZIP etc. *Populus alba* was a model plant but found some obstacles during regeneration after transgenesis. Some possible new approaches to skip *in vitro* recalcitrance of woody plants were further challenge, like protoplast extraction, *de novo* emerging transgenic plants on primed wild type rooted seedlings. Some of those improvements are expected to bring faster and more stable and reliable results in further research work with *in vitro* techniques.

POSTERS
WG1

Imidazole fungicides in fruit tree tissue culture: impact and potential applications

Tatjana Vujovic, Tatjana Marjanovic, Djrdjina Ruzic

Fruit Research Institute, Kralja Petra I No. 9, 32000, Cacak, Serbia
tvujovic@institut-cacak.org

Keywords: fruit tree rootstocks, multiplication, rooting, Prochloraz

Fungicides are mostly employed for preventing fungal contamination in *in vitro* culture, as fungi may interfere with the growth and development of plant cells and tissues. However, several imidazole fungicides can induce morphogenetic and organogenetic responses in *in vitro* plants through a cytokinin-like effect, by intensifying the effect of exogenous cytokinins, or by inhibiting endogenous gibberellic acid biosynthesis. The objective of this study was to see how the imidazole fungicide Prochloraz (PRO) affected the micropropagation of fruit tree species (pear rootstock Pyrodwarf and cherry rootstock Gisela 5) that had previously demonstrated poor multiplication and/or rooting capacity *in vitro*. It was used at concentrations of 1, 5, and 10 µM, either alone or in combination with BAP (4.4 µM), IBA (5 µM), and GA_3 (0.3 µM). PRO at 5 and 10 µM concentrations significantly increased the shoot-inducing effects of BAP in both genotypes, but no cytokinin-like effect was observed in cytokinin-free media. Furthermore, shoots of both rootstocks were the longest and had the largest fresh and dry weights on media containing this fungicide in combination with BAP and GA_3. PRO, either alone or in combination with IBA, also affects root induction *in vitro*. PRO alone stimulated the growth of lengthy roots; however, when combined with IBA, short, thick, white, radially spread roots emerged. These results strongly indicate that PRO could be recommended for micropropagation of these rootstocks.

In vitro propagation of lingonberry *Vaccinium vitis-idaea* L.

POSTERS
WG1

Signe Tomsone, Madara Lazdāne, Jeļena Kalniņa

Laboratory of Plant Biology, Botanical Garden, University of Latvia, Riga, LV-1083, Latvia

signe.tomsone@lu.lv

Keywords: Vaccinium vitis-idaea, in vitro shoot culture, propagation

The lingonberry *Vaccinium vitis-idaea* L. is a small evergreen perennial shrub producing valuable berries and contains components with health benefits. The crop is still mainly harvested in the wild. To achieve lingonberry commercial cultivation, plant propagation efficiency must be improved, therefore the application of tissue culture methods should be studied. The present work aims to establish and propagate *in vitro* shoot culture of lingonberry cultivars 'Koralle' and 'Runo Belawskie'. The stock plants were forced in heated greenhouse. The initiation of primary shoots *in vitro* from 2-3 node explants were affected by the duration of the stock plant forcing and cytokinines in media, and less by the duration of the explant disinfection in 1% NaOCl. The highest rate of primary shoot development was achieved from vegetative buds forced for 8 weeks. Anderson (1984) media supplemented by zeatine 0,75 mg/l was used to stimulate the origin of primary shoots as well as shoot proliferation and elongation. The cloning of the shoots was done after each 12 weeks. The studies showed that the collection of the shoots in a vessel significantly influenced their growth: healthier shoots developed when the microcuttings were alone in the test tubes, rather than in containers hosting 5-15 microcutings. This study improved lingonberry mocropropagation protocol.

POSTERS
WG1

Response of mature somatic embryos of hybrid larch to cold and desiccation treatments

[1]Kateřina Eliášová, [2,3]Parisa Savane, [1]Petre I. Dobrev, [1]Zuzana Vondráková, [1]Václav Motyka, [2]Caroline Teyssier, [2]Marie-Anne Lelu-Walter

[1]Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czechia
[2]INRAE, ONF, BioForA, UMR 0588, F-45075 Orléans, France
[3]Curent affiliation : Amatera Biosciences, 4 rue Pierre Fontaine, F-91000 Evry, France
eliasova@ueb.cas.cz

Keywords: somatic embryogenesis, Larix eurolepis, desiccation, phytohormones, histology

Somatic embryogenesis of conifers yields somatic embryos (SEs) that closely resemble fresh zygotic embryos (ZEs). Great efforts have been devoted to improving the biochemical composition of SEs and reducing their water content to bring SEs closer to fully mature zygotic embryos. The most common method to achieve this goal is the cold treatment of mature SEs or their desiccation at different relative humidity (RH).

Experiments were conducted with two embryogenic lines of hybrid larch (*Larix eurolepis*). Mature cotyledonary SEs, after 8 weeks of cultivation on media with abscisic acid (ABA), were either exposed to cold (+4 degrees) for a week or treated with 98% or 59% RH for a week or their combination (a week at 98% RH plus a week at 59% RH) at +4 degrees. After treatments, phytohormones were analysed in an LC/MS system coupled to a Triple Quadrupole Mass Spectrometer and histological analysis of SEs was performed to detect starch grains and storage proteins.

We focused on the changes in the content of ABA and its derivatives. The total amount of ABAs differed between the lines, but cold treatment and desiccation at 59% RH led to an increase in free ABA and total ABA content, respectively, in both lines. In contrast, desiccation at 98% RH and 98% + 59% RH caused a marked decrease in free ABA. Histological analysis revealed a reduction in starch grains after desiccation at 98% RH and 98% + 59% RH, but not after desiccation at 59% RH or cold treatment.

Cytokinin balance as a tool for improving plant regeneration *in vitro*: case study of somatic embryogenesis in spruce and micropropagation of Saharan cypress

[1]Hana Konrádová, [1]Nikola Štěpánová, [1]Lenka Hrušková, [2]Karel Doležal, [1]Helena Lipavská

[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 cytokinin application, carbohydrates and antioxidant balance in the context of important morphogenic processes - somatic embryogenesis and micropropagation.

Using unrelated embryogenic lines of Norway spruce, our work shows a positive effect of the substitution of widely used benzylaminopurine for meta-topolin in proliferation stage on subsequent somatic embryo (SE) maturation (higher yield of mature SE), although maturing culture is no longer exposed to cytokinin treatment. Using one selected line, we followed the markers of regular development and found similar pattern of carbohydrate dynamics in both cytokinin treatments, although the topolin treated variant exhibited increased ratio of sucrose and raffinose family oligosaccharides in mature and desiccated embryos, respectively. Notably, these compounds are known as multipotent protective substances, e.g. ROS scavengers. Moreover, preliminary data show that the positive effect of replacement may be related to changes in antioxidant capacity. Further, we have focused on critically endangered *Cupressus dupreziana*. Currently, no effective propagation method has been developed. We propose that recalcitrance cause is a physiological imbalance, which might be compensated for by changing the medium composition or physical cultivation conditions.

Funding: project LUC23150, Czech Ministry of Education, Youth and Sports.

POSTERS
WG1

Norway spruce somatic embryogenesis

Lucie Fischerová, Kateřina Eliášová, Jana Pavlíčková, Anastasia Revutska, and Zuzana Vondráková

Laboratory of Biologically Active Compounds, Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, Prague, Czech Republic
fischerova@ueb.cas.cz

Keywords: somatic embryogenesis, histochemistry, phytohormone analysis, expression analysis

One of the main objectives of COPYTREE is to share our expertise to tackle the challenges of woody plants *in vitro* cloning. Based on our recently published works, we present the methodological approaches we have implemented in studying Norway spruce somatic embryogenesis in our laboratory. Our expertise covers:

- methods of anatomy and histochemical detections (in combination with light, fluorescence, and confocal laser scanning microscopes). We use trypan blue or dual staining with fluorescein diacetate and propidium iodide to assess cell viability. To study cytology, localization of storage reserves, and to follow cell division we prepare sections stained with diverse dyes according to the structure we are interested in. Proteins of interest are visualized using whole-mount indirect immunofluorescence labeling.

- determination of the content of plant hormones (using LC-MS methods); these methods are conducted in cooperation with the analytical facility of IEB. We can detect cytokinins (free bases, ribosides, O-glucosides, N-glucosides, and CK phosphates), auxins and ABA with their derivatives as well as jasmonates and salicylates.

- expression analysis (qPCR). We applied qPCR to track the expression levels of polyamine biosynthetic enzyme genes, autophagy-related genes, and glucanase and chitinase genes.

Newly we are implementing methods of redox status assessment. According to the goal of knowledge transfer, we offer to share our expertise in the COPYTREE network.

Genetic investigation of adventitious rooting in olive

Fatih Sezer, Kemal Melih Taşkın

Department of Molecular Biology and Genetics, Faculty of Science, Çanakkale Onsekiz Mart University, Terzioğlu Campus, Çanakkale, Turkiye

fatihsezer@comu.edu.tr

Keywords: adventitious rooting, olive, DNA methylation

Our group has been working on the exploration of genetics controlling adventitious rooting in olive, a critical process for vegetative propagation and stress resilience in one of the world's oldest and economically important fruit crops. Our investigations have spanned a wide array of candidate genes and processes known to influence adventitious rooting, including the conversion of IBA to IAA, the interaction with auxin receptors, the metabolism of strigolactones, and the role of several key transcription factors. These studies have included gene identification, gene expression analyses, and functional studies like complementation tests using *Arabidopsis* mutants to validate our findings. We also tested several different approaches to increase rooting capabilities and improve micropropagation of micro-cuttings in olive. Despite yielding significant insights, our research is now turning towards omics technologies like whole genome DNA methylation analysis, with the aim of achieving a thorough understanding of the molecular mechanisms governing adventitious rooting in olive. In this presentation, we will share the current state of knowledge regarding the molecular control of adventitious rooting in olive, including our recent discoveries. Furthermore, here we outline our forward-looking research agenda, which is designed not only to advance our understanding of olive biology but also to enhance breeding strategies for improved rootstock development.

POSTERS
WG1

Testing mT and mTR to improve blackcurrant proliferation medium

Roberts Krūmiņš, Anna Korica, Rafaels Joffe, Līva Purmale

Bulduri Biotechnology center, Bulduri Technical School, Viestura street 6, Jurmala, Latvia

liva.purmale@bulduri.lv

Keywords: meta-topoline, meta-topoline-ribozide, in vitro, Ribes nigrum

Blackcurrant (*Ribes nigrum* L.) is tasty berry that is rich in vitamin C, anthocyanins, and other bioactive compounds. Although black currants are easy to propagate using stem cuttings, sometimes it is not an option if large quantities of plants are required. There are several protocols available but none of them showed satisfactory results. To improve proliferation rate meta-topoline (mT) and meta-topoline- ribozide (mTR) in concentrations 0,5 mg/L; 1 mg/L and 2 mg/L were tested for comparison MS medium containing 1 mg/L BAP was used (previously tested variations were also i) 1 mg/L BAP with 0,1 mg/L IAA; ii) 0,5 mg/L BAP; iii) 0,5 mg/L BAP with 0,5 mg/L GA3 and 0,1 mg/L IBA). To get clear insight plantlets were twice transferred to the testing medium 4 weeks apart before newly formed shoots were counted for each plantlet. On average blackcurrant formed 1,2 shoots on medium containing 1 mg/L BAP; 1,4 shoots on medium containing 0,5mg/L mT; 2,8 shoots- 1 mg/L mT; 1,8- 2 mg/L mT; 1,1- 0,5 mg/L mTR; 2,6- 1 mg/L mTR and 1,8 2 mg/L mTR. Although mT showed slightly better results than mTR , both 1 mg/L mT and 1 mg/L mTR is promising concentrations for further blackcurrant proliferation medium optimization.

Co-cultivation of *in vitro* trees with ectomycorrhizal fungi from former wildfire sides

Lea Möllhoff, René Jarling, Daniela Demski, Franka Thiesen, Ben Bubner

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

ben.bubner@thuenen.de

Keywords: ectomycorrhiza, co-cultures, reafforestation

In the course of climate change the chance of large fires in central European forest areas increases. Different management strategies for reafforestation after such a fire event are investigated within the project PYROPHOB. In this framework the potential of planting pre-mycorrhized trees with fire side specific fungi are discussed. Our aim is to develop and maintain respective fungal cultures from the PYROPHOB study area. This is realized with co-cultures of rooting in-vitro trees (aspen, oak, birch, beech). First results indicate that certain ectomycorrhizal fungi species, like *Boletus edulis, Hebeloma nanum* or *Cortinarius elegantissimus*, are more easily isolated together with a potential partner tree than without. Also, growth of the fungi is somehow promoted and some culture can be propagated and maintained until now under these conditions. Future experiments will show, whether these trees can grow on former fire sites and if fruitbodies of the respective fungi are produced.

POSTERS
WG1

Establish of walnuts (*Juglans regia* L.) variety 'Chandler' *in vitro* culture in Georgia

Maia Kukhaleishvili, Iveta Megrelishvili

Georgian Technical University, Biotechnology Center, Kostava st 77, 0171, Georgia
maia.kukh@gmail.com

Due to favourable climatic conditions, walnuts are widespread in most ecological zones in Georgia. However, local technologies for obtaining walnut seedlings often fail to meet farmers' demands, resulting in a shortage of high-quality walnut seedlings. This study aimed to establish walnuts in *in vitro* culture to facilitate the production of planting materials. Walnut cultivars 'Chandler' were collected in early spring from a 3-4-year-old orchard in the village of Dzevera, Gori region, Georgia.

In the initial stage, to prevent bacterial and fungal contamination, 20cm young stems with buds from dormant wood was treated with 3% Ridomil Gold, and 3% Captan solution. Afterwards, 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 until futrher use. Initiated explants was sterilized in 1) 0.8% (v/v) sodium hypochlorite (15% commercial bleach for 15-20 minutes and 2) 0.1% mercuric chloride for 5-6 minutes, in both case followed by dipped in 70% (v/v) ethanol solution for a short time and washed 4x times in sterile deionized water in both case.

Driver and Kuniyuki (DKW) media was used for initiation in vitro culture of (Juglans L) supplemented with growth regulators and additives: 1 mg/l bezinlaminopurine (BAP), 0.5 mg/l indole-3-butyric acid (IBA), 500 mg/l PVP, 40 g/l glucose, pH of the medium was set to 6.0 before autoclave.

Overall, the combination of 3% Captan and 1% mercuric chloride provided the highest survival rate (24.27%) among the treatments. This marks the first successful *in vitro* culture establishment of *Juglans regia* L. from open field sources, avoiding greenhouse phase, in Georgia.

Sipping from the genetic vine: unraveling the secrets of drought tolerance in grapevines

[1,2,3,4]Álvaro Vidal Valenzuela, [2]José Tomás Matus, [2]Antonio Santiago Pajuelo, [3]Felipe Gainza, [3]Alvaro Sequeida, [5]Olivier Zekri, [5]Pierre Videau, [1]Mickael Malnoy

[1]Research and innovation center, Biotechnology Vegetal Unit, Fondazione Edmund Mach, Via Mach 1, 38098 San michelle all'adige(TN), Italy

[2]Institute for Integrative Systems Biology (I2SysBio), Universitat de València-CSIC, Paterna, 46980, Valencia, Spain

[3]Center for Research and Innovation (CII), Viña Concha y Toro, Pencahue, Chile

[4]Center Agriculture Food Environment (C3A), University of Trento, via E. Mach 1, 38010 San Michele all'Adige, Italy

[5]Mercier Novatech, Le Champ des Noëls, 85770 – Le gué de velluire, France

alvaroignaciovidalvalenzuela@gmail.com

Keywords: transcriptome, abiotic stress, drought, RNA-seq, somatic embryogenesis

In the face of climate change and legislative restrictions on biotechnology, one of the paramount challenges for food security and sovereignty is the production of stress-tolerant trees without the introduction of foreign DNA. To meet this challenge, it's crucial to identify genes that can be manipulated to enhance plant stress tolerance, and to find faster techniques of somatic embryogenesis that avoid chimerism and inefficient transformations. To achieve this goal, we present an online tool for exploring the transcriptome of grapevines under water stress, which is one of the most significant abiotic stresses affecting viticulture. The tool is based on a comprehensive collection of RNA-seq data from 997 experiments, covering four different tissues (leaf, root, berry, and shoot), various levels of water stress, and diverse genetic backgrounds (cultivars and rootstocks) with different levels of tolerance to water stress. The tool allows us to compare the expression of all grapevine genes, using the V3 genome of 'PN40024' as a reference. With this app, we discovered genes that could boost the drought tolerance of grapevines through genome editing. These genes were transformed using a liquid culture-based protocol for somatic embryogenesis, which allows us to improve the efficiency of regeneration after a transformation of calli. This method was supported by the STSM programme of the COST Action CA21157 COPYTREE.

POSTERS
WG1

Enhancing *in vitro* rooting in *Eryngium maritimum* with dark shock treatment to suppress cytokinin accumulation

Irum Saadia Khan

Walter Blom Plants BV, Veenenburgerlaan 108A, 2182 DC Hillegom, the Netherlands
irum@walterblom.nl

Eryngium maritimum, better known as sea holly, is a valuable coastal plant of considerable ecological and economic importance. *In vitro* rooting of *E. maritimum* is often challenging due to the high accumulation of cytokinins, which inhibit root formation. We investigated the effect of auxins with a dark shock treatment on promoting in vitro rooting by suppressing cytokinin accumulation in *E. maritimum*. We examined the effects of duration of dark shock on rooting efficiency. The results suggested that dark shock treatment effectively reduced cytokinin levels, as root development in *E. maritimum* explants was effectively promoted. A two-day dark shock was good sufficient to initiate root formation in the plants, while after a seven-day dark shock, the plantlets etiolated too much, hindering *ex vitro* growth in a plug. Overall, the use of dark shock treatment with indole-3-acetic acid shows promising potential for improving *in vitro* rooting in *E. maritimum*, providing insight into new strategies for propagation and conservation of this important coastal plant species.

POSTERS
WG1

Effects of meta-topolin riboside and meta-methoxy topolin riboside on the *in vitro* micropropagation of *Pyrus communis* L.

[1]Nataliya Dimitrova, [1]Lilyana Nacheva, [1]Diyana Aleksandrova, [1]Marieta Nesheva, [2]Małgorzata Berova

[1]Fruit Growing Institute, Agricultural Academy, 12 Ostromila Str., 4004, Plovdiv, Bulgaria
[2]Agricultural University, Faculty of Agronomy, 12 Mendeleev Str., 4000 Plovdiv, Bulgaria
*lilyn@abv.bg

Keywords: tissue culture, cytokinins, plant growth regulators, shoot culture, photosynthetic pigments

The present study aimed to evaluate the effects of new meta–Topolin derivatives meta-Topolin Riboside (mTR) and meta-Methoxy topolin riboside (memTR) on the multiplication and subsequent rooting and *ex vitro* acclimatization of *Pyrus communis* L. ('OHF 333'). The cytokinins mTR and memTR were included in the nutrient medium in concentrations of 3μM, 6μM, 9μM, 12μM. A treatment without cytokinin was used as a control. In three passages of three weeks of culture, the following parameters of the plants grown on different nutrient media were evaluated: multiplication coefficient, fresh (FW) and dry (DW) mass (mg), average length of shoots (mm), average number of leaves, leaf length and width (mm). At the rooting stage data on the rooting percentage, number of roots per rooted micro-cutting and the length of roots were recorded 20 days after the beginning of the experiment. In the acclimatized plants, leaf area, FW and DW, the content of photosynthetic pigments were determined 40 days after the transfer to ex vitro conditions. Gas exchange rate and chlorophyll fluorescence were additionally measured for the control and the variants with 6 and 9 μM mTR and memTR. The plantlets grown on media supplemented with cytokinin showed a higher number of leaves compared to the control. Plantlets grown on nutrient media with 6 and 12 μM mTR were distinguished by the highest fresh and dry biomass. In these variants, the shoots were of the greatest length. The plants on medium with 6 μM mTR had the highest number of leaves. Control plants had larger leaves. The highest rooting percentage (70%) was achieved in plantlets grown with 9 μM mTR at the multiplication stage. A higher *ex vitro* acclimatization survival rate (76-100%) was found in all plants cultured with mTR or memTR compared to control plants (65%).

Acknowledgements: This study was financially supported by the Bulgarian National Science Fund (BNSF), project № КП-06-М 56/4 "Influence of natural aromatic cytokinin topolins on in vitro cultivation of fruit species").

POSTERS
WG1

The effect of topophysis on the *in vitro* development of *Handroanthus guayacan* and on its metabolism of meta topolin riboside

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

[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

grira.maroua@gmail.com

Keywords: cytokinin metabolites, Handroanthus guayacan, topophysis

A key factor affecting the uniformity of *in vitro* cultures is the topophysical position of the original explant. We investigated this phenomenon in *in vitro* culture of *Handroanthus guayacan*, a tropical tree. Shoots from established *in vitro* cultures were divided into upper, middle and basal sections and cultured on modified MS medium supplemented with meta-topolin riboside. After eight weeks, the middle section produced the most shoots, the longest shoots, and the highest number of nodes per plant. Shoots from the upper section were elongated but had the shortest internodes, while those from the basal section formed the largest callus. Analysis of endogenous cytokinin and auxin distribution revealed no clear correlation with the observed topophysical effects. However, the metabolism and distribution of the aromatic cytokinin could provide an explanation. The concentration of meta-hydroxy-substituted topolins was highest in shoots derived from the middle section. Aromatic N- and O-glucosides were much more concentrated in leaves than in stems. In conclusion, it is recommended to consider the explant's topophysis to avoid heterogeneity of *in vitro Handroanthus guayacan* and potentially other woody species.

Assessing cryotherapy's efficacy for virus eradication in raspberry *in vitro* cultures

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

[1]Plant Physiology and Cryobiology, Crop Research Institute, Drnovska 507, 16106 Prague 6, Czech Republic

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

[3]Biology Centre CAS, Ceske Budejovice, Czech Republic

bilavcik@vurv.cz

Keywords: Rubus idaeus L., cryoknife, virus

The aim of the National Cryobank in Prague Ruzyne, Czech Republic, is to serve as a safe backup facility for preservation of vegetatively propagated plants. One of its goals is to preserve healthy plant material. This study aims to evaluate the effectivity of cryopreservation cryoprotocol as a tool for virus eradication in selected positive genotypes raspberry (*Rubus idaeus* L.). Raspberry cultivation holds significant value in temperate regions, including the Czech Republic. As a perennial crop, it necessitates the maintenance of cultivars and elite breeding lines, either through field plant collections or *in vitro* cultures. However, both methods have drawbacks, such as susceptibility to viral and other diseases in field collections, as well as expenses and potential genetic variations associated with *in vitro* cultures. Consequently, cryopreservation is emerging as a promising approach, not only for the long-term preservation of genotypes without altering the stored material, but also for improving the health status of treated plant material. The efficacy of eliminating various viruses will be discussed.

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

POSTERS
WG2

Use of high throughput sequencing and cryopreservation in raspberry virus study in Norway

[1]Zhibo Hamborg, [1,2]Xiaoyan Ma, [1]Dag-Ragnar Blystad

[1]Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research (NIBIO), 1433 Ås, Norway

[2]College of Horticulture, Northwest A&F University, 712100 Yangling, Shaanxi, China

zhibo.hamborg@nibio.no

Keyword: cryopreservation, droplet-vitrification, in situ localization, raspberry, virus, shoot tips

Raspberry (*Rubus idaeus* L.) is an economically important crop widely grown in temperate regions worldwide. High-throughput sequencing (HTS) has significantly increased interest in the field of plant tissue culture sanitation, virus diagnostics, and plant health in Norway. Total RNA HTS has been applied to investigate the occurrence of raspberry viruses in Norway. Cryopreservation is considered the ideal method for long-term preservation of plant germplasm and has recently proven reliable for preserving plant viruses. We established an efficient droplet-vitrification method by optimizing shoot tip size, preculture media, and the duration of PVS2 treatment with *in vitro* cultures of raspberry cultivar 'Stiora.' Using this optimized procedure, we achieved 80-100% survival and 67-100% shoot regeneration in various raspberry cultivars. This droplet-vitrification method has also been applied to cryopreserve raspberry bush dwarf virus (RBDV), Rubus yellow net virus (RYNV), and Black raspberry necrosis virus (BRNV) in cryopreserved raspberry shoots. Our results indicate successful cryopreservation of BRNV, RBDV and RYNV. However, BRNV could have low preservation efficiency when cryopreserved using the same method. *In situ* hybridization was employed to localize the invasion of these viruses in plant shoot tips, providing insights into the differences in cryopreservation efficiency among these raspberry viruses. These results suggest that cryopreservation holds great potential for long-term preservation of raspberry germplasm and viruses.

Propagating of resistant specimen of *Ulmus glabra*

Liina Jürisoo

Linnaeus University, Forestry and Wood Technology, 351 95 Växjö, Sweden
liina.jurisoo@lnu.se

Keywords: Ulmus glabra, Dutch elm disease, resistance, propagation

Mostly elms were propagated from seeds. In case of Dutch elm disease as we need to propagate more resistant clones, there is a need for new methods.

To understand that a specimen is less susceptible to Dutch elm disease it takes at least 40 years or even more. It is known that variety 'Lutescens' had been propagated from cuttings but the plant, where are taken must be quite young. Then most important thing is how to propagate those trees from cuttings.

POSTERS
WG2

Effects of copper and silver ions on growth and biochemical characteristics of *Paulownia* plantlets at hydroponic cultivation

[1,2]Oksana V. Pasat, [1]Viktor V. Husak, [1]Angelika M. Pitukh, [1,2]Volodymyr I. Lushchak

[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

Keywords: Paulownia, hydroponic, growth, biochemical characteristics, oxidative stress

Paulownia, a deciduous tree with high growth potential, presents an opportunity for remediating polluted soils and is a perspective tree for bioenergetics. In Ukraine, where vast areas suffer from pollution of both technogenic and war-related origins, establishing sustainable solutions for land reclamation is crucial. This study focuses on the potential of utilizing *Paulownia* for phytoremediation purposes, particularly in regions like the Ivano-Frankivsk area, known for high copper contamination places. Given copper prevalence as a pollutant and its potential involvement in oxidative stress, copper sulfate was chosen as a model pollutant to assess its effects on selected *Paulownia* plantlets. Hydroponic incubation of plantlets with varying concentrations of copper sulfate for 3, 6, and 9 days revealed notable improvements in morphometric and biochemical characteristics, particularly increased starch content. In order to improve growth of *Paulownia* seedlings, we exposed them to silver nitrate. Such exposure significantly enhanced shoot length, stem mass, and leaf mass, increased the activities of catalase, ascorbate peroxidase, and ascorbate oxidase, whereas the activities of superoxide dismutase and glutathione-S-transferase, the concentrations of low molecular mass thiols, and carotenoids dropped. This research underscores the potential of *Paulownia* in phytoremediation efforts, particularly in regions facing copper contamination. Understanding the biochemical and growth responses of *Paulownia* to copper and silver ions can inform strategies for optimizing its effectiveness in remediating polluted soils, thus promoting environmental sustainability and ecosystem restoration.

Survey of viral and phytoplasma diseases of grapevine in Georgia

Iveta Megrelishvili, Zurab Khidesheli, Levan Ujmajurideze

Scientific Research Center of Agriculture, Marshal Gelovani Ave. 6, 0159, Tbilisi, Georgia
ivetameg@yahoo.com

The Integrated Pest Management department of the Scientific Research Center of Agriculture works on the detection of plant diseases in Georgia promotes improvement of the phytosanitary situation in the country.

The main objective of the proposed work was to study viral and phytoplasma diseases in local and wild varieties of grapevine distributed in Meskheti and Mtskheta-mtianeti regions of Georgia. Grapevine viral agents such as: Grapevine leaf roll associated virus-1, -2, -3 (GLRaV-1, -2, -3), Grapevine fleck virus (GFkV), Grapevine fanleaf virus (GFLV), Arabis mosaic virus (ArMV) and Grapevine virus A (GVA) were tested using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Molecular-TaqMan® triplex real-time PCR was used for the detection of two phytoplasma diseases Grapevine flavescence dorée (FD) and Grapevine Bois Noir (BN).

140 samples were collected from Mtskheta-Mtianeti and 90 samples from the Meskhetaregion. It was found that GLRaV-1, -2 (24.16%) infections were characterized by relatively high distribution in Mtskheta-Mtianeti region; following GFLV (12.8%) and GLRaV-3 (10.7%). Study revealed that GLRaV-3 (35.5%) is dominant virus in Meskheti region, following GLRaV-2 (17.7%), whereas GLRaV-1 and GFkV were estimated with relatively low prevalence. In addition, only 1 sample was positive for GFLV infection in Meskheti region. Among the tested samples, a minor infection rate was shown between GVA and ArMV (each 2.14%) viruses.

We have investigated the distribution of FD and BN phytoplasma diseases among Georgian and foreign grapevine varieties. Data from proposed study highlighted the fact that most of Georgian grapevine varieties are less susceptible to phytoplasmas associated with diseases of the GY complex. Based on the results obtained form the study a scheme for the diseases free plants was created, in order to promote the production of healthy planting material in Georgia.

POSTERS
WG2

Cryopreservation of dormant raspberry buds for further *in vitro* cultivation

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

[1]Plant Physiology and Cryobiology, Crop Research Institute, Drnovska 507, 16106 Prague 6, Czech Republic

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

helen.bobrova.77@gmail.com

The aim of this study was to develop a protocol for the low-temperature storage (cryoconservation) of dormant raspberry (*Rubus idaeus* L.) buds and their subsequent introduction into *in vitro* culture. One-year-old dormant canes of the Sanibelle and Willamette raspberry varieties were used in the experiments. Nodal segments with a single bud in the middle were previously frost-dehydrated until they reached a water content of 20 to 30% of fresh weight. At different stages of dehydration, the water state in the buds was monitored by measuring water activity, water content, and the percentage of crystallized water. The study revealed a high resistance of dormant raspberry buds to dehydration. The dehydrated raspberry buds were then frozen using a two-step cryoprotocol. After thawing, the cryopreserved raspberry buds were rehydrated. The buds were washed and sterilized in several stages and prepared for *in vitro* cultivation. Then, the buds with a portion of the twig were placed on a sterile semi-solid nutrient medium. A sufficient level to regenerate plants *in vitro* after cryopreservation of dormant buds was achieved. Therefore, cryopreservation of dormant buds of woody crops can be successfully utilized to preserve plant genetic resources. These cryopreserved buds can later be used at a convenient time for *in vitro* plant introduction for further multiplication and regeneration.

Acknowledgements: This study was partly funded through the MSCA4Ukraine project, which is funded by the European Union, and the project NOBERRYVIRUSCZ (TO01000295) carried out under the KAPPA funding programme, managed by the Technology Agency of the Czech Republic founded by a grant from Iceland, Liechtenstein and Norway through the EEA Grants.

Endogenous bacterial contamination of date palm during tissue cultures: Deglet Nour varieties

Amal Rabaaoui and Stefaan Werbrouck

Applied In Vitro Plant Biotechnology, Department of plants and crops, Faculty of bioscience engineering Ghent, Belgique

Keywords: in vitro plant cultures, palm date, bacterial contamination, Bacillus, 16S RNA sequencing

Bacterial contamination poses a persistent challenge in plant tissue culture, leading to substantial losses across academic and commercial laboratories. While surface disinfectants can eliminate many contaminants, they cannot address endogenous bacteria residing within plant tissues. This study delves into the challenges of bacterial contamination within *in vitro* date palm cultures.

Shoot cultures at the propagation stage were sampled, revealing cloudy, creamy white colonies. 16S RNA gene sequencing identified a complex of *Bacillus* species: *B. pumilus, B. safensis, B.inaquosorum,* and *B. mojavensis*. Interestingly, while all four species possess the potential for endophytic relationships, *B.pumilus* and *B. safensis* are particularly known for their endophytic and biostimulant capabilities.

These *Bacillus* spp. persisted within date palm meristematic tissue for over eight months, and up to a year in acclimated shoots, remarkably without causing harm. This suggests a potential beneficial role for these endogenous bacteria in date palm development. Potential benefits include enhanced nutrient uptake, disease resistance, and root development. Further research is crucial to gain a deeper understanding of the complex interactions between these endogenous bacteria and date palms.

POSTERS
WG2

In vitro forest trees: tissue culture as proper tool for breeding and conservation

[1]Anne-Mareen E. Eisold, [1]Franka Thiesen, [2]Tobias Brügmann, [1]Tina Ruhland, [1]Cornelia Bäucker, [1]Volker Schneck, [1]Ben Bubner

[1]Thuenen Institute of Forest Genetics, Eberswalder Chaussee 3a, D-15377 Waldsieversdorf, Germany

[2]Thuenen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany

anne-mareen.eisold@thuenen.de

Keywords: tissue culture, woody species

Breeding of trees adapted to problematic soil conditions as well as on changing climate is one of the most challenging responsibilities of forestry. Trees are long living organisms with long-term reproductive cycles. Modern breeding techniques rely on biotechnological methods, from which tissue culture is essential to produce clonal tree plantlets. Furthermore, tissue culture is suitable for rescuing endangered individuals or rarely distributed species. A small amount of vegetative explant material is sufficient to regenerate stable in vitro cultures, the source for future trees of value. Tissue culture techniques, therefore, play a crucial role as tools for breeding and conservation purposes.

The Thuenen Institute of Forest Genetics, Germany, has been working with *in vitro* cultures of woody species for decades. Here, we present an overview of all the species and current research topics, which depend on tissue culture techniques. The *in vitro* tree collection includes species of the genera *Ailanthus, Acer, Alnus, Betula, Fagus, Fraxinus, Larix, Picea, Populus, Quercus, Robinia, Salix, Taxus, Ulmus.*

POSTERS
WG2

Synthetic seeds conversion to plantlets in *Punica granatum* L. cv. Devedishe after storage in different temperature regimes

[1]Yllka Lala, [1]Klara Meta, [1]Brunilda Çuko, [1,2]Valbona Sota, [3]Carla Benelli, [3]Maurizio Lambardi, [2]Efigjeni Kongjika

[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

[3]IBE-CNR, Istituto per la BioEconomia, Consiglio Nazionale delle Ricerche, via Madonna del Piano 10, 50019 Sesto Fiorentino (Firenze), Italia

yllka.lala@qttbfushekruje.gov.al

Keywords: encapsulated explants, pomegranate, minimal growth techniques, ex situ conservation

Punica granatum L. (pomegranate) is an important fruit tree cultivated in Albania that has many uses in the food industry and for medical purposes. Several pomegranate cultivars in Albania are cultivated, showing specific characteristics and abilities to adapt to various environmental conditions. Due to climate change, agricultural systems face problems, and some cultivars of interest are at risk of extinction soon. For this reason, optimizing biotechnological methods for *ex situ* conservation of important pomegranate varieties is considered an emergency. This study aimed to preserve encapsulated explants of the Devedishe pomegranate variety for short and mid-term periods. The encapsulation was done using 3% sodium alginate, then dropping it into a 1.1 mM calcium chloride ($CaCl_2$) solution. The synthetic seeds were incubated in two different temperatures, specifically at 25°C and 4°C. Survival and conversion into plantlet rates were monitored and compared between treatments. A complete conversion into plantlets was observed two weeks after encapsulation for the synthetic seeds incubated at 25°C; thus, it cannot considered a suitable technique for germplasm storage. However, it can be beneficial for germplasm exchange. Meanwhile, the rate of conversion into plantlets of synthetic seeds incubated at 4°C was very high (93%) after 3 months of storage in these conditions. The micropropagation coefficient during further subcultures was not negatively affected. However, more extended conservation periods should be tested to optimize slow-growth conservation strategies for pomegranates further. Optimizing *ex situ* conservation protocols will make possible the sustainable utilization of pomegranate genetic resources cultivated in Albania, for further use in genetic improvement programs.

POSTERS
WG3

Endophytic bacteria in date palm tissue culture: impact on the large scale micropropagation using Rita Bioreactors and remedies

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

[1]Laboratory of Plant Biotechnology, Faculty of Sciences of Sfax- Route Sokra BP 1171, 3000 Sfax, Tunisia, University of Sfax, Tunisia

2 Cirad - DGDRS, Jakarta, Indonesia

lotfifki@yahoo.fr

Keywords: date palm, endophytic bacteria, large scale micropropagation, Rita Bioreactor

Endophytic bacteria expression during *in vitro* propagation of date palm is a major constraint which obstructs large scale propagation schemes using temporary immersion systems. This study has been designed to analyze the consequences of the anarchic proliferation of endophytic bacteria during date palm micropropagation and to develop a strategy to minimize culture losses. Endophytic bacteria expression was generally detected when cultures were under stress conditions such as temperature fluctuation and delayed subculture. Embryogenic cultures and bud clusters growing in Rita Bioreactors were seriously affected by the anarchic proliferation of endophytic bacteria. They slowly turned yellow and brown. Although some part of the tissue continued to form new shoot buds and somatic embryos, within 2 weeks all the tissues necrosed. It became impossible to continue cultures any longer. Only juvenile leaves could be used to establish relatively clean *in vitro* tissue cultures. Immaturity of vascular tissue in these explants may explain the low number of contaminated cultures. Defining physico-chemical conditions hampering bacterial growth, without affecting plant cell proliferation can be a promising way to overcome anarchic proliferation of endophytic bacteria. To sum up, we can conclude that endophytes have negative consequences on *in vitro* tissue cultures when they become out of plant cell control.

POSTERS
WG3

Effect of LEDs illumination on the *in vitro* micropropagation of peach rootstock GF 677 (*Prunus amygdalus* × *Prunus persica*)

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

[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

Keywords: LEDs, micropropagation, shoot culture, photosynthetic pigments

The rootstock GF 677 (*Prunus amygdalus* × *Prunus persica*) is the most commonly used rootstock for peach in Europe, propagated almost exclusively by tissue culture. The rootstock is tolerant to Fe deficiency and especially suited to soils with poor fertility, low water availability, and high $CaCO_3$ content.

In recent years light emitting diodes (LEDs) have become an up-to-date alternative to fluorescent lamps (FLs) source of light for plant tissue culture because of their low energy consumption, low heat emission, specific wavelength irradiation, etc. This study aimed at investigating the effects of different LED light regimes on the *in vitro* micropropagation 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 in our studies. Fluorescent lamps (FLs) were included as a control variant. Growth parameters and some physiological and biochemical characteristics of the plantlets were measured. Both mixed (WBR) and white (W) LEDs sources of illumination stimulated almost a twofold increase of the multiplication rate and biomass accumulation compared to the control (FL).

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

POSTERS
WG3

Unlocking the secrets of stable European Beech *in vitro* propagation

Franka Thiesen, Ben Bubner

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

franka.thiesen@thuenen.de

Keywords: in vitro propagation, forest tree breeding, beech, TIS, in vitro rooting

In vitro culture of beech trees is crucial in the face of changing climatic conditions in European forests, as beech is a key tree species. By using *in vitro* techniques, we can accelerate the breeding process and select for traits that enhance the tree's drought resistance and tolerance to pathogens.

By selecting various provenances and explant types, we have been able to establish over 500 *in vitro* clones of beech, despite it being classified as a recalcitrant species. We are currently able to stably propagate 58 clones. The optimisation of the propagation conditions led to multiplication rates between 1.5 and 2.00. Approaches to upscaling the propagation using temporary immersion system bioreactors are being implemented. Following the propagation step, first successful *in vitro* rooting was conducted with 11 clones. The subsequent *ex vitro* transfer and acclimatisation phase remains difficult due to the low growth rates of the plants in the greenhouse.

Our research on beech *in vitro* propagation is leaving an encouraging outlook for stable *in vitro* production.

POSTERS
WG3

Stem cutting propagation of *Sideritis* and *Salvia* spp. and survival rating under different rooting hormone concentrations

[1]T. Tselegkaridis, [2]Eirini Sarroy, [1]Sampson Panajiotidis, [1]Eleni Abraham

[1]School of Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124Thessaloniki, Greece

[2]Institute of Plant Breeding and Genetic Resources (IPB&GR), Hellenic Agricultural Organization Demeter, Thermi, 57001, Thessaloniki, Greece

eabraham@for.auth.gr

Keywords: medicinal plants, clonal propagation, rooting hormone

Stem cutting propagation accompanied by the use of rooting hormone is a well-known and popular way to propagate annual or perennial plants. However, the ideal concentration of rooting hormone that is required for propagation, is not yet known for every species. *Sideritis scardica, S. raeseri, Salvia officinalis* and *S. fruticosa* stem cuttings were propagated during autumn of 2022 and spring of 2023 in the Institute of Plant Breeding and Genetic Resources of the Hellenic Agricultural Organization-Demeter. Stem cuttings were placed in pots with a substrate of 1:1:1 perlite, terrahum and turf. In each period 144 stem cuttings for each species were equally divided in four groups depending on the amount of rooting hormone they were soaked in (control- no K-IBA, soaked in 250, 500 and 1000 ppm K-IBA). In autumn, the cuttings were placed in the greenhouse mist chamber for four weeks while in spring they were placed outside in cages for seven weeks due to high temperature. The percentage of cuttings that successfully developed a root system varies among species. In detail, *Sideritis scardica* in autumn achieved a total ratio (32/144 stem cuttings) of 22,22% which increased in spring to 28,27% (41/144). *Sideritis raeseri* had 0% success rate in autumn and 2,08% in spring. *Salvia fruticosa* had 13,19% (19/144) success rate in autumn, which decreased in spring to 9,72% (14/144) while *Salvia officinalis* had 0% success rate in autumn but notably increased to 37,5% (54/144) in spring. In *S. scardica* successful rooting ratio decreased from control to 1000 ppm block (37.5% (12/32), 28.12%, 18.75% and 15.63%). This trend was inversed in spring (14.63 (6/41), 21.95%, 21.95% and 41.47%). Further research is needed to improve the clonal propagation of the aforementioned species.

POSTERS
WG3

Application of topolin cytokinins in mutation-assisted breeding of *Coffea arabica*: role in somatic embryo germination and regeneration

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

[1]Laboratory for Applied In Vitro Plant Biotechnology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin vaerwyckweg 1, Schoonmeersen – C 9000 Ghent, Belgium

[2]Plant Breeding and Genetics Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA Laboratories Seibersdorf, International Atomic Energy Agency, Vienna International Centre, PO Box 100, A-1400 Vienna, Austria

radisras.nkurunziza@ugent.be

Keywords: coffee, genetic variation, mutation breeding, topolins, indirect somatic embryogenesis

Mutation breeding offers a powerful tool to increase genetic variation in perennial woody plants including *Coffea arabica*. Integration of random mutagenesis with advanced *in vitro* tissue culture techniques, such as somatic embryogenesis (SE) can reduce breeding time and induce adaptation to climate change and resilience to pests and diseases. In *C. arabica*, integration of indirect SE with temporary immersion is preferred due to its high efficiency for mass production. However, the process requires a series of different liquid media and frequent renewal. This increases the risk of somaclonal variants in the regenerated plants, a characteristic that should be avoided in mutation breeding. Moreover, the immersion of developing embryos in liquid media is also associated with hyperhydricity. To circumvent these bottlenecks, we tested the potential of meta-topolin riboside (mTR) and its fluorinated derivative (FmTR) to induce germination and maturation of embryogenic cells. In our study, the embryogenic cells of *C. arabica* cv. Venecia were cultured on MS-solid media supplemented with 5µM, 10µM and 20µM of either topolins. Our results indicated that topolins differentially induced embryo germination and maturation. FmTR induced early embryo germination at low concentration (5µM) whereas mTR induced early embryo germination at higher concentration (20µM). To generate a mutant population, FmTR (5µM) was selected. The mutagenized cells germinated and embryo regeneration through heart-shaped, torpedo and cotyledonary stages occurred without hyperhydricity.

Enhancing woody plant micropropagation: insights and strategies from progressive research at Deroose Plants

[1] Sofie Van Gijseghem, [1,2] Lucas Vanhaelewyn, [1] Filip Vandenbussche

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

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

sofie.vangijseghem@DeroosePlants.com

filip.vandenbussche@DeroosePlants.com

Keywords: Hevea, Eucalyptus, recalcitrance

The micropropagation of woody plants is gaining prominence in addressing challenges posed by climate change and evolving consumption patterns. Deroose Plants, a company specialised in young plant production of ornamental, fruit and vegetable plants and industrial crops, relies on a comprehensive approach in providing quality plant material, offering propagation-as-a-service to breeders and growers worldwide.

The company employs innovative techniques for plant initiation, propagation, rooting, and acclimation to overcome the challenges associated with recalcitrance in woody plants. Advanced tools like temporary immersion systems and a toolbox of diverse *in vitro* chemicals to pass barriers in phenology, rejuvenation, and somatic embryogenesis are applied.

Current propagation protocols feature a unique system for mass-producing *Hevea* clones, yielding enhanced growth and early latex tapping compared to grafted counterparts. In Oil Palm, Deroose Plants contributes to a sustainable multiplication program, selecting high-yielding, disease-resistant clones aligning with RSPO standards. Additionally, Deroose excels in micropropagation research, evidenced by high rooting rates in the recalcitrant *Eucalyptus gunnii* and cultivation of various woody plants, including *Vaccinium, Rubus, Actinidia*, and *Theobroma* species.

The advancements presented by Deroose Plants offer impactful contributions to the scientific, socio-economic, and environmental aspects of woody plant micropropagation.

POSTERS
WG3

Micropropagation of two commercial varieties of apple in bioreactors

[1]Simon Miranda, [1]Mickael Malnoy, [1]Stefano Piazza, [2]Anxela Aldrey, [2]Mª José Cernadas, [2]Conchi Sánchez, [2]Nieves Vidal

[1]Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via Mach 1, 38098, San Michele all'Adige, Italy

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

nieves@mbg.csic.es

Keywords: Golden Delicious, Royal Gala, hyperhydricity, rooting, temporary immersion

Plants micropropagated in liquid medium (LM) in bioreactors with forced ventilation can show enhanced proliferation and improved physiological status. Additionally, increased medium absorption in bioreactors can impact on development of technologies, such as improving genetic transformation or implementing a nanoparticle delivery system. Plant material was obtained from shoots cultured in semisolid (SS) medium in the FEMa laboratories that were transferred to the MBGb in a COPYTREE collaboration.

Axillary shoots of apple varieties - Golden Delicious (GD) and Royal Gala (RG) - were proliferated and rooted in LM in RITA and Plantform bioreactors.

We investigated the medium type, cytokinin type and concentration, immersion frequency, subculture duration, bioreactor type, supplementation with silver nitrate, including physical supports to hold explants in a vertical position. GD produced vigorous shoots in most of the treatments, whereas RG was more prone to produce hyperhydric shoots. Vigorous shoots cultivated in LM were rooted in ½MS with micronutrients supplemented with 4.9 µM indole butyric acid and 3% sucrose, either in SS medium or in bioreactors using temporary or continuous immersion (TIS/CIS).

Both apple varieties rooted more than 75% in all treatments. GD rooted 100% in Plantform using TIS whereas 100% rooting of RG occurred in CIS. Rooted shoots were successfully transferred to soilless media for acclimation.

Enhanced natural stilbene production in *Arachis hypogaea* callus culture through fungal elicitation

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

[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

Keywords: resveratrol, piceid, piceatannol, callus culture, elicitation

Stilbenes are a group of organic compounds characterized by a several biological properties. Peanuts are known for their health benefits, which are largely attributed to the presence of stilbenes such as resveratrol, piceatannol and piceid. However, limited research has focused on their production from *Arachis hypogaea*. The aim of this study was to investigate the production of these stilbenes using *in vitro* systems, specifically callus cultures. Only limited amounts of stilbenes have been produced from established peanut callus. To enhance production, biotic elicitors such as fungal culture filtrate (CF), mycelial extract (ME) and volatile organic compounds (VOCs) from three fungi (*Serendipita indica, Fusarium solani* and *Alternaria alternata*) were evaluated. Principal component analysis revealed that 5% v/v CF from *A.alternata* resulted in a significant increase in piceid production after 8 hours, while 5% v/v CF from *S. indica* showed a significant increase in resveratrol and piceatannol levels after 16 hours. PAL and TAL enzyme activity was also enhanced. The possibility of acquiring piceid, resveratrol and piceatannol through peanut callus culture and fungal elicitation remains a promising avenue for future exploration.

POSTERS
WG3

Optimizing propagation process of hybrid aspen *in vitro* cultures by the use of LEDs

Toms Kondratovičs, Mārtiņš Zeps

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

toms.kondratovics@silava.lv

Keywords: hybrid aspen, photomorphogenesis, full-spectrum LEDs

LEDs are powerful tool for the micropropagation of plants, as the light emitted by LED luminaires can be adjusted to match specific requirements. Light of different wavelengths is absorbed by specific photoreceptors, resulting in changes of physiology and morphology. Accordingly, by matching the emitted light to the absorption spectra of photoreceptors, increased photosynthesis, growth, and overall vitality of plants can be achieved. Studies of the effects of illumination have mainly focused on the *in vitro* propagation of herb and crop species, while perennial woody plants have received less attention. Moreover, the effects of illumination are often viewed from the perspective of monochromatic light, while overlapping absorption spectra of photoreceptors indicate, that the responses may be a result of interaction between several photoreceptors. Here we examined the effects of LEDs with different spectral compositions and illumination intensity on *in vitro* cultures of hybrid aspen – a popular tree species in the Baltic region. Our results indicated that illumination intensity determines culture development more than the spectral composition of light. Nonetheless, illumination with a wide spectral composition containing elevated levels of red light was more favorable than a narrow spectrum or light emitted by FL luminaires. However, the effects of light were mainly clone-specific and should be taken into account when establishing luminaires for propagation on industrial scale.

POSTERS
WG3

Physiological and biochemical responses of clonally propagated *Prunus* spp. rootstocks during water stress and recovery: molecular insights into GF677 post-stress responses

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

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

[2]Quality Plant - Investigation and Production in Plant Biotechnology, Lda., Coimbra, Portugal

[3]University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal

[4]InnovPlantProtect CoLab, Estrada de Gil Vaz, Elvas, Portugal

mjcorreia324@gmail.com

Keywords: rootstocks, water-stress, recovery

Prunus spp. are prized for their edible fruits, with most stone-fruit trees grafted on clonally propagated rootstocks. GF677 and GxN15, both the outcome of *Prunus dulcis* and *Prunus persica* hybridization, are widely used rootstocks known for their drought tolerance and therefore requested in large numbers by nurseries. This study aimed to evaluate the physiological and biochemical responses of both GF677 and GxN15 during recovery after stress. Clonally propagated rootstock plants underwent 18 days of drought (no watering) and logging (excessive watering), followed by a 30-day post-stress recovery period (regular watering). Parameters such as Relative Water Content (RWC), photosynthetic parameters, and pigment content were assessed after each period. Water deficit significantly reduced RWC, photosynthesis, and stomatal aperture. Yet, during recovery, there was an increase in net CO_2 assimilation rate, stomatal conductance, and transpiration rate, indicating the re-establishment of physiological performance, specifically for GF677. Logging showed a lot more damage for both rootstocks mainly for GxN15, showing more detriment in this variety. Analysis of gene expression was performed post-stress for GF766, particularly RBCS1 (coding for RuBisCO small subunit) and PIP1 (coding for a plasma membrane intrinsic protein), revealed lower expression values during drought stress, suggesting protein degradation. These findings validate the association of GF677 with water-deficit tolerance.

POSTERS
WG3

Strategy to produce stable clonal lines of *Dittrichia viscosa* for phytoremediation applications

Capaci Piergiorgio, Anglana Chiara, Barozzi Fabrizio, Stigliano Egidio, Gian Pietro Di Sansebastiano

Di.S.Te.B.A. (Department of Biological and Environmental Sciences and Technologies), University of Salento, Campus ECOTEKNE, 73100, Lecce, Italy
disansebastiano@unisalento.it

Keywords: Dittrichia viscosa, Nip1.1, arsenic, cadmium, phytoremediation

Dittrichia viscosa uptake and translocation of the metalloid As is known but not fully understood. This is not a hyperaccumulator plant, but can grow in high drought conditions while still producing large biomass, even tolerating significant concentrations of As3+ and As5+ accumulating, in the end, good quantities of As. We established experimental clonal populations with variable performances in As uptake and proved that these are inherited in aclonal population independently from selective pressure. We propose a strategy to select a clonal population of *D. viscosa* with a defined phenotype related to As tolerance based on the reduced NIP1.1 expression levels for phytoremediation applications. The procedure allowed to propagate genetically stable tolerant clonal lines with good uptake of As and Cd. The plants, mass-produced with the developed in vitro protocol, were able to maintain their acquired abilities and are potentially able to be later applied in phytoremediation.

Micropropagation of *Philodendron Birkin* using temporary immersion system

POSTERS
WG4

Soner Yağ, Bora Onur Hallaç

FSB Biotech Company, Adana, Türkiye
soneryag@gmail.com

Keywords: Philodendron, micropropagation, ornamental

Philodendron Birkin originates from Brazil. It can develop very quickly under appropriate care conditions. It has eye-catching leaves with white and thin striped patterns. Most varieties of the *Philodendron* plant are propagated by tissue culture method. Almost all commercial production is carried out under semi-solid culture conditions. In our project, studies were carried out using bioreactors with temporary immersion systems as an alternative to semi-solid culture conditions. In this way, higher tillering coefficient and higher quality *in vitro* plant materials were obtained at lower cost. Shoot tips of 20 cm sized plants were used in the studies. The outer leaves were cut and the 2-3 cm long shoots were sterilized for 10 minutes using 1% sodium hypochlorite and 2ml/l polysorbate 20. To obtain the first shoots, MS medium, 6 g/l agar and 6-Benzyladenine at a concentration of 0.2 mg/l were used and the pH was adjusted to 5.8. Work has continued with the temporary immersion system since the first shoots were obtained. In the studies, trial groups were created using 0.5 mg/l, 1mg/l and 2 mg/l 6-Benzyladenine. While the reproduction coefficient was 2 plants per explant in semi-solid culture, 14.3 plants per explant were obtained in the bioreactor system containing 2mg/l 6-Benzyladenine. As for the rooting medium, trial groups containing 0.2 mg/l NAA, 0.5 mg/l NAA and 1mg/l NAA were created in the medium containing ½ MS medium, and 100% root formation was observed in all trial groups. Compared to solid culture, the temporary immersion system has been shown to have superior properties, especially for quickly reaching high quantities in commercial production. 95% acclimatization rate was achieved in the adaptation of the obtained plants to greenhouse conditions.

POSTERS
WG4

How to capture thousands of genotypes – initiation of SE in Norway spruce

Saila Varis, Jaanika Edesi, Mikko Tikkinen, Tuija Aronen

Natural Resource Institute Finland, Vipusenkuja 5, 57200 Savonlinna
tuija.aronen@luke.fi

Keywords: cryo-bank, embryogenic tissue, Picea abies, mass-propagation

For scaling up SE, the best genotypes for the purpose must be found. This means, not just finding the genotypes which are easy to propagate but also the genotypes that perform well on the field. To achieve this, numerous initiations are needed to increase the number of available genotypes in the cryo-bank and to maintain genetic diversity in reforestation following testing of propagation characteristics and field performance. We analyzed the data from the SE initiations of Norway spruce (*Picea abies*) from six different years, including 126 families and almost 13,000 initiations, and used several genetic (including allele PaLAR3B improving *Heterobasidion* resistance), environmental, and operational variables to explain initiation and cryopreservation success. Overall, the cone collection date was the best and most comprehensive single variable for predicting the initiation success and the number of cryopreserved ET samples in the logistic regression models. PaLAR3B allele did not interfere with SE initiation or cryopreservation. In the optimal scenario, cones would be collected in southern Finland during early July (in approximately 800 d.d.) from seed orchards or greenhouse and delivered quickly to the laboratory, and the cones would be cold stored for five days or less before initiations onto mLM media. Lower initiation frequencies in some families can be compensated by increasing the number of explants to some extent — however, taking operational limitations into account.

Micropropagation of some *Anthurium* varieties for commercial *in vitro* production

[1]Yeşim Yalçın Mendi, [1]Yıldız Aka Kaçar, [2]Belgin Biçen, [1]Melike Cengiz, [3]Çağlar Yıldız

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

[2]YK Technopark Company, Adana, Türkiye

[3]Department of Biotechnology, University of Çukurova Adana, Türkiye

ymendi@gmail.com

Keywords: ornamental, Anthurium, micropropagation

Turkey has an advantageous position for the cultivation of ornamental plants for reasons such as favorable conditions, proximity to markets and cheap labor. Biotechnological methods, especially plant tissue cultures and molecular techniques come to the fore to support breeders and producers in meeting the demands of the next century in the ornamental plants sector. *Anthurium* plant is an important species in the world cut flower trade. In addition to classical production methods, biotechnological methods are used to meet the demand of *Anthurium*, which is an indoor plant. Within the scope of the present study, micropropagation and rooting studies were carried out using tissue culture in the commercially important *Anthurium*, "Debby" and '"Lilian" genotypes. In the use of Murashige and Skoog (MS) medium (1/2) and 20% sucrose, 0.1 mg L-1 1-Naphthylacetic acid (NAA) was constant in every media and different concentrations/combinations with 6-Benzylaminopurine (BAP) and N6-furfuryladenine (Kinetin) were tested. The best micropropagation result was the combination of NAA (0.1 mg L-1) and BAP (0.5 mg L-1) for Debby, and the combination of NAA (0.1 mg L-1) and Kinetin (1.0 mg L-1) for Lilian. In rooting experiments, different concentrations of NAA and Indole-3-butyric acid (IBA) were tried in the medium containing 1/2 MS, and the best rooting results for both genotypes were obtained from 2 mg L-1 IBA. The results of micropropagation and rooting have been found to be successful in both genotypes.

POSTERS
WG5

Joint project OPAL in Germany: development and evaluation of methods for the efficient production of hybrid larch and fir clones

Juliane Raschke, Jana Seifert, Madlen Walther, Emma Ehmke, Andrea Rupps

Dept. of Plant Evolution and Biodiversity, Arboretum, Institute of Biology, Humboldt-Universität zu Berlin, Späthstr. 80/81, 12437 Berlin, Germany
andrea.rupps@hu-berlin.de

Keywords: German joint project OPAL, somatic embryogenesis of Larix and Abies species and hybrids, transfer into practice

In the frame of the national project OPAL, we are investigating, optimizing and applying somatic embryogenesis (SE) in order to mass propagate individual tree genotypes of *Larix* x *eurolepis* and of several *Abies* species and hybrids with relevance for forestry.

Our aim is to transfer our knowledge into small and medium-sized enterprises for ready-to-market large-scale production. To that end we are adapting the single steps of the SE *in vitro* chain, collaborating tightly with tree nurseries and a seed trader - as we all share the aim to ensure the availability of resilient plant material for a robust and economic forestry.

Our starting material are zygotic embryos, on which we initiate SE using plant growth regulator-induced stress. This initiation step was successfully achieved for multiple *Abies* and *Larix* species and hybrids. Due to continued research on *A. nordmanniana*, the effectiveness is now up to 30%, depending on seed availability and quality. At this stage, the somatic embryos can be cryopreserved. By now, we have established a clone bank that holds *in vitro* cultures of more than 800 characterized *Abies* spp. genotypes. Subsequently, during the maturation step, the early embryos develop further into cotyledonary embryos and are then ready for conversion/germination into seedlings on a PGR-free culture medium. Accordingly, we were already able to plant several test plots – unless the acclimatisation of in vitro trees is still challenging for several genotypes.

In vitro culture used in biology of forest tree species - new research opportunities

[1]Teresa Hazubska-Przybył, [1]Paweł Chmielarz, [1]Mikołaj Wawrzyniak, [1]Joao Paulo Rodrigues Martins, [1]Joanna Kijowska-Oberc, [1]Hanna Fuchs, [1]Ewelina Ratajczak, [2]Agnieszka Szuba, [3]Leszek Karliński, [4]Konrad Forysiak, [4]Jarosław Sęktas

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

[2]Dept. of Genetics and Environmental Interactions, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

[3]Dept. of Symbiotic Associations, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

[4]Prof. Stefan Białobok Forest Arboretum, Syców Forest District, Leśna 6, 56-504 Stradomia Dolna, Poland

hazubska@man.poznan.pl

Keywords: anatomy, cryopreservation, metabolomics, mycorrhiza, phytoremediation

The main goal of our research is to develop effective protocols for the multiplication of selected, economically important species of coniferous trees (*Picea abies, P. sylvestris*) and deciduous trees (*Quercus robur, Fagus sylvatica, Populus* spp.) and to understand changes in the anatomical structure, biochemical and molecular aspects related to the in vitro cultivation of plants. The possibilities of using *in vitro* methods in the propagation of endangered trees (*P. omorika*), and centuries-old specimens (*Q. robur*) are being explored. Some of the research focuses on developing innovative procedures for cryopreservation of embryogenic cultures of conifers species (*Picea, Pinus, Abies* genera), among others, based on the method of gradual dehydration (without the use of DMSO). The impact of specific breeding factors on the development and growth of plants under various *in vitro* culture conditions is studied. A noteworthy topic is research on the influence of ectomycorrhization of *Populus* x *canescsens* seedlings with the fungus *Paxillus involutus* on the growth intensity of colonized seedlings. These studies are based on high-throughput metabolomic or proteometabolomic analysis. We are interested in improving the growth and tolerance of poplars to stress related to heavy metals, with a view to using vegetative seedlings for afforestation and phytoremediation of polluted areas. Our research form the basis for innovative solutions in e.g. forest management, environmental protection.

POSTERS
WG5

The wonderful forest world inside a glass jar: micropropagation as an educational tool

Puri Covelo, Conchi Sánchez, Mª José Cernadas, Anxela Aldrey, Ricardo Castro, Carmen Fuentes, Nieves Vidal

Dept. of Plant Production, Misión Biológica de Galicia (MBG), CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain
nieves@mbg.csic.es

Keywords: dissemination, children, hands on, public, trees

Trees, as all photosynthetic organisms, perform unique biochemical processes which sustain life in our planet. They have essential roles in biogeochemical cycles, soil and water quality preservation and biodiversity conservation. We perform educational and dissemination activities taking *in vitro* trees out of the laboratory, in glass jars. These "portable" or "traveling" forests allow us to present scientific concepts such as photosynthesis, genetic diversity, gene expression, as well as current challenges as climate change and germplasm conservation. We take them to classrooms, theatres, streets, hospitals, gardens and fairs. We use jars that are kept tightly closed to prevent microbial contamination, so they can be admired or given as special gift to schools or associations, whereas others will be available for opening and touching, smelling and starting the questions of how life is possible in a glass jar. We also take others with media but without plants (so people can touch the agar), mineral salts, agar and sugar, small twigs with dormant buds, a kitchen sieve that acts as a laminar flow cabinet, colourful playdough shaped as microbes, forceps plus scalpels, a sprayer, a little pot with peat, a plastic soft drink or water bottle with the top cut off and used to make a small greenhouse, a sun shaped cushion pillow and a laser sword. Our goal is that children and adults experiment and play, make questions and share ideas enjoying the beauty of *in vitro* trees.

The history and present status of *in vitro* culture research at the institute of nature and forest research (INBO)

Hannes Wilms, Marijke Steenackers, Linda Meiresonne, Bart De Cuyper

Research Institute for Nature and Forest (INBO), Herman Teirlinckgebouw, Havenlaan 88 bus 73, 1000, Brussel, Belgium

hannes.wilms@inbo.be

Keywords: forestry, seed orchards, poplar, Prunus, Fraxinus

In 1948, the precursor of the Institute of nature and forest research (INBO), then called the "Instituut voor populierenteelt" (Institute for poplar cultivation), was founded, and focussed on the improvement and propagation of poplars. In 1999 the institute started its first *in vitro* project: "The development and application of an efficient method to propagate selected materials of wild cherry (*Prunus avium* L.) and European ash (*Fraxinus excelsior* L.)." This project was meant to create more plant material that could be used in clonal tests. From these tests 45 genotypes of wild cherry were selected, from which a clonal orchard was established in 2012. By 2001, the institute was also maintaining an *in vitro* collection of 20 grey poplar (*Populus* × *canescens*) and 13 Elm clones. Besides, maintaining collections, techniques such as embryo rescue, virus eradication and micropropagation were used in poplar breeding, allowing them to create more virus free material, complementary to classical breeding approaches. In the following 5 years, the collection size increased greatly. However, the *in vitro* plants proved to be too expensive for commercialization. This combined with the gradual phase out of tree breeding at the institute resulted in the stop of on site *in vitro* activities, as the final poplar *in vitro* test fields, were planted in 2008 and evaluated in 2016. However, small *in vitro* collaborations with other partners continued as the *in vitro* expertise remains at the institute.

List of authors

AUTHORS

A

B

C

D

E

F

G

H

I

J

K

AUTHORS

L

M

N

O

P

R

S

T

U

V

AUTHORS

W

X

Y

Z

Bulduri Technical school,
Jūrmala, Latvia, 2024

Design: Lauma Ķeire