



From lab to forest and orchard

The Final CopyTree Conference

Book of Abstracts

14-16 September, 2026
Tirana, Albania

Hosted by
Academy of Sciences of Albania





From lab to forest and orchard: Final Conference of CopyTree

COST ACTION CA21157

European Network for Innovative Woody Plant Cloning

Book of Abstracts

**Organized by
Academy of Sciences of Albania**

Supported by



**Under the auspices of Acad. Prof. Dr. Skënder Gjinushi
President of the Academy of Sciences of Albania**

**ACADEMY OF SCIENCES OF ALBANIA
TIRANA, ALBANIA
September 14 - 16, 2026**

Title: From lab to forest and orchard: Final Conference of CopyTree – Book of Abstracts

Disclaimer: Every issue related to the content of the abstracts is under the responsibility of the authors

ISBN: 9789928873255

Acknowledgements:

This book is based upon work from COPYTREE - CA21157 (www.copytree.eu), supported by COST (European Cooperation in Science and Technology).

COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation. www.cost.eu

Printed in the Republic of Albania

Publisher: Academy of Sciences of Albania

Table of Content

Scientific Committee	15
Local Organizing Committee.....	16
Research Topics	17
Opening Speech.....	18
Conference Program.....	20
Working Group 1.....	26
<i>Recalcitromics: what recalcitrance means in the omics era</i>	27
<i>Jesús M. Vielba</i>	
<i>Single-cell regeneration from apple suspension cultures for DNA-free genome editing in a recalcitrant woody species</i>	28
<i>Giovanni Broggin, Jonathan Ohnmacht, Xavier Gaudet, Bruno Studer</i>	
<i>In-planta transformation combined with developmental genes, a strategy to produce edited non-transgenic woody fruit trees?</i>	29
<i>Lorenzo Burgos, Juan Debernardi, Francisco Javier Alfosea Simón, Marina Martín-Valmaseda, Nuria Alburquerque</i>	
<i>Development of an in vitro micrografting system to study anatomical and molecular processes associated with graft healing in olive (Olea europaea L.)</i>	30
<i>Nourhene Jouini, Jorge Canhoto, Aparna S Balan, Irene Granata, Francesco Paolo Marra, Tiziano Caruso, Annalisa Marchese</i>	
<i>Enhancing rooting efficiency and ex vitro survival in Shirvanshah grapevine through gradient based auxin stratification in culture medium.....</i>	31
<i>Aygun Sadigova, Mahira Mammadova</i>	
<i>An efficient micropropagation protocol to overcome recalcitrance in adult Argania spinosa</i>	32
<i>Pablo Piñeiro, M^aTeresa Martínez, Javier Sampedro, Elena Corredoira</i>	
<i>Almond micropropagation: Hyperhydricity characterisation and rooting assays.....</i>	33
<i>Tércia Lopes, Mariana Correia, Elsa Baltazar, Cláudia Marinho, Mónica Zuzarte, Ricardo Costa, Liliana Marum, Sandra Correia, Jorge Canhoto</i>	
<i>Epigenetic regulation of woody plant in vitro culture: From mechanisms, challenges, opportunities to experimental results</i>	34
<i>Elena Carneros, Vera Inácio, Isabelle Le Jan, Alexandre Mary, Jean-François Trontin, Celia Miguel, Pilar S. Testillano, Stéphane Maury</i>	

DCPE vs IBA: Early gene activation drives improved adventitious rooting in chestnut.....	35
Saleta Rico, Bruno Fernández-Moreira, Jesús M. Vielba, Nieves Vidal, María José Cernadas, Hajer Darouez, Stefaan P.O. Werbrouck, Conchi Sánchez	
Results of the Ring Test: A quick overview.....	36
Dicle Dönmez	
From initiation to plant conversion: testing a new cytokinin oxidase/dehydrogenase inhibitor in conifer somatic embryogenesis	37
Miroslav Pernis, Lucie Fischerova, Tuija Aronen, Saila Varis, Sakari Välimäki, Itziar A. Montalbán, Ander Castander-Olarieta, Paloma Moncaleán, Emma Ehmke, Madlen Walthe, Andrea Rupps, Teresa Hazubska-Przybył, Mikołaj Krzysztof Wawrzyniak, Katarína Klubíková, Zuzana Vondráková, Jaroslav Nisler	
From inhibition of cytokinin degradation to auxin protection	38
Jaroslav Nisler, Federica Brunoni	
CKX – what about rooting stimulation?.....	39
Vladislava Galović, Florin Adrian Huiban, Jaroslav Nisler, Branislav Kovačević	
Phenotyping and genotyping of genetically transformed and CRISPR-Cas gene edited recalcitrant crops.....	40
Olha Yaroshko	
Protoplast isolation and PEG-mediated transient transfection in the recalcitrant Pistacia UCB1	41
Sara Yasemin, Saba Taheri, Chaima Ben Othmen, Stefaan Werbrouck	
Characterisation of the proteome of extracellular vesicles from Juniperus communis	42
Ján Jurčík, Miroslav Perniš, Zuzana Kozovská, Maksym Danchenko, Katarína Klubíková	
Determining the effects of different bud sizes and nutrient media on anther culture in Rosa damascena.....	43
İlknur Eskimez, Mehmet Polat	
Improving adventitious rooting in UCB-1 pistachio rootstock: Comparative effects of novel auxin prodrugs and auxin combinations	44
Chaima Ben Othmen, Taoufik Bettaieb, Stefaan P.O. Werbrouck	
Optimizing in vitro propagation of blackberry: Effect of permeable lids and sucrose concentration.....	45
Luca Regni, Arianna Cesarini, Raffaella Brigante, Laura Marconi, Sara Zollini, Nieves Vidal, Primo Proietti	
Epigenetic and non-coding RNA-mediated regulation of plant regeneration: Insights from tamarillo (Solanum betaceum Cav.) for woody plants.....	46
Sandra Correia, André Caeiro, Daniela Cordeiro, Mariana Neves, Miguel Rito, Ricardo Ferraz, Jorge Canhoto	

Exploiting the untapped potential of fruit tree wild diversity for sustainable agriculture (FruitDiv Project)	47
Vlatko Andonovski, Nikolco Velkovski, Viktorija Brndevska Stipanović	
Use of in-vitro-culture to decipher the transcriptomic answer of alder to <i>Phytophthora</i> infection.....	48
Ben Bubner, Sebastian Horn, Ana-Paula Montalvão	
Getting more resilient conifers: What we have learned by modifying environmental conditions during the somatic embryogenesis process	49
Isabel Arrillaga, M. Amparo Pérez-Oliver, Álvaro Rodríguez-Cuesta, Diana C. López-Álvarez, M. Carmen González-Mas, Esther Asensio ⁴ , Ester Sales	
Enhancing transformation of recalcitrant woody plants through a modular morphogenic regulator–Geminivirus-Based Replicon System.....	50
Ashkan Hodaiei, Laurens Pauwels, Stefaan P.O. Werbrouck	
CRISPR/Cas9-based strategies for enhancing disease resistance in holm oak.....	51
Manuel Caamaño-Mayo, M ^a Teresa Martínez, Pablo Piñeiro, Javier Sampedro, Elena Corredoira	
Optimisation of an effective sterilization protocol for the micropropagation of GF677 rootstock	52
Melina Roboçi, Brunilda Çuko, Efigjeni Kongjika, Valbona Sota	
CRISPR/Cas9-mediated mutagenesis of the susceptibility gene CsDMR6 in European chestnut	53
Vera Pavese, María Teresa Martínez, Pablo Piñeiro, Silvia Valladares, José Luis Couselo, Manuel Caamaño, Lorenzo Antonio Marino, Martina Ferrero, Daniela Torello Marinoni, Javier Sampedro, Roberto Botta, Andrea Moglia, Elena Corredoira	
Kinase inhibitors as novel chemical promoters of somatic embryogenesis in European chestnut and holm oak.....	54
María Teresa Martínez, Cristina Buceta, Fátima Mosteiro, Pablo Piñeiro, Elena Carneros, Alfonso García-Rubia, Carmen Gil, Ana Martínez, Pilar S. Testillano, Elena Corredoira	
Effects of PDE inhibitors on somatic embryogenesis induction in European chestnut and holm oak.....	55
María Teresa Martínez, Cristina Buceta, Fátima Mosteiro, Pablo Piñeiro, Elena Carneros, Alfonso García-Rubia, Carmen Gil, Ana Martínez, Pilar S. Testillano, Elena Corredoira. 55	
In vitro propagation of the Greek endemic species <i>Sideritis euboea</i> and <i>Saponaria aenesia</i>: A biotechnological approach for conservation and sustainable exploitation	56
Eirini Sarrou, Aikaterini Grigoriadou, Eleni Abraham	
Investigation of the possibilities of propagating the orchid (<i>Serapias vomeracea</i>) plant in different nutrient media.....	57
İlknur Eskimez, Mehmet Polat	

Effects of different red - blue light ratios on growth and photosynthetic parameters of silver birch (<i>Betula pendula</i>) cultured in vitro.....	58
<i>Ineta Samsonė, Toms Kondratovičs, Arnis Gailis</i>	
Study of Sensitivity of Georgian <i>Vitis vinifera</i> L. varieties to grapevine yellows diseases	59
<i>Iveta Megrelishvili, Zurab Khidesheli, Nino Maziashvili</i>	
Effect of Cytokinin Oxidase/Dehydrogenase Inhibitors on in vitro regeneration in <i>Daphne cneorum</i> L.	60
<i>Jana Hanzal Šedivá, Zuzana Rottová, Michaela Pekařová, Daniel Zahradník, Jaroslav Nisler</i>	
Optimisation of light intensity for in vitro propagation of silver birch (<i>Betula pendula</i>) clones	61
<i>Katrīna Eglīte, Ineta Samsonė, Toms Kondratovičs</i>	
Effect of LED light on in vitro cultivation of Persian walnut (<i>Juglans regia</i> L.)	62
<i>Lilyana Nacheva</i>	
Initiation of in vitro culture of <i>Vaccinium angustifolium</i>.....	63
<i>Anna Korica, Karina Denisova, Elga Ence, Rafaels Joffe, Anta Sparinska, Liva Purmale-Trasune</i>	
Uncovering the mode of action of aromatic cytokinins in Norway spruce embryogenic cultures	64
<i>Lucie Fischerová, Zuzana Vondráková, Karel Doležal, Daria Penzina, Marzieh Mohri, Kateřina Eliášová</i>	
Initiation of Georgian grapevine (<i>Vitis vinifera</i> L.) a Georgian grapevine variety under different sterilization techniques.....	65
<i>Maia Kukhaleishvili, Iveta Megrelishvili, Tamar Chipashvili</i>	
Targeting ABA to overcome adventitious rooting recalcitrance in chestnut and oak microshoots.....	66
<i>Jesús M. Vielba, Nieves Vidal, Ricardo Castro-Camba, Saleta Rico, María José Cernadas, Anxela Aldrey, Conchi Sánchez</i>	
Advancing in the establishment of a micropropagation protocol for the endemic Mediterranean seagrass <i>Posidonia oceanica</i>.....	67
<i>Ricardo Castro-Camba, Laura Guerrero-Meseguer, Inés Castejón, María Rubio, Jorge Terrados, Carlos Encina</i>	
Improving in vitro shoot multiplication of temperate fruit species using novel CKX inhibitors	68
<i>Tatjana Anđelić, Mirjana Mičević, Jaroslav Nisler, Tatjana Vujović</i>	
Initial establishment of in vitro cultures of sweet chestnut (<i>Castanea sativa</i> Mill.) and dog rose (<i>Rosa canina</i> L.).....	69
<i>Vanja Daničić, Syjetlana Zeljković</i>	

WGCNA identification of gene modules and transcription factors relevant for adventitious rooting in chestnut	70
Jesús M. Vielba, Ricardo Castro-Camba, Nieves Vidal, Saleta Rico, Conchi Sánchez	
Impact of vacuum infiltration on the efficiency of temporary <i>Agrobacterium</i> transformation in different ornamental sunflower explant types	71
Şükran Yıldız, Ashkan Hodaiei, Sara Yasemin, Dilek Tekdal, Stefaan P.O. Werbrouck	
Small motif, big function: A 19 amino acid signal links chloroplast targeting to viral replication.....	72
Yajiao Wang, Richita Saikia, Andreas Maurice Grant, Dianqiu Lv, Xifeng Wang, Robert C. Wilson, Huanbin Zhou, Carl Spetz	
From explant stress to regeneration competence in recalcitrant woody species: physiological responses and emerging epigenetic perspectives.....	73
Paula Oros, Corina Cătană	
WB3: Possibilities for using somatic embryogenesis for scale production of Scots pine (<i>Pinus sylvestris</i> L.)	74
Sara Raggi, Malin Abrahamsson, Magnus Hertzberg	
Working Group 2.....	75
Cryopreservation and in vitro culture of meristems for the conservation of forest genetic resources ex situ	76
Paweł Chmielarz, João Paulo Rodrigues Martins, Juan Manuel Ley-López, Mikołaj Krzysztof Wawrzyniak, Marcin Michalak, Małgorzata Pałucka, Urszula Wasileńczyk	
Tiny allies and hidden threats: Bacteria in woody plant micropropagation.....	77
Stefaan P.O. Werbrouck	
Transgrafting: a biotechnological solution for plant virus control	78
Nuria Alburquerque, Lidia Faize, Francisco Javier Alfosea Simon, Marina Martín-Valmaseda, Elena Yelo, Lorenzo Burgos	
From virome to clean planting stock: Cost-effective NGS-guided sanitation for in vitro propagation of heritage grapevine	79
Sebastián Gómez Talquenca, Humberto Debat, Raul Cuello, Diego Zavallo, Rocio Torres ¹ , Sebastian Asurmendi	
Establishing a Cryobank for Your Tree Species.....	80
Bart Panis	
In vitro conservation and propagation of endangered Mediterranean thymes for sustainable genetic resource valorisation	81
Daniela Duarte, Bernardo Onofre, Jorge Canhoto, Paula Castro, Sandra Correia	
Understanding the antiviral effects of silver nanoparticles through omics tools in apricot plants cultured in vitro	82

Francisco Javier Alfosea-Simón, Alejandro Castro-Cegrí, Leilei Zhang, Luigi Lucini, Lorenzo Burgos, Nuria Alburquerque¹

How long is too long? Preservation of *Pinus radiata* embryogenic cultures at -80°C and evaluation of alternative freezing methods 83

Katherine P. Sandoval, Ander Castander-Olarieta, Paloma Moncaleán, Itziar A. Montalbán

Assessing Cryotherapy as a Sanitation Tool for Hop Stunt Viroid in Apricot 84

Marina Martín-Valmaseda, Zhibo Hamborg, Lydia Faize, Elena Yelo, Lorenzo Burgos, Nuria Alburquerque

New technological tools for improving chestnut stress tolerance 85

Vera Pavese, Lorenzo Antonio Marino, Paola Ruffa, Eric Mozzanini, Davide Lucien Patono, Alessandra Sereno, Martina Ferrero, Anita Fuda, Guglielmo Lione, Paolo Gonthier, Silvia Sabbadini, Luca Capriotti, Bruno Mezzetti, Roberto Botta, Daniela Torello Marinoni, Andrea Moglia

From poplar transcriptomics to CRISPR validation: Functional characterisation of a Key Gene for metal-toxicity tolerance in trees 86

Kubilay Yildirim, Şükriye Coşkun

Enhanced viral detection in grapevine via exome depletion and next-generation sequencing (NGS) 87

Raúl Cuello, Diego Zavallo, Pablo Vera, Agustín Sattle, Andrea Puebla, Humberto Debat, Sebastián Gómez Talquenca, Sebastian Asurmendi

Plant biotechnology in support of the Albanian certification system for seedlings of autochthonous fruit tree cultivars: From scientific research to market.....88

Efigjeni Kongjika, Valbona Sota, Magdalena Cara, Jordan Merkuri, Ariola Bacu, Raimonda Sevo, Elektra Papakosta, Maurizio Lambardi, Carla Benelli

The Sycamore Maple Clone Collection – when Minis grow up 89

Anne-Mareen Eisold, Diana Morgenroth, Volker Schneck

Tissue culture techniques in the diagnosis of viral tree diseases 90

Anne-Mareen E. Eisold, Marius Rehanek, Ben Bubner

CKX20 as effective booster for shoots formation in wild service tree (*Sorbus torminalis* L.) explants 91

Eva Pokorná, Pavlína Máchová, Helena Cvrčková, Jaroslav Niesler, Jozef Lacek, Peter I. Dobrev, Daniel Haisel, Petr Soudek

Endophyte-assisted cold hardening and cryopreservation of apple microshoots in vitro 92

Rytis Rugienius, Jurgita Vinskienė, Inga Tamošiūnė, Olha Posunko, Šarūnė – Morkūnaitė Haimi, Perttu Juhani Haimi

Safeguarding Albanian grapevine germplasm: phytosanitary assessment of Shesh i Bardhë and Shesh i Zi 93

Adriatik Çakalli, Doriana Bode, Magdalena Cara, Klevis Hoxhallari, Jordan Merkuri

Cryotherapy as a tool for virus elimination in selected blueberry genotypes	94
<i>Barbora Tunklová, Jana Fráňová, Miloš Faltus, Jaroslava Přibyllová, Igor Koloniuk, Olena Bobrova, Lesia Golosna, Alois Bilavčík</i>	
Establishment of an endangered <i>Cydonia oblonga</i> collection in vitro	95
<i>Alois Bilavčík, Barborá Tunklova, Olena Bobrova, Lesia Golosna, Miloš Faltus</i>	
Integrating pesticide registration and food safety surveillance: An in silico framework for mode-of-action analysis and prioritisation in Albania	96
<i>Magdalena Cara, Florida Gashi, Anjeza Bekolli, Klevis Hoxhallari</i>	
Phytosanitary selection and molecular testing of the Albanian grapevine cultivar <i>Kallmet</i> in support of plant material certification	97
<i>Magdalena Cara, Efigjeni Kongjika, Valbona Sota, Klevis Hoxhallari, Orges Cara, Elektra Papakosta, Adriatik Cakalli, Jordan Merkuri</i>	
Medium-term conservation of olive synthetic seeds using different carbohydrate sources, light conditions and abscisic acid.....	98
<i>Doaa S. Elazab, Claudia Ruta, Maurizio Lambardi</i>	
Cryopreservation as a tool for the long-term conservation of edited embryogenic cultures in European chestnut.....	99
<i>M^oTeresa Martínez, Cristina Buceta, Elena Corredoira</i>	
Cryopreservation and micropropagation of <i>Prunus padus</i> L.....	100
<i>Essi Risu, Leena Yrjänä, Tuija Aronen, Sakari Välimäki</i>	
Improvement of in vitro propagation of ‘Gisela 5’ (<i>Prunus cerasus</i> × <i>P. canescens</i>) cherry rootstock using the ElecTIS bioreactor	101
<i>Elektra Papakosta, Efigjeni Kongjika, Magdalena Cara</i>	
Identification of promising grape varieties under Lithuanian agroclimatic conditions and the introduction of healthy propagation material.....	102
<i>Birutė Frercks, Ingrida Mažeikienė</i>	
Development of a cryopreservation protocol for dormant buds of Georgian hazelnut (<i>Corylus avellana</i> L.) cultivars	103
<i>Mariam Gaidamashvili</i>	
High-resolution DNA methylation profiling and transcriptomic analysis of a wheat-barley 7H addition line	104
<i>Norbert Hidvégi, István Molnár, Andrea Gulyás</i>	
Occurrence of Plum Pox Virus (PPV) in <i>Prunus</i> species in Azerbaijan and its effects on oxidative stress and antioxidant responses	105
<i>Nargiz Sultanova</i>	
Cadmium effects on ion balance, leaf structure, and antioxidant regulation in micropropagated <i>Paulownia</i> plantlets.....	106

Viktor Husak, Ulyana Kilchytska, Yurii Vakiv, Viktoriia Nykolyshyn, Yuliia Savchuk, Viktoriia Zadarko, Volodymyr Lushchak

Biotechnological approaches of the OMIBREED project for sustaining olive genetic resources: In vitro conservation by slow growth storage.....107

Anna De Carlo, Waed Tarraf

Comparative evaluation of aluminum cryo-plate methods for the cryopreservation of autochthonous plum (*Prunus domestica* L.) genotypes in Albania 108

Yllka Lala, Tatjana Vujović, Efigjeni Kongjika, Valbona Sota

Innovative cryopreservation of dormant buds of apple and pear to safeguard genetic resources and commercial cultivars (DormaCryo).....109

Zhibo Hamborg, Linn Borgen Nilsen, Lill-Iren Hansen, Dag-Ragnar Blystad

Working Group 3.....110

Artificial intelligence for large-scale and data-driven optimisation of micropropagation: Towards open-access AI platforms.....111

Pedro Pablo Gallego, Tomás A. Arteta

Machine learning tools reveal the effect of topolin-type cytokinins on the induction of Norway spruce embryogenic cultures 112

Marzieh Mohri, Pedro Pablo Gallego, Lucie Fischerová, Zuzana Vondráková, Kateřina Eliášová

Biophysics meets plant morphogenesis - monitoring callus growth and somatic embryogenesis with electrical signals.....113

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

Somatic embryogenesis and image-based deep learning approaches in *Coffea arabica* 114

Ceren Unek, Sena Melis Tutar, Ipek Baz, Bahar Sogutmaz Ozdemir

Biochemical and infrared spectroscopic characterisation of *Pinus radiata* somatic embryos during maturation 115

Caroline Teyssier, Jamie Bridson, Nina Eichler, Mark West, Cathie Reeves, Ilena Isak, Celine Mercier, Jana Krajnakova

Application of somatic embryogenesis to increase the range of conifer tree species used for forestry in Finland.....116

Saila Varis, Mikko Tikkinen, Sakari Välimäki, Tuija Aronen

Characterisation of embryogenic lines of *Abies pinsapo* Boiss.....117

Laura Cabero-Moreno, Carolina Sánchez-Romero

Mimicking drought in germination of somatic hybrid larch (*Larix × eurolepis*) by inducing mild and sublethal osmotic stress in vitro.....118

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

Establishing an efficient in vitro micropropagation protocol for <i>Fraxinus excelsior</i> tolerant to ash dieback disease	119
Karuna Shrestha, Oliver Gailing, Dheeraj Singh Rathore	
Date palm artificial seeds: current results and perspectives	120
Lotfi Fki	
Automation in tissue culture – transforming the transplanting and hardening process of tissue cultured plants	121
Michiel van Bennekom	
In vitro multiplication of <i>Quercus robur</i> in the RITA system.....	122
Teresa Hazubska-Przybył, Joao Paulo Rodrigues Martins, Mikołaj Krzysztof Wawrzyniak, Maciej Rajek, Agata Obarska, Paulina Pilarz, Bruce Christie, Nieves Vidal, Paweł Chmielarz	
Temporary immersion bioreactors as a promising approach for large-scale micropropagation and sustainable supply of high-value terpenes in <i>Myrtus communis</i>	123
Waed Tarraf, Carla Benelli, Francesca Ieri, Gabriele Cencetti, Anna De Carlo	
ADAM: Advanced Design and AI-Driven Modeling for plant tissue culture media optimisation.....	124
Hans Bethge, Traud Winkelmann, Tomás A. Arteta, Esmaeil Nezami, Marco Pepe, Mohsen Hesami, Andrew Maxwell Phineas Jones, Mariana Landin, Pedro P. Gallego	
In vitro somatic embryogenesis and phytochemical profiling of the endangered <i>Hyphaene thebaica</i> for conservation and sustainable utilization	125
Doa'a Jamal Abu-Darwish	
In vitro Callus Induction and Metabolic Profiling of <i>Melaleuca alternifolia</i>.....	126
Muhammad Mudasir	
Influence of different LED light wavelengths and auxin dependence on rhizogenesis induction in <i>Prunus amygdalus</i> Batsch (Rosaceae)	127
Dawid Kocot, Antonina Gluch, Tércia Lopes	
In vitro establishment of European Ash (<i>Fraxinus excelsior</i>) for a clonal trial on ash dieback resistance	128
Franka N. Thiesen, Ben Bubner	
Preserving historic trees for Pückler Park Branitz, Germany by in vitro propagation	129
Juliane Raschke, Aisha Mehar, Liis Viljak, Anika Dreilich, Christian Boehm, Juliane Ribbeck-Lampel, Andrea Rupps	
Influence of silicon on the micropropagation of peach rootstock GF 677.....	130
Lilyana Nacheva, Diyana Aleksandrova, Ivaylo Tsvetkov	
WB3: A highly automated somatic embryogenesis based method for plant production - latest developments	131
Malin Abrahamsson, Magnus Hertzberg	

<i>Integrating micropropagation into conservation strategies for endangered field elm (<i>Ulmus minor</i> Mill.) populations in Croatia</i>	132
<i>Sanja Bogunović, Doroteja Bitunjac, Mia Marušić, Ivana Sirovica, Marko Bačurin</i>	
<i>Fifteen years of research on hybrid larch somatic embryogenesis: Progress, pitfalls and perspectives for forestry</i>	133
<i>Andrea Rupps, Emma Ehmke, Madlen Walther, Juliane Raschke, Ute Tröber, Ricardo Joffe, Silvan Liebsch, Thomas Hoppe, Birgit Reiche, Sophie Maiwald, Anna Rudzinski, Tony Heitkam</i>	
<i>Evaluation of CKX inhibitors in the presence of trans-zeatin during micropropagation of <i>Prunus domestica</i> L. cv “Tropojane”</i>	134
<i>Brunilda Çuko, Ledina Shkëmbi, Stefaan P.O. Werbrouck, Maurizio Lambardi, Jaroslav Nisler, Efigjeni Kongjika, Valbona Sota</i>	
<i>Adaptation of a temporary immersion bioreactor system for facilitating ex vitro transfer of micropropagated blueberry plants</i>	135
<i>Olena Bobrova, Barborá Tunklova, Miloš Faltus, Lesia Golosna, Alois Bilavčík</i>	
<i>Multiplication of <i>Melaleuca alternifolia</i> shoots in temporary immersion: effect of genotype, cytokinin and bioreactor type</i>	136
<i>Bruce Christie, Anxela Aldrey, Conchi Sánchez, Jesús M. Vielba, M José Cernadas, Saleta Rico, Nieves Vidal</i>	
<i>Effect of bioreactor type and explant density on multiplication and shoot quality of <i>Corylus avellana</i> L. cultured by temporary immersion</i>	137
<i>Conchi Sánchez, Anxela Aldrey, Jesús M. Vielba, Saleta Rico, M José Cernadas, Bruce Christie, Nieves Vidal</i>	
<i>Temporary immersion system as a valid method to isolate extracellular vesicles from in vitro-cultivated hop (<i>Humulus lupulus</i> L.) plantlets</i>	138
<i>Benedetta Chiancone, Angela Paterna, Valeria Gianguzzi, Estella Rao, Samuele Raccosta, Paola Gargano, Sabrina Picciotto, Giorgia Adamo, Antonella Bongiovann, Valeria Guarrasi, Mauro Manno</i>	
<i>Comparing the potential of somatic embryogenesis and organogenesis in Rosaceae species</i>	139
<i>Simón Miranda, Maddalena Nesler, Mickael Malnoy</i>	
<i>Effect of PSK and CAP6 peptides on somatic embryogenesis of <i>Cryptomeria japonica</i> (Sugi, Japanese cedar)</i>	140
<i>Tsuyoshi E. Maruyama, Jesús Jiménez, Momi Tsuruta, Saneyoshi Ueno, Yoshinari Moriguchi</i>	
<i>Somatic embryogenesis potential in <i>Hyphaene thebaica</i> (L.) Mart. via in vitro callus induction from female inflorescences</i>	141
<i>Doa'a Jamal Abu-Darwish</i>	

Meta-topolin applied in proliferation increases the yield of Norway spruce somatic embryos.....	142
<i>Hana Konrádová, Nikola Švorc Štěpánová, Lukáš Ležal, Monika Kofroňová, Václav Motyka, Karel Doležal, Helena Lipavská</i>	
Outcome of COPYTREE training school: Utilising the Plantform™ temporary immersion system at IBISS.....	143
<i>Sladana Jevremović, Tatjana Vujović, Snežana Zdravković-Korać</i>	
RING Test study: The effect of three CKX inhibitors on somatic embryo germination of Aesculus hippocastanum	144
<i>Sladana Jevremović, Snežana Zdravković-Korać, Jaroslav Nisler</i>	
Salt tolerance of Artemisia maritima L. under in vitro conditions: a comparison of agar-solidified and TIS cultivation systems	145
<i>Liva Purmale-Trasune, Ksenija Nikitina, Gederts Ievinsh</i>	
Influence of light regimes and plant growth regulators on in vitro shoot proliferation of Arbutus unedo L. toward scale-up	146
<i>Mariana Correia, João Martins, Mónica Zuzarte, Sandra Correia, Jorge Canhoto</i>	
Micropropagation of Quercus suber shoots in RITA® and Plantform™ bioreactors	147
<i>Conchi Sánchez, Anxela Aldrey, Jesús M. Vielba, M José Cernadas, Saleta Rico, Beatriz Cuenca, Bruce Christie, Nieves Vidal</i>	
A protocol for somatic embryogenesis induction in argan using zygotic embryos....	148
<i>Pablo Piñeiro, MªTeresa Martínez, Beck Costas, Fátima Mosteiro, Sol Campaño, Cristina Buceta, Clara González, Javier Sampedro, Elena Corredoira</i>	
Application of somatic embryogenesis to Norway spruce special forms.....	149
<i>Saila Varis, Mikko Tikkinen, Sakari Välimäki, Teijo Nikkanen, Tuija Aronen</i>	
Nanoparticle-induced modulation of antioxidant potential and polyphenol biosynthesis in in vitro shoot cultures of Brassica rapa var. japonica (mizuna).....	150
<i>Aleksandra Łukaszyk, Inga Kwiecień, Magdalena Kniaziewicz, Eliza Blicharska, Małgorzata Tatarczak-Michalewska, Katarzyna Czarnek, Agnieszka Szopa</i>	
Phytochemical composition and antioxidant potential of Schisandra rubriflora biomass derived from in vitro cultures.....	151
<i>Grzegorz Kos, Angelika Warzecha, Kubica Paweł, Michał Dziurka, Agnieszka Szopa</i>	
Machine-learning-assisted evaluation of genotype-specific drought responses in myrtle (Myrtus communis L.) under in vitro conditions.....	152
<i>Tolga İzgü, Ümit Bektaş, Musab A. Isak, Taner Bozkurt, Dicle Dönmez, Mehmet Tütüncü, Özhan Simsek</i>	
The effect of embryo orientation on the in vitro germination of coconut in semi-solid medium	153
<i>Hannes Wilms, Bart Panis</i>	

Working Group 4	154
Strategies for improving the phytosanitary and genetic quality of micropropagated materials. The strategic role of Conservation Centers	155
Marco Pancaldi	
Commercialisation of new SE automation – origins of Lauka Biotech	156
Sakari Välimäki, Elmar Bernhardt, Jonne Pohjankukka, Elina Havia, Saila Varis, Jaakko Salminen, Tuija Aronen, Mikko Tikkinen	
Business models and commercialization strategies for woody micropropagation: From lab to forest, orchard and market impact	157
Rober Ahmad Kreimech	
Effect of Plantform on commercial micropropagation of <i>Paulownia fortunei</i> and <i>Paulownia catalpifolia</i>	158
Ademi Fahri Pirhan, Basar Sevindik, Hasan Yıldırım	
In vitro propagation of elite <i>Hevea brasiliensis</i>	159
Filip Vandenbussche, Lucas Vanhaelewyn	
Micropropagation of <i>Alocasia bambino</i> via tissue culture	160
Soner Yağ, Bora Onur Hallaç, Cansu Ece Sari, Yeşim Yalçın Mendi	
Micropropagation of <i>Caladium</i> ‘Sunfire’ via tissue culture	161
Bora Onur Hallaç, Soner Yağ, Cansu Ece Sari, Yeşim Yalçın Mendi	
Working Group 5	162
Public attitude toward new technologies in FRM production – case study Bosnia and Herzegovina	163
Branislav Cvjetković	
Mapping the CopyTree ecosystem: A complexity science approach to knowledge sharing and digital impact in woody plant biotechnology	164
Buhara Yücesan	
Propagation pathways in <i>radiata</i> pine: A comparative analysis of production performance and biochemical profiles	165
Itziar A. Montalbán, Ander Castander-Olarieta, Javier Herrero, Iker Domínguez-Basterretxea, Caroline Teyssier, Paloma Moncaleán	
Growth Promotion of Pepper (<i>Capsicum annuum</i> L.) by <i>Bacillus halotolerans</i> Sp405 Inoculation: Phenotypic and Whole-Genome Characterization	166
Ibtisem Khalifa, Radhouane Gdoura, Stefaan P.O. Werbrouck	
Strengthening communication and technology transfer for innovative woody plant cloning in Albania	167
Rozarta Nezaj, Efigjeni Kongjika, Valbona Sota	

SCIENTIFIC COMMITTEE

Stefaan Werbrouck – Ghent University, Belgium; *Chair of CopyTree*

Maurizio Lambardi – National Research Council (CNR), IBE-Institute of BioEconomy, Italy; *Vice-Chair of CopyTree*

Lucie Fischerová – Institute of Experimental Botany of CAS, Czech Republic; *Grant Holder Scientific Representative of CopyTree*

Tuija Aronen – Natural Resources Institute Finland, Finland; *Grant Awarding Coordinator of CopyTree*

Sandra Correia – University of Coimbra, Portugal; *Leader of WG1*

Itziar Aurora Montalbán – NEIKER-BRTA, Spain; *Co-Leader of WG1*

Elif Aylin Ozudogru – İstinye University Istanbul, Turkey; *Leader of WG2*

Claudia Ruta – University of Bari, Italy; *Co-Leader of WG2*

Nieves Vidal – Misión Biológica de Galicia (MBG-CSIC), Spain; *Leader of WG3*

Liva Purmale-Trasune – Bulduri Technical School, Latvia; *Co-Leader of WG3*

Neslihan Yeşim Yalçın Mendi – Cukurova University, Turkey; *Leader of WG4*

Barbara Ruffoni – Research Centre for Vegetable and Ornamental Crops, Italy; *Co-Leader of WG4*

Valbona Sota – University of Tirana, Albania; *Leader of WG5 & Science Communication Coordinator of CopyTree*

Branislav Cvjetković – University of Banja Luka, Bosnia and Herzegovina; *Co-Leader of WG5*

Dicle Dönmez – Cukurova University, Turkey; *Scientific Innovation Network Coordinator of CopyTree*

Tobias Breugmann – Technical University Freiberg, Germany, formerly Thünen Institute; *Webinar Coordinator of CopyTree*

LOCAL ORGANIZING COMMITTEE

- **Valbona Sota** – ¹Research Center of Biotechnology and Genetics, Academy of Sciences of Albania; ²University of Tirana, Albania
- **Efigjeni Kongjika** – Research Center of Biotechnology and Genetics, Academy of Sciences of Albania
- **Anila Hoda** – General Secretary of the Academy of Sciences of Albania
- **Neki Frashëri** – Head of the Section of Natural and Technical Sciences, Academy of Sciences of Albania
- **Genc Sulçebe** – Director of the Research Center of Biotechnology and Genetics, Academy of Sciences of Albania
- **Ilia Mikerezi** – Research Center of Biotechnology and Genetics, Academy of Sciences of Albania
- **Ruzhdi Kuçi** – Administrator, Academy of Sciences of Albania
- **Brunilda Çuko** – University of Tirana, Albania
- **Kela Misha** – Administrative Sector, Academy of Sciences of Albania
- **Arben Hoxha** - Administrative Sector, Academy of Sciences of Albania
- **Mimoza Monka** – Financial Sector, Academy of Sciences of Albania
- **Linda Luarasi** – University of Tirana, Albania
- **Yllka Lala** – University of Tirana, Albania
- **Eljora Sema** – Administrative Sector, Academy of Sciences of Albania
- **Blerina Shkreta** – Administrative Sector, Academy of Sciences of Albania

Contact:

valbona.sota@fshn.edu.al

copytree21157@gmail.com

RESEARCH TOPICS

WG1: Recalcitrance

Insights into genetics, epigenetics, and protein networks. New chemical and physical tools that might break recalcitrance. Bio-imaging systems.

WG2: Diagnosis, sanitation, and conservation

Diagnosis of viruses, viroids, and bacteria, sanitation protocols for clean stock production, mid- and long-term storage of clean stocks and germplasm.

WG3: Scaling up and automation

Initiation, multiplication, preservation, germination, and conversion of somatic embryos of new species. Temporary immersion systems. Fully automatic micro-propagation systems combining robotics, laser cutting, machine learning, and artificial intelligence.

WG4: Technological risk assessment, public acceptance, legislation, and commercialisation

Risk assessment of plant cloning, strategies to stimulate technology awareness and stakeholder acceptance, stimulating investment and commercialisation.

WG5: Communication, dissemination, and technology transfer

Communication, dissemination, and technology transfer.

Opening Speech

Good morning, dear colleagues, dear friends. It is both a great pleasure and a privilege to welcome you to Tirana for the final conference of the COST Action CopyTree, entitled “From Lab to Forest and Orchard.”

I would first like to express our sincere gratitude to the Academy of Sciences of Albania for hosting this conference, and to Acad. Prof. Dr. Skënder Gjinushi, President of the Academy, under whose auspices this event is being held. I also warmly thank Valbona Sota and the entire local organizing team for their commitment, hospitality, and considerable efforts in bringing us together for these three important days.

When CopyTree began, our ambition was clear: to bring together a fragmented but highly skilled community working on the cloning, propagation, conservation, and sustainable use of woody plants. We wanted to create a network in which researchers, commercial producers, policymakers, students, and other stakeholders could exchange knowledge, share facilities and expertise, and develop solutions that no institution or country could achieve alone. Today, as we meet for our final conference, we can look back with pride on how that ambition has developed into a dynamic and collaborative international community.

Woody plants are fundamental to our societies and ecosystems. Their importance is increasing as we face climate change, emerging diseases, the erosion of genetic resources, and a growing demand for resilient and high-quality planting material. Yet woody plants are often among the most difficult species to propagate under *in vitro* conditions. This challenge has been at the heart of CopyTree.

Our work begins in the laboratory, where we study cells, tissues, microorganisms, genes, hormones, culture media, and developmental processes. However, it cannot end there. Scientific advances must ultimately produce healthy plants, resilient forests, productive orchards, sustainable businesses, conserved genetic resources, and benefits for society.

During this conference, we will hear about new approaches to overcoming recalcitrance, the use of omics and genome-editing technologies, pathogen diagnosis and sanitation, cryopreservation, somatic embryogenesis, temporary immersion systems, digital phenotyping, robotics, machine learning, and artificial intelligence. We will also examine quality assurance, commercialization, regulation, public perception, communication, and the transfer of research results into practice.

CopyTree has also been a network of people. Its most valuable results are not limited to publications, protocols, databases, conferences, or scientific missions. They can also be found in the professional relationships that have been created: between established scientists and young researchers, between laboratories and companies, and between colleagues from different countries who might otherwise never have worked together.

I am particularly proud of the opportunities that the Action has provided for young researchers. Through conferences, training activities, working-group meetings, and Short-Term Scientific Missions, they have gained access to new techniques, laboratories, mentors, and international collaborations. Their energy and scientific curiosity give us confidence that the work initiated through CopyTree will continue well beyond the formal lifetime of the Action.

Our most important knowledge output is the Springer book volume ‘Next-Generation *In vitro* Strategies in Woody Plant Cloning’. It will help preserve and extend the knowledge generated through our collaboration, and it will serve as a lasting memory. We extend our thanks to Valbona Sota for being the driving force behind the editorial team.

I would like to thank our complete Core Group. Your enthusiasm, leadership, patience, creativity, and voluntary commitment have sustained this Action. My sincere thanks also go to COST and to the officers and administrative colleagues who have guided us throughout this journey.

Above all, I thank every active CopyTree participant. Whether you organized an event, hosted a scientific mission, contributed to a publication, reviewed a manuscript, presented a poster, shared a protocol, mentored a young colleague, or simply participated actively in our discussions, you have helped shape this network.

Although this is called our final conference, it should not be understood as an ending. A successful network does not disappear when its official funding period concludes. It continues through joint projects, publications, exchanges, friendships, new consortia, and a shared scientific vision.

Let us therefore use these three days not only to present what we have achieved, but also to decide what comes next. Let us identify the questions that remain unanswered, the technologies that need further development, and the partnerships required to carry our work from experimental systems into forests, orchards, nurseries, conservation programs, and commercial production.

I wish you all an inspiring, productive, and enjoyable conference. May our discussions be open, our collaborations ambitious, and our shared commitment to the future of woody plants stronger than ever.

It is my great pleasure to declare the Final Conference of CopyTree officially open.

Thank you.

**Stefaan Werbrouck,
Chair of CopyTree**

Conference Program

FIRST DAY - September 14, 2026	
08.00 – 08.30	Registration of participants
09.00 – 09.30	CONFERENCE OPENING - Welcome Speech

WORKING GROUP 1 - RECALCITRANCE	
Moderators: Sandra Correia, Itziar A. Montalbán	
09.30 – 10.15	Keynote Speaker: Jesús María Vielba Villegas Recalcitromics: what recalcitrance means in the omics era
10.15 – 10.30	Giovanni Broggin Single-cell regeneration from apple suspension cultures for DNA-free genome editing in a recalcitrant woody species
10.30 – 10.45	Lorenzo Burgos Ortis In-planta transformation combined with developmental genes, a strategy to produce edited non-transgenic woody fruit trees?
10.45 – 11.30	Coffee Break & Poster session
11.30 – 11.45	Nourhene Jouini Development of an <i>in vitro</i> micrografting system to study anatomical and molecular processes associated with graft healing in olive (<i>Olea europaea</i> L.)
11.45 – 12.00	Aygun Sadigova Enhancing rooting efficiency and <i>ex vitro</i> survival in <i>Shirvanshah</i> grapevine through gradient-based auxin stratification in culture medium
12.00 – 12.15	Pablo Piñeiro An efficient micropropagation protocol to overcome recalcitrance in adult <i>Argania spinosa</i>
12.15 – 12.30	Tércia Lopes Almond micropropagation: Hyperhydricity characterisation and rooting assays
12.30 – 12.45	Stéphane Maury Epigenetic regulation of woody plant <i>in vitro</i> culture: from mechanisms, challenges, opportunities to experimental results
12.45 – 13.00	Conchi Sanchez DCPE vs IBA: Early gene activation drives improved adventitious rooting in chestnut
13.00 – 14.15	Lunch Break

14.15 – 14.30	Dicle Dönmez Results of the Ring Test: A quick overview
14.30 – 14.45	Miroslav Perniš From initiation to plant conversion: testing a new cytokinin oxidase/dehydrogenase inhibitor in conifer somatic embryogenesis
14.45 – 15.00	Jaroslav Nisler From inhibition of cytokinin degradation to auxin protection
15.00 – 15.15	Branislav Kovačević CKX – What about rooting stimulation?

WG1 FLASH TALKS

15.15 – 15.22	Ján Jurčík Characterisation of the proteome of extracellular vesicles from <i>Juniperus communis</i>
15.22 – 15.29	İlknur Eskimez Determining the effects of different bud sizes and nutrient media on anther culture in <i>Rosa damascena</i>
15.29 – 15.36	Chaima Ben Othmen Improving adventitious rooting in UCB-1 pistachio rootstock: Comparative effects of novel auxin prodrugs and auxin combinations
15.36 – 15.43	Luca Regni Optimising <i>in vitro</i> propagation of blackberry: Effect of permeable lids and sucrose concentration
15.43 – 16.30	Coffee Break & Poster session

SHORT TERM SCIENTIFIC MISSION SESSION

16.30 – 16.45	<u>Tuija Aronen</u> Overview of CopyTree Short Term Scientific Missions
16.45 – 17.00	Hans Bethge ADAM: Advanced Design and AI-Driven Modeling for Plant Tissue Culture Media Optimization (<i>Working Group 3</i>)
17.00 – 17.15	Sara Yasemin Protoplast isolation and PEG-mediated transient transfection in the recalcitrant <i>Pistacia</i> UCB1 (<i>Working Group 1</i>)
17.15 – 17.30	Olha Yaroshko Phenotyping and genotyping of genetically transformed and CRISPR-Cas gene edited recalcitrant crops (<i>Working Group 1</i>)

SECOND DAY - September 15, 2026	
08.30 – 09.00	Registration of participants

WORKING GROUP 3 - AUTOMATION	
Moderators: Nieves Vidal; Liva Purmale-Trasune	
09.00 – 09.45	Keynote Speaker: Pedro Pablo Gallego Artificial intelligence for large-scale and data-driven optimisation of micropropagation: Towards open-access AI platforms
09.45 – 10.00	Marzieh Mohri Machine learning tools reveal the effect of topolin-type cytokinins on the induction of Norway spruce embryogenic cultures
10.00 – 10.15	Jorge Canhoto Biophysics meets plant morphogenesis - monitoring callus growth and somatic embryogenesis with electrical signals
10.15 – 10.30	Ceren Unek Somatic embryogenesis and image-based deep learning approaches in <i>Coffea arabica</i>
10.30 – 11.00	Coffee Break & Poster session
11.00 – 11.15	Caroline Teyssier Biochemical and infrared spectroscopic characterisation of <i>Pinus radiata</i> somatic embryos during maturation
11.15 – 11.30	Saila Varis Application of somatic embryogenesis to increase the range of conifer tree species used for forestry in Finland
11.30 – 11.45	Carolina Sánchez-Romero Characterisation of embryogenic lines of <i>Abies pinsapo</i> Boiss.
11.45 – 12.00	Emma Ehmke Mimicking drought in germination of somatic hybrid larch (<i>Larix x Eurolepis</i>) by inducing mild and sublethal osmotic stress <i>in vitro</i>
12.00 – 12.15	Karuna Shrestha Establishing an efficient <i>in vitro</i> micropropagation protocol for <i>Fraxinus excelsior</i> tolerant to ash dieback disease
12.15 – 12.30	Lotfi Fki Date palm artificial seeds: current results and perspectives

WG3 + WG2 FLASH TALKS

	Doa'a Jamal Abu-Darwish <i>In vitro</i> somatic embryogenesis and phytochemical profiling of the
--	--

12.30 – 12.37	endangered <i>Hyphaene thebaica</i> for conservation and sustainable utilization
12.37 – 12.44	Brunilda Çuko Evaluation of CKX inhibitors in the presence of trans-zeatin during micropropagation of <i>Prunus domestica</i> L. cv “Tropojane”
12.44 – 12.51	Vera Pavese New technological tools for improving chestnut stress tolerance
12.51 – 12.59	Kubilay Yildirim From poplar transcriptomics to CRISPR validation: functional characterisation of a key gene for metal-toxicity tolerance in trees
13.00 – 14.15	Lunch Break
14.15 – 14.30	Michiel van Bennekom Automation in tissue culture – transforming the transplanting and hardening process of tissue cultured plants
14.30 – 14.45	Teresa Hazubska-Przybył <i>In vitro</i> multiplication of <i>Quercus robur</i> in the RITA system
14.45 – 15.00	Waed Tarraf Temporary immersion bioreactors as a promising approach for large-scale micropropagation and sustainable supply of high-value terpenes in <i>Myrtus communis</i>

WORKING GROUP 4 and 5 - RISK ASSESSMENT AND COMMUNICATION	
Moderators: Yesim Yalçın Mendi; Branislav Cvjetković	
15.00 – 15.45	Keynote Speaker: Marco Pancaldi Strategies for improving the phytosanitary and genetic quality of micropropagated materials. The strategic role of conservation centers
15.45 – 16.15	Coffee Break & Poster session
16.15 – 16.30	Sakari Välimäki Commercialisation of new SE automation – origins of Lauka Biotech
16.30 – 16.45	Rober Ahmad Kreimech Business models and commercialization strategies for woody micropropagation: from lab to forest, orchard and market impact
16.45 – 17.00	Basar Sevindik Effect of Plantform on commercial micropropagation of <i>Paulownia fortunei</i> and <i>Paulownia catalpifolia</i>
17.00 – 17.15	Branislav Cvjetković Public attitude toward new technologies in FRM production – case study Bosnia and Herzegovina

17.15 - 17.30	Buhara Yücesan Mapping the CopyTree ecosystem: A complexity science approach to knowledge sharing and digital impact in woody plant biotechnology
19.30	SOCIAL DINNER

THIRD DAY - September 16, 2026	
08.30 – 09.00	Registration of participants

WORKING GROUP 2 - SANITATION and CONSERVATION Moderators: Elif Aylin Ozudogru; Claudia Ruta	
09.00 – 09.45	Keynote Speaker: Pawel Chmielarz Cryopreservation and <i>in vitro</i> culture of meristems for the conservation of forest genetic resources <i>ex situ</i>
09.45 – 10.00	Stefaan P.O. Werbrouck Tiny allies and hidden threats: bacteria in woody plant micropropagation
10.00 – 10.15	Nuria Alburquerque Transgrafting: a biotechnological solution for plant virus control
10.15 – 10.30	Sebastián Gómez Talquenca From virome to clean planting stock, cost-effective NGS-guided sanitation for <i>in vitro</i> propagation of heritage grapevine
10.30 – 11.15	Coffee Break & Poster session
11.15 – 11.30	Bart Panis Establishing a cryobank for your tree species
11.30 – 11.45	Daniela Duarte <i>In vitro</i> conservation and propagation of endangered Mediterranean thymes for sustainable genetic resource valorisation
11.45 – 12.00	Francisco Javier Alfosea-Simón Understanding the antiviral effects of silver nanoparticles through omics tools in apricot plants cultured <i>in vitro</i>
12.00 – 12.15	Ander Castander-Olarieta How long is too long? Preservation of <i>Pinus radiata</i> embryogenic cultures at -80°C and evaluation of alternative freezing methods
12.15 – 12.30	Marina Martín-Valmaseda Assessing cryotherapy as a sanitation tool for hop stunt viroid in apricot

12.30 – 13.00	Closing Talk + Group picture
13.00 – 14.30	Lunch Break
14.30 – 16.30	Management Committee Meeting
16.30 – 18.30	Guided walking tour

Working Group 1

Recalcitrance

Insights into genetics, epigenetics, and protein networks. New chemical and physical tools that might break recalcitrance. Bio-imaging systems.

Keynote speaker

Recalcitromics: what recalcitrance means in the omics era

Jesús M. Vielba

*Department of Plant Production, Misión Biológica de Galicia (CSIC), Avda de Vigo
s/n, 15705 Santiago de Compostela, Spain*

jmvielba@mbg.csic.es

Abstract

Despite the totipotency inherent to plant cells, a recalcitrant response often arises when plants, particularly woody ones, are treated to induce specific developmental processes, such as adventitious rooting or embryogenesis. The nature of these responses remains elusive, although significant progress has been made in recent years. The adoption of omics techniques provides researchers with a valuable opportunity to expand their toolkit in order to characterise, understand and revert recalcitrant responses in plants. This offers the chance to design specific, tailored protocols that may help to overcome this recalcitrant nature. In this research field, a complete understanding of the biological processes taking place in the tissues under study is essential for the proper scientific and economic exploitation of woody species. This understanding can be achieved through transcriptomics, epigenomics, metabolomics, hormonomics, proteomics and other available techniques, along with their integration through systems biology approaches and the generation of correlation networks. When applied in proper working models, in which other factors such as the genotype or previous hormonal treatments are minimized, it increases the ability to modify current protocols and achieve improved results. This talk will provide a comprehensive analysis of what is currently known about recalcitrance at the molecular level, focusing on its relationship with cellular competence and the capacity of cells to alter their developmental trajectory. Particular emphasis will be placed on adventitious rooting, which will be used as a proxy for characterising plant recalcitrant responses.

Keywords: *cellular competence, data integration, dedifferentiation, omics, recalcitrance*

Oral presentation**Single-cell regeneration from apple suspension cultures for DNA-free genome editing in a recalcitrant woody species****Giovanni Broggini, Jonathan Ohnmacht, Xavier Gaudet, Bruno Studer***Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich,
Universitaetstrasse 2, 8092, Switzerland*giovanni.broggini@usys.ethz.ch**Abstract**

Genome editing offers improvement of specific traits in established cultivars of perennial fruit crops such as apple (*Malus domestica* Borkh.). Conventional approaches based on stable transformation and segregation of CRISPR/Cas9 constructs are limited in apple, as sexual recombination disrupts cultivar-specific trait combinations. To generate genome-edited cultivars free of transgenic sequences, DNA-free ribonucleoprotein (RNP)-mediated editing must be combined with plant regeneration from single cells to ensure genetic uniformity. However, regeneration efficiency from apple protoplasts remains low. Here, we investigate apple suspension cultures as an alternative source of single cells for genome editing and regeneration. These cultures provide rapidly proliferating, dedifferentiated cells with high regenerative potential. Unlike protoplasts, they retain their cell wall, limiting uptake of genome-editing components. We report a protocol for regeneration of plantlets from suspension culture-derived single cells across cultivars. We further assess cell-penetrating peptide-mediated delivery of GFP-labeled RNPs and quantify editing efficiencies in cell pools using sequencing. This work establishes a basis for non-transgenic, single-cell-based genome editing in apple, addressing a key bottleneck in clonal crop improvement and enabling precise, cultivar-preserving trait modification in woody species.

Keywords: *apple, DNA-free editing, single-cell regeneration, suspension cultures*

Oral presentation

In-planta transformation combined with developmental genes, a strategy to produce edited non-transgenic woody fruit trees?

**Lorenzo Burgos¹, Juan Debernardi², Francisco Javier Alfosea Simón¹,
Marina Martín-Valmaseda¹, Nuria Alburquerque¹**

¹Group of Fruit Tree Biotechnology, Department of Plant Breeding, CEBAS-CSIC,
Campus de Espinardo, 25, 30100 Murcia, Spain

²Plant Transformation Facility. UC Davis

burgos@cebas.csic.es

Abstract

A revised classification of some new biotechnological techniques (NBTs), concretely of the edited non-transgenic plants, is being discussed within the European Union that will eliminate the need for strict regulation and opens new possibilities to apply these NBTs to breeding. Fruit trees are generally recalcitrant to regeneration and/or transformation and therefore some approaches, such as in-planta transformation, search to improve them and have been demonstrated to overcome regeneration bottleneck. The idea of infecting meristematic cells but applied to microshoots combines powerful *in vitro* techniques with in-planta transformation. Additionally, different genes involved in plant development (PDG) have been characterized and are used to improve transformation of many recalcitrant species. In this work, we compare the results of combining the transformation of meristematic cells with constructions harbouring different PDGs or combinations of them on the regeneration of transformed and non-transformed shoots. All the constructions used harbour a nptII gene for kanamycin resistance and dsRed for visual identification of transformed shoots as well as one or more of the following genes: IPT, WUS, PLETHORA 5, GRF and GIF. Consequently, the combination of CRISPR-Cas technology with the optimized PDG constructs identified in this work offers a powerful strategy to generate edited, non-transgenic fruit trees that can fully align with the upcoming NGT regulations.

Keywords: *meristems, developmental genes, transformation, edited plants*

Acknowledgements: This work was partially supported by Salvador de Madariaga project number PRX24/00227 for short scientific visits to foreign research institutions.

Oral presentation**Development of an *in vitro* micrografting system to study anatomical and molecular processes associated with graft healing in olive (*Olea europaea* L.)**

**Nourhene Jouini¹, Jorge Canhoto², Aparna S Balan¹, Irene Granata¹,
Francesco Paolo Marra¹, Tiziano Caruso¹, Annalisa Marchese¹**

¹Department of Agricultural, Food and Forestry Sciences, University of Palermo,
Palermo, Italy

²Center for Functional Ecology, Associate Laboratory Terra, Department of Life
Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra,
Portugal

nourhene.jouini@unipa.it

Abstract

This study aimed to develop an efficient *in vitro* micrografting (IVM) system using clonal rootstocks and to characterize the anatomical and molecular events associated with graft healing in olive. Micropropagation protocols were optimized for two olive cultivars (Galega and Arbequina) and two genotypes (G35 and G26). The effects of trans-zeatin, trans-zeatin riboside, and the cytokinin oxidase inhibitor JN2 were evaluated to improve micropropagation protocols. Graft healing was monitored through morphological observations and histological analyses at 7, 14, 21, 30, 45, and 60 days post IVM. Moreover, RNA sequencing analyses were performed to investigate molecular changes during graft development. Successful grafting was strongly influenced by dimensional compatibility between scion and rootstock, and the optimized protocol achieved 100 % graft success rates. Anatomical analyses revealed a coordinated healing sequence involving callus formation, parenchymatic tissue proliferation, and vascular tissue differentiation. Preliminary transcriptomic results at 0, 2, and 7 weeks post-grafting identified 3,009 differentially expressed genes, with the greatest transcriptional reprogramming observed at 7 weeks. Functional enrichment analyses highlighted pathways related to hormone signaling, cell wall remodeling, and vascular development, providing new insights into the mechanisms regulating graft union formation in olive.

Keywords: *clonal rootstock, gene expression, graft healing, vascular connection*

Oral presentation

Enhancing rooting efficiency and *ex vitro* survival in Shirvanshah grapevine through gradient based auxin stratification in culture medium

Aygun Sadigova, Mahira Mammadova

Institute of Molecular Biology, Ministry of Science and Education of the Republic of Azerbaijan, Baku, Azerbaijan

aygunsadigova06@gmail.com

Abstract

Woody plant micropropagation is often limited by the inability of regenerants to form a stable and functional root system, especially in *Vitis vinifera* L. genotypes. This study evaluated a three-component gradient culture medium for staged regulation of rooting in the Shirvanshah technical grapevine cultivar, a valuable indigenous genetic resource of Azerbaijan. The system combines a hormone-free basal layer, a low-auxin zone and a high-auxin layer to regulate root induction, initiation and morphogenesis within one cultivation unit. Among the tested variants, Variant B, containing indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) under reduced nitrogen conditions, showed the best response in complete microshoots, with $74.0 \pm 5.0\%$ rooting, 8.6 ± 0.3 lateral roots per explant and 7.8 ± 0.2 cm mean root length. Complete microshoots performed better than single-node explants, probably due to preserved morphophysiological integrity and endogenous regulatory potential. A peat–perlite substrate combined with gradual hardening supported successful *ex vitro* acclimatisation. The proposed protocol can be applied for mass clonal propagation, *in vitro* collection maintenance and conservation of valuable woody grapevine genotypes.

Keywords: *in vitro*, *ex vitro*, a three-component gradient

Oral presentation**An efficient micropropagation protocol to overcome recalcitrance in adult *Argania spinosa*****Pablo Piñeiro¹, M^aTeresa Martínez¹, Javier Sampedro², Elena Corredoira¹**¹Grupo de Biotecnología y Mejora Forestal, Misión Biológica de Galicia (MBG-CSIC),
Avd Vigo s/n, 15705, Santiago de Compostela, La Coruña, Spain²Departamento de Biología Funcional, Facultad de Biología, Rúa Lope Gómez de
Marzoa, s/n. Campus Sur, 15782 Santiago de Compostela, A Coruña, Spainpablomanuel.pineiro@mbg.csic.es**Abstract**

Argan (*Argania spinosa*) is a forest species endemic to Morocco, with high social, ecological, and economic importance. Overgrazing and overexploitation driven by increased demand for argan oil are threatening this species, which is notoriously difficult to propagate. The development of reliable micropropagation protocols is essential for both conservation of argan forests and establishment of new plantations. This study describes an efficient protocol for micropropagation from adult material via axillary bud proliferation. Shoots were obtained from flushing cuttings of three adult genotypes and established on Murashige and Skoog (MS) medium supplemented with 2 mg L⁻¹ meta-topolin (mT). Efficient proliferation required frequent transfer to fresh media. After two cultures of 3 weeks on MS medium supplemented with 5 mg L⁻¹ mT and 0.5 mg L⁻¹ indole-3-acetic acid (IAA), shoots were transferred to media with reduced mT concentration for 2 weeks. In genotypes with many axillary buds, *in vitro* pruning was necessary to promote shoot elongation. The addition of liquid medium was evaluated as a cost-effective alternative to full renewal. For rooting, several auxin treatments and basal mineral media were tested. The highest rooting efficiency was achieved with 7 mg L⁻¹ indole-3-butyric acid applied for 8 weeks. Responses to the mineral media were dependent on genotype. Half-strength MS and half-strength Driver and Kuniyuki Walnut medium produced the best results.

Keywords: *Argania spinosa*, axillary bud proliferation, *in vitro* rooting, meta-topolin, shoot pruning

Funding: This work was partially funded by the Spanish Ministry of Science, Innovation and Universities (MICIN, Spain) through project PID2024-156422OB-C33, and by the intramural CSIC project 202540E137. Pablo Manuel Piñeiro Insua received an FPU fellowship (Formación de Profesorado Universitario, FPU23/00921) from the MICIN.

Oral presentation**Almond micropropagation: Hyperhydricity characterisation and rooting assays**

Tércia Lopes¹, Mariana Correia¹, Elsa Baltazar¹, Cláudia Marinho², Mónica Zuzarte³, Ricardo Costa^{4,5}, Liliana Marum^{2,6}, Sandra Correia¹, Jorge Canhoto¹

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

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

³QualityPlant – Investigation and production in Plant Biotech., Lda. Coimbra, Portugal

⁴Chemical Engineering and Renewable Resources for Sustainability (CERES), Faculty of Sciences and Technology, University of Coimbra, 3030-790 Coimbra, Portugal

⁵Laboratório Associado para a Química Verde/Rede de Química e Tecnologia (LAQV/REQUIMTE), Molecular Physical-Chemistry R&D Unit, Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal

⁶MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, CEBAL, 7801-908 Beja, Portugal

tercia.lobes@student.uc.pt

Abstract

Almond tree (*Prunus amygdalus*) has great socio-economic and environmental importance in Mediterranean regions. *In vitro* propagation is an important tool for the preservation and multiplication of elite genotypes, producing disease-free plant material. However, the efficiency of this technique remains limited by physiological disorders such as hyperhydricity and by the low rooting capacity of regenerated shoots. In this study, the histological and chemical alterations associated with hyperhydricity were investigated using scanning electron microscopy and light microscopy. Hyperhydric plants exhibited marked changes in the organisation of stem and leaf tissues compared to control, including significant structural alterations and modifications in tissue chemical composition. Variations in lignin content were also detected, suggesting that hyperhydricity interferes with lignification processes and may reduce the mechanical stability of plant tissues. In parallel, several *in vitro* and *ex vitro* rooting assays were carried out to optimize plant regeneration. The most effective results were obtained under *ex vitro* conditions using the commercial rooting formulation Rhizopon AA, which promoted root formation and showed potential for application in almond micropropagation protocols. Overall, these findings improve current understanding of hyperhydricity-related disorders and provide useful information for the optimization of almond *in vitro* propagation and conservation strategies.

Keywords: *Prunus amygdalus*, micropropagation, hyperhydricity, rooting, recalcitrance

Oral presentation**Epigenetic regulation of woody plant *in vitro* culture: From mechanisms, challenges, opportunities to experimental results**

Elena Carneros¹, Vera Inácio², Isabelle Le Jan³, Alexandre Mary³, Jean-François Trontin⁴, Celia Miguel², Pilar S. Testillano¹, Stéphane Maury³

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

²Faculdade de Ciências, Biosystems and Integrative Sciences Institute, Universidade de Lisboa, Lisboa 1749-016, Portugal

³P2e, Université d'Orléans, INRAE, EA 1207 USC 1328, Orléans 45067, France

⁴BEF, INRAE, UR 1138, 54280 Champenoux, France

stephane.maury@univ-orleans.fr

Abstract

The efficiency and reliability of *in vitro* culture of woody plants are strongly shaped by epigenetic regulation, which governs both the stability of clonal identity and the plasticity needed for developmental reprogramming. Here (Carneros/ Inácio et al., in press), we examine the central role of DNA methylation, histone modifications, and non-coding RNAs in controlling totipotency, (de)differentiation and rejuvenation for *in vitro* propagation. Emphasis is placed on recalcitrance, a major limitation in woody species, where (epi)genetic bottlenecks restrict morphogenetic competence. We explore how targeted approaches, epimutants, chemical demethylation and stress priming, can help overcome these barriers, and how predictive epigenetic biomarkers may assist in identifying responsive genotypes and optimal culture conditions. Clonal fidelity, sanitation, and long-term storage are additional challenges, since somaclonal variation and (epi)genetic drift can compromise culture stability and the production of high-quality plants. We also integrate applied perspectives, addressing scalability, risks and biosafety concerns associated with unintended epigenetic changes, as well as the need for clear communication about epigenetic versus genetic modification. Finally, we will illustrate the potential of epigenetic by reporting our recent experimental results obtained using poplar epitypes and chemicals including the Ring test of COPYTREE.

Keywords: epitypes, epigenetics, poplar, ring test

Carneros E, Inácio V, Miguel CM, Testillano P, Trontin J-F, Maury S (in press). Epigenetic regulation of woody plant *in vitro* culture: mechanisms, challenges, and opportunities. In: Sota V, Lambardi M, Vidal N, Werbrouck S (eds) *Next-Generation in vitro Strategies in Woody Plant Cloning*. Springer, Cham

Oral presentation

**DCPE vs IBA: Early gene activation drives improved
adventitious rooting in chestnut**

**Saleta Rico¹, Bruno Fernández-Moreira¹, Jesús M. Vielba¹, Nieves Vidal¹,
María José Cernadas¹, Hajer Darouez², Stefaan P.O. Werbrouck², Conchi
Sánchez¹**

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

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

conchi@mbg.csic.es

Abstract

Adventitious rooting is a major limitation for clonal propagation of chestnut (*Castanea sativa* Mill.), a rooting recalcitrant species. Indole-3-butyric acid (IBA) is commonly used to induce rooting in chestnut. Auxin prodrugs, such as dichlorprop ester (DCPE, commercialized as Corasil®), allow controlled auxin release and can improve rooting performance. This study compared the effect of DCPE and IBA on adventitious rooting of chestnut microshoots at both phenotypic and transcriptomic levels. Rooting assays were conducted using different DCPE concentrations (1, 2.5 and 5 μ M) and exposure times (24, 48 and 72 h). IBA treatments were conducted for 72 h. Additionally, long-read sequencing was used to analyze gene expression at 24 and 72 hours after 0 (controls) and 2.5 μ M IBA or DCPE treatments. Under equivalent conditions, rooting rates were higher in shoots treated with DCPE than in those treated with IBA. Furthermore, DCPE produced vigorous rooted plantlets that were prone to acclimatization. Transcriptomic analysis revealed an earlier and stronger gene activation under DCPE, involving auxin signalling, other hormones pathways (ethylene, ABA, brassinosteroids) and cell wall modification-related processes. These findings suggest that controlled auxin release enhances early molecular responses associated with rooting competence, leading to improved rooting performance. This approach represents a promising strategy to overcome recalcitrance in chestnut propagation.

Keywords: *auxin signalling, controlled auxin release, transcriptomic analysis*

Acknowledgements: This research was partially funded by the Spanish Ministry of Science, Innovation and Universities (PID2024-162781-OB100).

Oral presentation – Ring Test**Results of the Ring Test: A quick overview****Dicle Dönmez***Biotechnology Research and Application Center, Çukurova University, 01250 Sarıçam, Adana, Turkey*dicledonmez4@gmail.com**Abstract**

As part of the COPYTREE project's objectives to promote collaboration through joint experiments (Ring Test), data sharing, and joint publications, four high-performing compounds (CP19, CP20, CP52, and CP77), primarily derived from diphenylurea and synthesized by Dr. Jaroslav Nisler, were selected and distributed to project members. These compounds were evaluated for their activity as cytokinin oxidase/dehydrogenase (CKX) inhibitors in micropropagation, somatic embryogenesis, endosperm culture, and regeneration of woody plant species. The compounds were distributed to COPYTREE members across 19 countries. Each participating laboratory evaluated their efficacy using its own woody plant species (e.g., *Prunus* spp., *Pinus* spp., *Pyrus* spp., and *Picea* spp.). Because CKX inhibitors are generally most effective when used in combination with endogenous cytokinins (iP, iPR, zeatin, and zeatin riboside), the compounds were tested either alone or together with commonly used plant growth regulators (PGRs). By inhibiting CKX activity, these compounds reduce cytokinin degradation and help maintain endogenous cytokinin homeostasis. The results of the Ring Test demonstrated that CKX inhibitors generally enhance cytokinin-dependent growth responses; however, the magnitude of the effect strongly depends on the plant species and the specific PGR combination used. Overall, these compounds improved micropropagation efficiency in several woody species, including carob, blueberry, and *Prunus* spp.

Keywords: CKX inhibitors, cytokinin homeostasis, micropropagation, woody plants

Oral presentation – Ring Test**From initiation to plant conversion: testing a new cytokinin oxidase/dehydrogenase inhibitor in conifer somatic embryogenesis**

Miroslav Pernis¹, Lucie Fischerova², Tuija Aronen³, Salla Varis³, Sakari Välimäki³, Itziar A. Montalbán⁴, Ander Castander-Olarieta⁴, Paloma Moncaleán⁴, Emma Ehmke⁵, Madlen Walther⁵, Andrea Rupps⁵, Teresa Hazubska-Przybyl⁶, Mikołaj Krzysztof Wawrzyniak⁶, Katarína Klubíková¹, Zuzana Vondráková², Jaroslav Nisler²

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

²Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 00 Prague, Czechia

³Natural Resources Institute Finland, Vipusenkuja 5, FI-57200, Savonlinna, Finland

⁴Dep. Forestry Sciences, NEIKER-BRTA, Basque Institute for Agricultural Research and Development, Campus Agroalimentario de Arkaute, Ctra N-104 km 355, Arkaute, 01192 Álava, Spain

⁵Humboldt-Universität zu Berlin, AG Plant Evolution and Biodiversity, Späth-Arboretum, Späthstr. 80/81, 12437 Berlin, Germany

⁶Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

miroslav.pernis@savba.sk

Abstract

Within the CopyTree Ring test, six European laboratories evaluated whether a new cytokinin oxidase/dehydrogenase inhibitor (compound 77) could improve somatic embryogenesis (SE) initiation rates and affect subsequent developmental stages in *Picea abies*, *Pinus sylvestris*, *P. radiata*, *P. nigra* and *Larix x eurolepis*. Compound 77 was applied during initiation and proliferation, and its effects on later SE stages were evaluated. Endogenous phytohormone analysis in *P. abies* revealed that the application of compound 77 did not affect the auxin levels, while it increased the levels of both active and inactive forms of cytokinins in proliferating cultures. However, this metabolic effect did not translate into a consistent improvement of SE efficiency. Across the tested conifers, compound 77 did not lead to an overall increase in initiation rates. Embryo yield and plant conversion showed no consistent improvement, although seedling growth was affected in some systems. These results indicate that compound 77 alters cytokinin metabolism but, as a stand-alone medium supplement, does not broadly enhance conifer SE under the tested protocols, highlighting the need to better understand the role of cytokinins traditionally used in conifer SE.

Keywords: cytokinin homeostasis, cytokinin oxidase inhibitors, conifers, initiation rate somatic embryogenesis

Acknowledgements: This work was supported by the Slovak Grant Agency VEGA, Project No. 2/0014/26; Project LUC24054 of the Czech Ministry of Education, Youth and Sports; the German Federal Ministry of Food and Agriculture, BMELEH, project no. 2222NR005A; projects PID2020-112627RB-C32 and PID2024-156422OB-C32 funded by MICIU/AEI/10; Institute of Dendrology, PAS statutory funding.

Oral presentation – Ring Test**From inhibition of cytokinin degradation to auxin protection****Jaroslav Nisler¹, Federica Brunoni²**

¹*Isotope Laboratory, Institute of Experimental Botany, Czech Academy of Sciences, Czech Republic*

²*Laboratory of Growth Regulators, Faculty of Science, Palacký University & Institute of Experimental Botany, The Czech Academy of Sciences, Czech Republic.*

jaroslav.nisler@gmail.com

Abstract

Compounds 19, 52, 77, and 82, originally developed by Nisler and co-workers as inhibitors of cytokinin oxidase/dehydrogenase (CKX), were found to also inhibit members of the GRETCHEN HAGEN 3 (GH3) family of amidosynthetases responsible for auxin (indole-3-acetic acid, IAA) inactivation. This additional activity is consistent with the stimulation of root growth observed in several plant tissue culture systems. IAA inactivation occurs through conjugation with amino acids, and here we show that compound 19 inhibits this process by targeting the Arabidopsis enzymes AtGH3.6 and AtGH3.17. In contrast, compounds 77 and 82 did not inhibit these GH3 isoforms. However, hormone profiling suggested that they may affect GH3.11 (JAR1), which catalyses the formation of the bioactive jasmonate conjugate jasmonoyl-isoleucine (JA-Ile). Because JA-Ile is a known inhibitor of root growth, reduced JA-Ile formation may contribute to the root-promoting effects of these compounds. Similar responses were observed in both monocotyledonous and dicotyledonous species, suggesting that CKX inhibitors promote root growth through distinct but related mechanisms involving different GH3 isoforms. These findings indicate that the enhanced rooting and shoot proliferation observed in plant tissue cultures result from the combined modulation of cytokinin, auxin, and jasmonate homeostasis.

Keywords: CKX inhibitor, GH3 inhibitor, cytokinin, auxin.

Oral presentation – Ring Test

CKX – what about rooting stimulation?

Vladislava Galović¹, Florin Adrian Huiban², Jaroslav Nisler³, Branislav Kovačević¹

¹*University of Novi Sad, Institute of Lowland Forestry and Environment*

²*University of Life Sciences “King Mihai I” Timisoara, Romania*

³*Palacký University, Olomouc: Olomouc, CZ*

branek@uns.ac.rs

Abstract

CKX Inhibitors of cytokinin oxidases are established as growth promoters in agricultural species. However, in this study we examined their ability to stimulate rooting of one white poplar (LBM) and one eastern cottonwood (S6-7) clone. The hardwood cuttings were treated with powder formulation of different contents of two CKX inhibitors: 52 and 77. Two and a half months after the treatment and planting, the two shoots and six rooting parameters that describe initial growth and development of rooted cuttings were measured. Treatment 52/3 (0.001%) powder formulation showed some inhibitory effect on shoot height in S6-7, while 77/1 (0.1%) achieved stimulatory effect on number of leaves in LBM. The reaction by rooting parameters include stimulatory effect of 77/1 (0.1%) powder formulation on number of roots on basal part of cutting (RN5) in LBM, but inhibitory effect on rooting on middle part of cutting by 52/2 (0.01%), 77/3 (0.001%) and 77/4 (0.0001%) in LBM. There was stimulatory effect of 52/3 (0.001%) on rooting on upper part of cutting in LBM, but inhibitory effect on rooting on the same part of the cutting by 77 in all concentrations. There was stimulatory effect of 52/3 (0.001%) on rooting on upper part of cutting in S6-7 too. The stimulatory effect was also notified 77/1 (0.1%) on rooting on lower part with basal cut of cutting in LBM, but inhibitory effect of 77/4 (0.0001%) on total number of roots in the same clone. Obtained results suggest that there is potential for use of CKX in rooting stimulation in poplars, supporting future research in this field.

Keywords: CKX, rooted cuttings, poplar

Acknowledgement: This study has been financed partly by CopyTree Cost Action CA21157 and partly by Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Project No: 451-03-33/2026-03/ 200197).

STSM presentation**Phenotyping and genotyping of genetically transformed and CRISPR-Cas gene edited recalcitrant crops****Olha Yaroshko***Institute of Cell biology and Genetic engineering NAS of Ukraine, Acad. Zabolotnoho, 148, 03143, Kyiv, Ukraine**90tigeryaroshko90@gmail.com***Abstract**

Genetic transformation and gene editing of recalcitrant plants remain a major challenge in crop biotechnology. This study aimed to evaluate the efficiency of *Agrobacterium*-mediated transformation and CRISPR-Cas9 gene editing for improving shoot regeneration in *Amaranthus hypochondriacus*, orphan crop with high regeneration and transformation recalcitrance. A double transformation was applied by co-agroinjection of apical, axillary meristems with two *A. tumefaciens* suspensions: one carrying vector pMKV057 (containing *ZmWUS2*, *IPT*, *Luc*, and *nptII* genes, to enhance shoot regeneration) and one carrying vector pMGS37 (encoding Cas9 and guide RNAs targeting betalain biosynthesis genes *AhCYP76AD2* and *AhCYP76AD5*). A total of 460 plants were treated. Tumour formation was detected in 22.17% of plants, with 5.1% efficiency per injected area. One plant showed *de novo* multiple shoot formation. Coloration changes in all injected meristems confirmed activation of betalain synthesis. These results demonstrate that double agroinjection is a promising method for transformation and gene editing of recalcitrant species. The developed phenotyping protocols and obtained data contribute to advancing biotechnology tools for orphan crops and sustainable agriculture.

Keywords: *Agrobacterium*-mediated transformation, *Amaranthus hypochondriacus*

STSM presentation**Protoplast isolation and PEG-mediated transient transfection in the recalcitrant *Pistacia* UCB1****Sara Yasemin¹, Saba Taheri³, Chaima Ben Othmen^{2,3}, Stefaan Werbrouck³**¹Department of Horticulture Siirt University, Faculty of Agriculture, Siirt, Türkiye²Laboratory of Horticultural Sciences, National Agronomic Institute of Tunisia, University of Carthage, Tunis 1082, Tunisia³Laboratory for Applied In vitro Plant Biotechnology, Ghent University, Ghent, Belgiumsarayaseminbenelli@gmail.com**Abstract**

Pistacia spp. are recalcitrant woody crops for *in vitro* manipulation, limiting the application of functional genomics and genome-editing tools. Here, we developed a reproducible protocol for protoplast isolation and PEG-mediated transient transfection in the *Pistacia* UCB1 genotype. *In vitro* leaves, callus tissues and regenerated shoot tissues were compared as starting material, together with cellulase–macerozyme enzyme mixtures. Leaf tissues produced low and inconsistent yields, whereas shoot-derived materials gave the highest recovery after optimisation of tissue source and enzyme composition. The optimised protocol yielded up to 1.0×10^6 protoplasts mL⁻¹, with approximately 70% viability as determined by fluorescein diacetate staining. Transient delivery of a GFP-expressing plasmid by PEG treatment resulted in detectable fluorescence in protoplasts, with an estimated transfection efficiency of approximately 40%. Positive and negative controls confirmed that fluorescence resulted from plasmid-mediated GFP expression. To our knowledge, this represents one of the first reproducible protoplast isolation and PEG-mediated transient transfection systems reported for *Pistacia*, providing a practical platform for future functional genomics and genome-editing studies in this recalcitrant woody crop.

Keywords: *Pistacia* sp., protoplast biotechnology, transient transformation, CRISPR platform, woody crop recalcitrance

Flash talk presentation**Characterisation of the proteome of extracellular vesicles from
*Juniperus communis*****Ján Jurčík¹, Miroslav Perniš¹, Zuzana Kozovská², Maksym Danchenko¹,
Katarína Klubicová¹**¹*Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center p. r. i., Slovak Academy of Sciences, Akademická 2, P.O. Box 39A, 95007 Nitra, Slovakia*²*Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovakia*jan.jurcik@savba.sk**Abstract**

Extracellular vesicles (EVs) are nano-sized membrane particles secreted by cells, including plant cells. They transport proteins, DNA, RNA, lipids, and secondary metabolites, thereby contributing to cell communication and immunity. Our laboratory studies somatic embryogenesis (SE) in selected conifers and has initiated research into the possible role of EVs in this process. To support this aim, we optimised a protocol for isolating EVs from conifer plant material for proteomic analysis. As a pilot study, we analyzed the EV proteome of *Juniperus communis* berries. The berries were pooled, homogenized, clarified by differential centrifugation, concentrated by ultrafiltration, and purified by size-exclusion chromatography (SEC). EV fractions were evaluated by nanoparticle tracking analysis (NTA), and their protein cargo was analyzed by LC-MS/MS. Functional annotation of proteins highlighted EV-relevant pathways associated with trafficking, stress-defense responses, signaling, and cell-wall/carbohydrate metabolism. The analysis also revealed proteins lacking a canonical signal peptide. These results provide the first proteomic overview of EVs from *J. communis* berries and support their relevance for studying unconventional protein secretion through plant-derived EVs. The optimised workflow yielded EV fractions with consistent and sufficient concentrations for downstream LC-MS/MS analysis.

Keywords: *extracellular vesicles, EVs, SEC, somatic embryogenesis, Juniperus communis, LC-MS/MS*

Acknowledgements: This work was supported by the Slovak Grant Agency VEGA, Proj. No. 2/0014/26.

Flash talk presentation

Determining the effects of different bud sizes and nutrient media on anther culture in *Rosa damascena*

İlknur Eskimez, Mehmet Polat

Isparta University of Applied Sciences, Faculty of Agriculture, Horticulture, Isparta, Türkiye

ilknureskimez01@gmail.com

Abstract

Widely cultivated for essential oil production and as an ornamental plant, the rose (*Rosa damascena* Mill.) is an important crop with high economic value. Increasing commercial demand highlights the importance of research aimed at developing new genotypes with superior characteristics and obtaining standard propagation material. In this context, anther culture, used in plant biotechnology, stands out as an important method enabling the evaluation of genotypes under *in vitro* conditions and the development of regeneration systems. However, studies on anther culture in *Rosa damascena* are quite limited, and there is a need to determine the appropriate explant development stage and nutrient medium for a successful regeneration system. This study aimed to determine the effects of culturing anthers obtained from flower buds of different sizes in *Rosa damascena* in various nutrient media on callus formation, embryogenesis, and plant regeneration. In the study, anthers isolated from buds representing different developmental stages were cultured in nutrient media containing different plant growth regulators; callus induction rate, embryo formation, and regeneration success were evaluated comparatively. The findings are expected to contribute to the development of an effective and reproducible anther culture protocol for *Rosa damascena* and to provide a basis for future tissue culture and biotechnology studies.

Keywords: *Rosa damascena*, anther culture, medium

Flash talk presentation**Improving adventitious rooting in UCB-1 pistachio rootstock:
Comparative effects of novel auxin prodrugs and auxin
combinations****Chaima Ben Othmen^{1,2}, Taoufik Bettaieb¹, Stefaan P.O. Werbrouck²**¹*Laboratory of Horticultural Sciences, National Agronomic Institute of Tunisia,
University of Carthage, 43 Av. Charles Nicolle, Tunis 1082, Tunisia*²*Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, Valentin
Vaerwyckweg 1, 9000 Ghent, Belgium*Chaimabenothmenn11@yahoo.fr**Abstract**

Adventitious rooting remains a major constraint in the clonal propagation of UCB-1 pistachio rootstock. This study evaluated DCPE, a novel auxin ester prodrug, and its hydrolysis product DCP as potential alternatives to indole-3-butyric acid (IBA), the standard auxin used for root induction. Microshoots were treated with 5, 10, 15, and 20 μM of each compound in POM medium supplemented with 1 mM phloroglucinol as a rooting enhancer. After a 1 h induction pulse, explants were incubated in darkness for 1 week at 26 °C and then transferred to fluorescent light. In a second experiment, mOCF3-2HE (coded JN2), an auxin oxidation and GH3 inhibitor, was tested individually and in combination with IBA or the benzoxazole auxin stimulator Arinole (ARI) at 0.1 and 1 μM , together with a hormone-free control, to evaluate whether modulation of auxin homeostasis could improve rooting. DCP showed very limited rooting capacity, with a maximum rooting rate of 6.7% at 5 μM . DCPE performed better, reaching 13% rooting at 5 and 10 μM , whereas higher concentrations were inhibitory. In the second experiment, JN2, IBA, and ARI applied individually at 0.1 or 1 μM did not induce rooting. Among the combination treatments, only JN2 + ARI at 1 μM each promoted rooting, with a rooting rate of 16%. Although rooting remained limited overall, DCPE and the JN2 + ARI combination showed measurable activity and may provide useful leads for further optimisation of rooting protocols in UCB-1 pistachio rootstock.

Keywords: *rootstock, micropropagation, adventitious rooting, auxin prodrug, GH3 inhibitor, DCPE*

Flash talk presentation

Optimizing *in vitro* propagation of blackberry: Effect of permeable lids and sucrose concentration

Luca Regni¹, Arianna Cesarini¹, Raffaella Brigante², Laura Marconi², Sara Zollini³, Nieves Vidal⁴, Primo Proietti¹

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

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

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

⁴*Misión Biológica de Galicia, Consejo Superior de Investigaciones Científicas, Avda de Vigo s/n, 15705 Santiago de Compostela, Spain*

luca.regni@unipg.it

Abstract

The increasing demand for blackberries has highlighted the need for efficient propagation systems, making micropropagation the preferred technique. This study evaluated the effects of vessel ventilation (plastic containers with or without filters in the lid) and sucrose concentration (0, 7.5, 15, and 30 g L⁻¹) on the micropropagation of blackberry cv. 'Thornfree'. Sucrose significantly affected all parameters, including shoot number, shoot length, fresh weight, callus incidence, callus weight, and total dry weight, whereas ventilation influenced only shoot number and callus weight. In particular, filters enhanced shoot proliferation and callus development. Sucrose-free medium reduced shoot number, shoot length, callus incidence, and callus weight. The highest callus weight and total dry weight were observed in ventilated vessels containing 15 and 30 g L⁻¹ sucrose, while the highest shoot fresh weight was obtained with 7.5 and 15 g L⁻¹ sucrose. A non-destructive smartphone-based 3D reconstruction approach (iPhone + ViDoc) was further applied to explants grown in ventilated vessels, enabling the extraction of structural and geometric traits from point clouds, including shoot height, projected area, volume, and structural density indices. The reconstructed models highlighted differences in shoot spatial organization and compactness among sucrose treatments. These results provide initial insights into optimising blackberry micropropagation through the use of ventilated vessels and reduced sucrose concentrations in the culture medium.

Keywords: *Rubus spp*, shoot proliferation, photoautotrophic micropropagation, culture media optimisation

Poster presentation**Epigenetic and non-coding RNA-mediated regulation of plant regeneration: Insights from tamarillo (*Solanum betaceum* Cav.) for woody plants****Sandra Correia, André Caeiro, Daniela Cordeiro, Mariana Neves, Miguel Rito, Ricardo Ferraz, Jorge Canhoto**

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

sandraimc@uc.pt

Abstract

Plant regeneration is essential for propagation, genetic transformation, and genome editing. However, many important crops and resilient genotypes are difficult to regenerate *in vitro*, hindering large-scale breeding and efficient biotechnological applications. We have investigated the molecular regulation of somatic embryogenesis and organogenic responses in woody and horticultural crops to elucidate the mechanisms controlling regeneration competence and developmental plasticity under *in vitro* conditions. In this context, we have focused on epigenetic and post-transcriptional regulation, including chromatin remodelling dynamics and non-coding RNA (ncRNA)-mediated regulatory networks, as key drivers of cellular reprogramming. Most of this work has used the woody solanaceous species tamarillo (*Solanum betaceum* Cav.) as a model system, owing to its intrinsic regenerative capacity. Our results consistently link regeneration efficiency to extensive epigenetic reprogramming associated with the transition from somatic identity to embryogenic and/or organogenic competence. Moreover, integrative multi-omics analyses have revealed regulatory networks involving auxin and ethylene perception and signalling, carbohydrate metabolism, and ribosomal RNA methylation, all of which contribute to the control of cellular totipotency and regeneration success. This work emphasises the integration of epigenetic and ncRNA regulation to understand woody plant regeneration.

Keywords: *epigenetic reprogramming, organogenic competence, somatic embryogenesis*

Poster presentation**Exploiting the untapped potential of fruit tree wild diversity for sustainable agriculture (FruitDiv Project)****Vlatko Andonovski, Nikolco Velkovski, Viktorija Brndevska Stipanović***Ss. Cyril and Methodius University in Skopje, Hans Em Faculty of Forest Sciences,
Landscape Architecture and Environmental Engineering**vandonovski@sf.ukim.edu.mk***Abstract**

The domestication of fruit trees has significantly reduced the genetic diversity of modern cultivars, limiting their resilience to climate change-related abiotic and biotic stresses. Crop Wild Relatives (CWR) of major pome and stone fruits, including apple, pear, cherry, and plum, represent an important but underutilized source of genetic variation for traits such as drought tolerance, disease resistance, and environmental adaptation. The Horizon Europe project FRUITDIV (2024–2028) aims to monitor, characterise, conserve, and utilize the diversity of fruit tree CWR to improve the sustainability and climate resilience of European fruit production systems. The project brings together 27 partners from 14 countries and applies a multidisciplinary approach integrating inventory development, genotypic and phenotypic characterisation, multi-omics analyses, and pre-breeding strategies. FRUITDIV will catalogue wild fruit genetic resources conserved in repositories and natural habitats, assess their diversity using molecular markers, and establish multispecies core collections. The project will also generate phenotypic, environmental, and genomic data, including a pangenome linking wild and cultivated genomes, to support targeted breeding strategies and identification of adaptive alleles. FRUITDIV will deliver publicly accessible resources, including genomic datasets, phenotyping data, pre-breeding materials, and conservation frameworks, contributing to the sustainable use and long-term preservation of wild fruit genetic resources. By strengthening the link between conservation and breeding, the project will support the development of resilient fruit varieties and enhance the sustainability and productivity of European agriculture.

Keywords: *genetic diversity, abiotic and biotic stress, fruit tree conservation, multi-omics, pangenome, Horizon Europe*

Poster presentation**Use of in-vitro-culture to decipher the transcriptomic answer of alder to *Phytophthora* infection****Ben Bubner, Sebastian Horn, Ana-Paula Montalvão***Thuenen Institute of Forest Genetics, Eberswalder Chaussee 3a, 15377
Waldsiedersdorf, Germany**ben.bubner@thuenen.de***Abstract**

Phytophthora disease of alder (*Alnus glutinosa*) has threatened European riparian forests since the 1990s. It is caused by the hybrid oomycete species, *Phytophthora* × *alni*. Because this waterborne pathogen spreads efficiently in wet habitats yet survives poorly in dry soils or without host tissue, controlled studies and pathogen recovery from field material can be challenging. The underlying host responses associated with infection and potential resistance to this pathogen therefore remains insufficiently understood. Here, our objective was to establish a controlled *in vitro* infection system and used it to generate an exploratory transcriptomic resource to characterize alder responses to *P. × alni* and identify a core set of infection-responsive genes across genetic backgrounds. We infected in-vitro-plants from genotypes of two species (*Alnus glutinosa* and *A. incana*) with a *Phytophthora* × *alni* isolate from northeastern Germany and profiled gene expression by RNA-seq. Accounting for genotype effects, we identified 41 significant infection-associated expression changes. Induced genes were enriched for defense-related processes, including an FMO1-like transcript implicated in systemic acquired resistance, whereas downregulated genes were enriched for growth- and photosynthesis-associated functions. Overall, this work provides a reproducible experimental framework and shows the usefulness of in-vitro-cultures to support future studies linking transcriptional responses to phenotypes and informing resistance screening and breeding.

Keywords: *Phytophthora* × *alni*, RNAseq, systemic acquired resistance

Poster presentation

Getting more resilient conifers: What we have learned by modifying environmental conditions during the somatic embryogenesis process

Isabel Arrillaga¹, M. Amparo Pérez-Oliver¹, Álvaro Rodríguez-Cuesta², Diana C. López-Álvarez³, M. Carmen González-Mas¹, Esther Asensio⁴, Ester Sales²

¹*Instituto de Biotecnología y Biomedicina (BiotecMed) y Dpto. Biología Vegetal, Universidad de Valencia (UV). Facultad de Farmacia, Av. Vicent Andrés Estellés 22, 46100 Burjassot, Spain*

²*Dpto. Ciencias agrarias y del Medio natural, Instituto de Investigación en Ciencias Ambientales (IUCA), Universidad de Zaragoza. Escuela Politécnica Superior, Ctra. Cuarte s/n, 22005 Huesca, Spain*

³*Dpto. Ciencias Biológicas, Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia. Sede Palmira, Ctra. 32 # 12 -00, Palmira, Colombia*

⁴*Dpto. Analytical Chemistry, Faculty of Veterinary Sciences, University of Zaragoza, C/ Miguel Servet 177, 50013 Zaragoza, Spain*

Isabel.arrillaga@uv.es

Abstract

Our last year's work is being devoted to produce maritime pine trees adapted to abiotic stress by applying priming treatments during the somatic embryogenesis process. We analyzed physiological, genetic and metabolic changes in the embryogenic masses and in the derived somatic plants. Applying a heat pulse to megagametophytes at the induction phase upregulated some transcription factors related to stress that were maintained in the derived plants that showed a certain degree of tolerance to heat and cross-tolerance to drought in greenhouse trials. Decreasing water availability during the proliferation phase produced osmolyte accumulation in plants that recovered better after drought stress, whereas repetitive short heat pulses altered the transcriptome towards increased expression of genes encoding PPR proteins, in both embryogenic masses and derived plants. Decreasing temperature by 5 °C during the maturation phase, also modified transcriptome upregulating genes involved in epigenetic changes, whereas a 5 °C increase in temperature upregulated genes involved in heat stress. All treatments altered phenol and terpene profiles of plants. In summary, modifying environmental conditions during the somatic embryogenesis process has been proved to be an efficient tool to produce maritime pine plants coping with adverse conditions derived of climate change.

Keywords: *maritime pine, somatic embryogenesis, priming, resilience*

Supported: PID2024156422OBC31/MICIN/AEI/10.13039/501100011033/FEDER; GVA/CIAICO/2023/005; EU grant to ARC and UV grant to MAPO.

Poster presentation**Enhancing transformation of recalcitrant woody plants through a modular morphogenic regulator–Geminivirus-Based Replicon System****Ashkan Hodaei¹, Laurens Pauwels^{2,3}, Stefaan P.O. Werbrouck¹**¹*In-vitro Plant Biotechnology Lab, Department of Plants & Crops, Faculty of Bioscience engineering, Ghent University, Ghent, Belgium*²*Laboratory for Plant Transformation & Gene Editing, Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium*³*VIB Center for Plant Systems Biology, Ghent, Belgium*ashkan.hodaei@ugent.be**Abstract**

Genetic transformation of woody plants remains a major bottleneck in plant biotechnology due to genotype dependency, limited regeneration competence, and inefficient recovery of transformed tissues. These constraints continue to restrict functional genomics and precision breeding applications such as CRISPR across woody and perennial crops. Recent advances in developmental regulator (DR) technologies have shown that morphogenic regulators (MRs) can substantially enhance regeneration and transformation efficiency. Key regulators, including WUSCHEL (WUS), BABY BOOM (BBM), GRF-GIF modules, and the cytokinin regulator ISOPENTENYL TRANSFERASE (IPT), stimulate somatic embryogenesis, shoot regeneration, and cellular reprogramming. Complementarily, Geminivirus-Based Replicon Systems (GBRS) increase transient transgene copy numbers and expression during early transformation, enabling high accumulation of genetic cargo in plant cells. We present a modular ~20 kb plasmid platform for flexible CRISPR construct assembly in woody and recalcitrant species. The vector features interchangeable modules allowing rapid gRNA replacement via restriction digestion and simple insertion of selectable or visual marker cassettes through Golden Gate assembly. The system integrates MRs with a GBRS to enhance transient developmental signaling during early dedifferentiation and regeneration, improving recovery of transformed cells. We welcome academic and industrial collaborations to evaluate this platform across diverse crops and tree species.

Keywords: *CRISPR, plant transformation, woody plants, recalcitrant species, regeneration competence*

Poster presentation**CRISPR/Cas9-based strategies for enhancing disease resistance in holm oak**

Manuel Caamaño-Mayo^{1,2}, M^a Teresa Martínez¹, Pablo Piñeiro¹, Javier Sampedro², Elena Corredoira¹

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

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

manuel.caamano.mayo@rai.usc.es

Abstract

Quercus ilex is a species of major ecological, economic, and social importance in Spain, particularly in Mediterranean dehesa ecosystems. However, in recent decades, it has been severely affected by “oak decline” disease, primarily caused by *Phytophthora cinnamomi*. In this context, genome editing emerges as a promising strategy for the development of new genotypes with enhanced resistance to this pathogen. This study investigated the application of CRISPR/Cas9 technology to inactivate the susceptibility gene *PMR4* (*Powdery Mildew Resistant 4*). This gene encodes a callose synthase and its mutation has conferred tolerance to fungal pathogens in other species. Somatic embryos from two lines, E2 and Q8, were transformed using *Agrobacterium tumefaciens* AGL-1 with a CRISPR construct that produces four gRNAs targeted to *PMR4*. Editing efficiency was assessed 10 weeks after transformation by inducing frameshift mutations in the marker gene *GFP* (*Green Fluorescent Protein*). Fluorescence was detected in 11.8% and 8.3% of explants for E2 and Q8, respectively, with no significant differences between genotypes. Sequencing of *PMR4* in fluorescent embryos showed successful inactivation in all the samples that could be amplified, with numerous large deletions and high editing efficiencies for all 4 gRNAs. Furthermore, preliminary tolerance assays suggest that *PMR4* silencing may reduce embryo susceptibility to the pathogen compared with wild-type embryos.

Keywords: *GFP*, oak decline, *Phytophthora cinnamomi*, *Quercus ilex*, susceptibility genes

Poster presentation**Optimisation of an effective sterilization protocol for the micropropagation of GF677 rootstock****Melina Roboçi¹, Brunilda Çuko¹, Efigjeni Kongjika², Valbona Sota^{1,2}**¹*Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania*²*Research Center of Biotechnology and Genetics, Academy of Sciences of Albania, Tirana, Albania*melina.roboci@fshnstudent.info**Abstract**

GF677 (*Prunus amygdalus* × *Prunus persica*) is a hybrid rootstock commonly used in pomology due to its iron chlorosis resistance and adaptability to calcareous soils. It is usually propagated vegetatively by cuttings, but *in vitro* micropropagation offers an effective alternative for the mass production of uniform plants. However, the success of micropropagation is strictly dependent on the surface sterilization of plant material collected from the field. In this context, this study aimed to optimise a sterilization protocol for GF677 apical and lateral buds. Plant material was collected from a nursery in Albania in April 2026. In order to remove external materials, the explants were washed under running tap water and then they were dipped in 70% ethanol. Afterwards, they were treated with 0.01% mercuric chloride (HgCl₂) under varying exposure durations (10, 15 and 20 minutes). The explants were then rinsed with successive washes of sterile distilled water to remove any possible residues of the mercuric compound before inoculation into tubes with LP nutrient medium. Within one week of culture establishment, two main types of contamination were detected: bacterial halos around the base of the explant and fungal growths of filamentous mycelium. The results showed that a treatment of 0.01% HgCl₂ for 15 minutes followed by a series of washes with sterile distilled water was the most effective in reducing contamination to a minimum level without inducing tissue necrosis, thereby establishing a controlled protocol for subsequent GF677 *in vitro* culture.

Keywords: GF677, micropropagation, surface sterilization, mercuric chloride, contamination, tissue necrosis

Poster presentation**CRISPR/Cas9-mediated mutagenesis of the susceptibility gene *CsDMR6* in European chestnut**

Vera Pavese¹, María Teresa Martínez², Pablo Piñeiro², Silvia Valladares^{2*}, José Luis Couselo², Manuel Caamaño^{2,3}, Lorenzo Antonio Marino¹, Martina Ferrero¹, Daniela Torello Marinoni¹, Javier Sampedro³, Roberto Botta¹, Andrea Moglia¹, Elena Corredoira¹

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

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

³Departamento de Biología Funcional, Facultad de Biología, Rúa Lope Gómez de Marzoa, s/n. Campus Sur, 15782 Santiago de Compostela, A Coruña, Spain

elenac@mbg.csic.es

Abstract

Castanea sativa Mill. is a tree species with great ecological, economic, and social importance in Europe. However, its long-term sustainability is severely threatened by ink disease, mainly caused by the oomycete *Phytophthora cinnamomi* (Pc), whose incidence has increased under current climate change conditions. In this context, CRISPR/Cas9 genome editing has emerged as a powerful tool for the development of improved genotypes with enhanced resilience to Pc, as well as increased adaptive capacity to heat and drought stress. Therefore, the main objective of this study was to assess the potential of CRISPR/Cas9-mediated targeted mutagenesis of the susceptibility gene *DMR6* (*Downy Mildew Resistance 6*), a negative regulator of plant immune responses, to enhance resistance-associated responses against Pc in European chestnut. Somatic embryos were transformed using an *Agrobacterium*-mediated approach to introduce a CRISPR/Cas9 construct targeting *DMR6*. Eleven transgenic embryogenic lines resistant to the selective agent were successfully generated. Sanger sequencing confirmed the presence of mutations at the target locus, demonstrating efficient genome editing. Editing efficiencies ranged from 85.9% to 98.3% for the first guide RNA and from 43.9% to 51.1% for the second guide RNA. Furthermore, *in vitro* infection assays with Pc showed that three edited lines displayed significantly reduced susceptibility compared with the corresponding non-edited control lines.

Keywords: ink disease, *Phytophthora cinnamomi*, *Castanea sativa*, somatic embryos, susceptibility genes

Funding: This work was supported by Ministerio de Ciencia, Innovación y Universidades (MICIN, Spain) through the project PID2024-156422OB-C33 and by the Italian National Recovery and Resilience Plan (PNRR) – M4C2 Inv. 1.4, Project Agritech CN00000022 (CUP:D13C22001330005).

Poster presentation**Kinase inhibitors as novel chemical promoters of somatic embryogenesis in European chestnut and holm oak**

María Teresa Martínez¹, Cristina Buceta¹, Fátima Mosteiro¹, Pablo Piñeiro¹, Elena Carneros², Alfonso García-Rubia³, Carmen Gil³, Ana Martínez³, Pilar S. Testillano², Elena Corredoira¹

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

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

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

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

Abstract

Somatic embryogenesis (SE) is widely recognized as the most efficient regeneration pathway for the clonal propagation of forest species. However, the induction of SE remains a major challenge in many woody species, including European chestnut (*Castanea sativa*) and holm oak (*Quercus ilex*), particularly when explants are derived from adult trees. The aim of this study was to identify novel compounds capable of enhancing SE induction through the screening of chemical libraries originally developed for pharmaceutical applications and to evaluate their effectiveness in these two recalcitrant forest species. Recent studies have shown that small-molecule inhibitors of mammalian glycogen synthase kinase 3 β (GSK3 β) and leucine-rich repeat kinase 2 (LRRK2) can stimulate the initiation of SE, highlighting the potential of chemical biology approaches to improve plant regeneration. Leaves and shoot apices excised from axillary shoot cultures of chestnut, and shoot apices from holm oak, were used as explants for SE induction. Among the compounds tested, LRRK inhibitors consistently outperformed GSK3 β inhibitors in both species. In particular, compounds IGS 4.25 and IGS 4.75 showed the greatest ability to enhance SE induction, resulting in the highest embryogenic responses and demonstrating their potential as effective tools for improving regeneration in recalcitrant woody species.

Keywords: *Castanea sativa*, cell reprogramming, *Quercus ilex*, small molecule inhibitors

Funding: Work partly supported by projects PID2023-146824OB-I00 (funded by MCIN/AEI/10.13039/501100011033) and PID2024-156422OB-C33 (funded by MCIN/AEI).

Poster presentation**Effects of PDE inhibitors on somatic embryogenesis induction in European chestnut and holm oak**

María Teresa Martínez¹, Cristina Buceta¹, Fátima Mosteiro¹, Pablo Piñeiro¹, Elena Carneros², Alfonso García-Rubia³, Carmen Gil³, Ana Martínez³, Pilar S. Testillano², Elena Corredoira¹

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

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

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

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

Abstract

European chestnut and holm oak are highly recalcitrant to somatic embryogenesis (SE), particularly when mature tissues are used as explants. In this study, we aimed to identify novel chemical compounds capable of enhancing SE induction by screening chemical libraries originally developed for pharmaceutical applications. Among the compounds evaluated, phosphodiesterase (PDE) inhibitors emerged as promising candidates. These small molecules prevent the degradation of cyclic AMP (cAMP), a key signaling molecule involved in cellular reprogramming in mammalian systems. Although PDE inhibitors have been widely used to enhance cellular reprogramming in animals, their potential role in plant cell reprogramming and SE remains largely unexplored, with the exception of our previous findings (1). Leaves of European chestnut and shoot apices of holm oak, both excised from axillary shoots, were used as explants for SE induction. Explants were cultured on induction media supplemented with seven different PDE inhibitors. Among the compounds tested, IGS 1.18 significantly enhanced SE induction in both species compared with the control treatment, highlighting its potential as a novel chemical enhancer of SE in woody plants. These findings provide new insights into the potential involvement of cAMP-mediated signaling in plant cellular reprogramming and suggest that PDE inhibitors may represent a promising strategy for improving SE induction in recalcitrant woody species.

Keywords: *Castanea sativa*, cell reprogramming, *Quercus ilex*, small molecule inhibitors

Funding: Work partly supported by projects PID2023-146824OB-I00 (funded by MCIN/AEI/10.13039/501100011033) and PID2024-156422OB-C33 (funded by MCIN/AEI).

Poster presentation***In vitro* propagation of the Greek endemic species *Sideritis euboica* and *Saponaria aenesia*: A biotechnological approach for conservation and sustainable exploitation****Eirini Sarrou¹, Aikaterini Grigoriadou¹, Eleni Abraham²**¹*Hellenic Agricultural Organization Demeter, Institute of Plant Breeding and Genetic Resources, Thermi, 57001, Thessaloniki, Greece*²*Aristotle University of Thessaloniki, Faculty of Forestry and Natural Environment, Laboratory of Range Science, 54124, Thessaloniki, Greece*eabraham@for.auth.gr**Abstract**

The conservation of endemic plant species with restricted natural distribution has become increasingly important due to habitat degradation, climate change, and anthropogenic pressures. *Sideritis euboica* and *Saponaria aenesia* are two Greek endemic taxa of high ecological and medicinal value, characterised by limited natural populations and propagation constraints. Plant tissue culture offers an efficient alternative for rapid clonal propagation, *ex situ* conservation, and the production of high-quality planting material, contributing to biodiversity preservation and future cultivation efforts. Seeds collected from natural populations from Evoia and Cefalonia (Greece) were surface sterilized and established under aseptic conditions. Shoot proliferation was evaluated on Murashige and Skoog (MS) medium supplemented with different concentrations of plant growth regulators. Multiplication efficiency and the number of regenerated shoots were recorded after successive subcultures. Root induction was investigated using auxin-enriched media. Morphological observations and survival rates were assessed throughout the propagation process. Optimised culture media promoted efficient shoot multiplication and healthy shoot development, while rooting was successfully induced under selected auxin treatments. The developed micropropagation protocols demonstrate the feasibility of large-scale propagation of both endemic species and provide a valuable tool for germplasm conservation, restoration initiatives, and the sustainable exploitation of native Greek flora.

Keywords: *endemic plants, micropropagation, plant tissue culture, ex situ conservation, medicinal plants, biodiversity*

Poster presentation

**Investigation of the possibilities of propagating the orchid
(*Serapias vomeracea*) plant in different nutrient media**

İlknur Eskimez, Mehmet Polat

*Isparta University of Applied Sciences, Faculty of Agriculture, Horticulture, Isparta,
Türkiye*

ilknureskimez01@gmail.com

Abstract

This study aims to successfully propagate Orchis species (commonly known as salep orchids) using plant tissue culture techniques. Bulbous orchid species cannot be efficiently propagated using traditional cultivation methods due to their slow growth rates, ecologically sensitive structures, and dependence on specific symbiotic relationships in their natural habitats. Tissue culture, however, provides a controlled, sterile, and reproducible environment that minimizes disease risk while preserving genetic integrity, enabling the rapid propagation of high-quality plant material. This project aims to determine the optimal medium composition by propagating samples taken from bulb tissues in different nutrient media. The findings are expected to contribute to the conservation of endangered salep orchid species, reduce excessive harvesting pressure on natural populations, and support the sustainable production of the species for commercial and medicinal purposes.

Keywords: *Serapias vomeracea*, medium, orchid, in vitro

Poster presentation**Effects of different red - blue light ratios on growth and photosynthetic parameters of silver birch (*Betula pendula*) cultured *in vitro*****Ineta Samsone, Toms Kondratovičs, Arnis Gailis***Latvian State Forest Research Institute "Silava", Riga street 111, Salaspils LV-2169, Latvia**ineta.samsone@silava.lv***Abstract**

Birch micropropagation requires high-quality lighting. The most important regions of the light spectrum for plant growth and development are the red and blue wavelengths. The combination of these spectral ranges should be adjusted to the requirements of specific plant species. The aim of this study was to evaluate the optimal ratio of red and blue light for birch micropropagation at a fixed photosynthetic photon flux density of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Four lighting treatments with red : blue (R:B) spectrum ratios of 4:1, 3:1, 1:1, and 1:3 were compared. The results showed that the ratio of R:B light significantly affected the growth and development of birch microshoots. A high proportion of blue light (R:B ratio 1:3) negatively affected the growth of the main shoot, internode length, and the multiplication index. The longest shoots were observed under the R:B ratio of 4:1; however, this lighting regime seemed to cause excessive elongation, leading to the formation of fragile shoots unsuitable for micropropagation. This was confirmed by physiological measurements, as both chlorophyll content and the performance index indicated that the plants were under physiological stress and not in optimal condition. Therefore, for the micropropagation of birch, the R:B light ratio of 3:1 can be recommended, as it results in a higher multiplication index without adversely affecting photosynthetic performance.

Keywords: *micropropagation, birch, LED lighting, photosynthesis*

Poster presentation**Study of Sensitivity of Georgian *Vitis vinifera* L. varieties to grapevine yellows diseases****Iveta Megrelishvili, Zurab Khidesheli, Nino Maziashvili***LEPL Scientific-Research Center of Agriculture, Tbilisi, Georgia**ivetameg@yahoo.com***Abstract**

During field surveys in the eastern part of Georgia, including Kakheti (Kondoli, Napareuli, Tsinandali villages) and Kartli (Jigaura and Mukhrani villages) regions, carried out from 2019 to 2025, typical GY symptoms were observed in both Georgian and international varieties. The susceptibility of 12 selected Georgian varieties to FD and BN (CaPsoI) was tested using TaqMan® triplex real-time PCR using TaqMan® MGB™ probes (Applied Biosystems). In addition, nested PCR was used to detect different 16SrV phytoplasma strains, including FD-associated phytoplasmas, and BN phytoplasma from the 16SrXII-A subgroup. Based on the results obtained in this study, the presence of BN and 16SrV phytoplasmas was confirmed using two molecular methods in the eastern part of Georgia. Interestingly, the results highlighted the first detection of 16SrV phytoplasmas in 11 grapevine varieties in mother stock and collection vineyards and four varieties in commercial vineyards. Monitoring of GY phytoplasma infections should be continued using molecular techniques like triplex real-time PCR. Data from these studies highlighted that most Georgian varieties are less susceptible to phytoplasmas associated with diseases of the GY complex indicating tolerance to the infection. Further investigations are needed to study their molecular interactions with GY phytoplasmas, and to identify the genetic traits related to their low susceptibility.

Keywords: *phytoplasma, diseases, FD, BN*

Poster presentation**Effect of Cytokinin Oxidase/Dehydrogenase Inhibitors on *in vitro* regeneration in *Daphne cneorum* L.**

Jana Hanzal Šedivá¹, Zuzana Rottová¹, Michaela Pekařová¹, Daniel Zahradník¹, Jaroslav Nisler²

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

²Institute of Experimental Botany, Isotope Laboratory, The Czech Academy of Sciences, Videňská 1083, 142 20 Prague, Czech Republic

jana.sediva@vuk.gov.cz

Abstract

Daphne cneorum L. (fragrant daphne) is a small, ground-covering shrub of the Thymelaceae family, which occurs naturally in many European countries, including the Czech Republic, where it is a critically endangered species. A significant reduction in the number of localities occurred in the second half of the twentieth century. The reasons were changes in landscape management and the plundering of natural localities by rock gardeners. In natural conditions in the Czech Republic, *D. cneorum* reproduces mainly vegetatively, as seed production is low. It is a recalcitrant plant species; propagation in culture is problematic. Micropropagation offers advantages for the conservation of endangered species in that it is based on a minimum amount of plant material. One of the possibilities for improving *in vitro* shoot production in recalcitrant plant species is a new group of chemical compounds (CKX inhibitors) that protect cytokinins in *in vitro* culture from degradation. The aim of our study was to evaluate the effect of cytokinin oxidase/dehydrogenase (CKX) inhibitors (diphenylurea derivatives) on multiplication in *D. cneorum*. We tested two CKX inhibitors, compounds 52 and 77 alone or in combination with isoprenoid cytokinin 2iP. CKX inhibitors had a significant effect on shoot formation compared to the control. The highest multiplication effect was achieved with the CKX inhibitor 77 compared to the CKX inhibitor 52 and cytokinin 2iP, both alone and in combination with cytokinin.

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

Poster presentation

Optimisation of light intensity for *in vitro* propagation of silver birch (*Betula pendula*) clones

Katrīna Eglīte, Ineta Samsone, Toms Kondratovičs

*Latvian State Forest Research Institute “Silava”, Riga street 111, Salaspils LV-2169,
Latvia*

katrina.eglite@silava.lv

Abstract

This study investigated the effects of light intensity on the growth and propagation efficiency of *in vitro* cultures of silver birch clones cultivated under LED lighting, with the aim of identifying the optimal light intensity for improving micropropagation efficiency. Three birch clones were grown under three lighting treatments with photosynthetic photon flux densities of 20, 30, and 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a constant red and blue light ratio of 3:1. Morphological and physiological traits of the plantlets, including shoot length, internode development, multiplication index, chlorophyll concentration, and chlorophyll fluorescence parameters Fv/Fm and PI, were assessed after six weeks of cultivation. The results showed that the lowest light intensity (20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) resulted in the most balanced growth, producing longer shoots with a higher number of internodes and greater propagation potential, whereas higher light intensities reduced photosynthetic efficiency and increased physiological stress indicators. Clone-specific responses to lighting conditions were also observed, with fast-growing clones showing greater sensitivity to light variation than slower-growing clones. These findings demonstrate that a light intensity of 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ improves birch micropropagation efficiency and plant quality.

Keywords: *micropropagation, silver birch, LED lighting, light intensity*

Poster presentation**Effect of LED light on *in vitro* cultivation of Persian walnut (*Juglans regia* L.)****Lilyana Nacheva**

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

lilyn@abv.bg

Abstract

The Persian walnut (*Juglans regia* L.) is one of the most valuable nuts cultivated in the world due to its valuable nutritional composition and health benefits. The micropropagation of walnut trees is difficult and presents some complications, such as culture establishment, irregular and low multiplication or rooting rates, and a high number of losses during *ex vitro* acclimatisation of plantlets to soil. The present work focuses on opportunities to improve the *in vitro* cultivation of walnut using LED light. The plants studied were cultivated on DKW medium supplemented with BAP (0, 2.5 or 5 µM) under LED lighting with four spectral regions: white (W), red (R), blue (B) and mixed (W:R:B:far-red = 1:1:1:1). The control plantlets were grown under fluorescent lamps (FL) and the PPFD (photosynthetic photon flux density) of all treatments was set at $87 \pm 7.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. The effect of different light sources on some growth parameters of plantlets was monitored. Mixed light (WBR) stimulated the development of explants and the accumulation of biomass. Excellent results were also obtained with plants cultivated under white light (W) on a nutrient medium with the highest concentration of BAP (5 µM). The red light (R) had a beneficial effect on stem length.

Keywords: *micropropagation, tissue culture, light, recalcitrant, woody species*

Acknowledgements: This study is based upon work from COST Action CA21157 “European Network for Innovative Woody Plant Cloning”, supported by COST (European Cooperation in Science and Technology) and financially supported by the Bulgarian National Science Fund (projects KP-06-COST/17 “CopyFruitTree: Innovative approaches for woody fruit species cloning”).

Poster presentation

Initiation of *in vitro* culture of *Vaccinium angustifolium*

Anna Korica¹, Karina Denisova², Elga Ence¹, Rafaels Joffe¹, Anta Sparinska², Liva Purmale-Trasune¹

¹*Bulduri Biotechnology Center, Bulduri Technical School, 6 Viestura Str., LV-2010 Jurmala, Latvia*

²*Institute of Soil and Plant Sciences, Faculty of Agriculture and Food Technology, Latvia University of Life Sciences and Technologies, 2 Liela Str., LV-3001 Jelgava, Latvia*

liva.purmale@bulduri.lv

Abstract

Lowbush blueberry (*Vaccinium angustifolium* Aiton) is a shrub with delicious and highly valuable berries. Mechanical harvest is complicated, resulting in costly berry production. A commercial grower in Latvia has identified a plant from a wild population with desired properties for mechanical berry collection and reported that propagation with conventional methods is slow; thus, an option to use micropropagation must be explored. As explants, 120 shoots with axillary buds were collected and surface sterilized. 12 different media were tested to identify a potentially suitable one for *in vitro* culture initiation (10 explants per treatment, individual test tubes). For the experiment, Anderson's medium and BM-C (cranberry tissue culture medium) supplemented with 0.5 or 1 mg/L 2iP, mT or mTR were used. Growth conditions were a 16 h photoperiod provided by fluorescent lamps, 22 °C. 50—80% of the initial explants in each treatment survived. Plants were transferred to fresh medium every 4 weeks. Promising results were obtained using medium BM-C supplemented with 2iP 0.5 mg/L, Anderson's medium supplemented with mT and mTR 0.5 mg/L although further evaluation is required to determine the optimal medium for this lowbush blueberry clone.

Keywords: *lowbush blueberries, medium optimization, micropropagation*

Acknowledgement: This research was supported by project No. 25-00-C0LA1601-000008, InARA

Poster presentation**Uncovering the mode of action of aromatic cytokinins in Norway spruce embryogenic cultures**

Lucie Fischerová, Zuzana Vondráková, Karel Doležal, Daria Penzina, Marzieh Mohri, Kateřina Eliášová

Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, Prague, Czech Republic

fischerova@ueb.cas.cz

Abstract

In protocols for conifer somatic embryogenesis (SE), *N*⁶-benzyladenine (BA) is the dominant cytokinin (CK). However, BA and its degradation products are stable and, in many *in vitro* plant propagation systems, cause adverse effects. Alongside the traditionally used BA and its combination with kinetin, we applied aromatic topolin-type CKs, *meta*-Topolin (*mT*), *meta*-Methoxy topolin (*MemT*), and *meta*-Topolin riboside (*mTR*) during the proliferation stage of Norway spruce SE. The effect of different CKs on somatic embryo development was assessed. In embryogenic lines with a contrasting growth response, endogenous phytohormone content was analysed in proliferating cultures following the application of CKs and in mature somatic embryos. The endogenous phytohormone content was strongly genotype-dependent; however, the dynamics of both auxin and cytokinin content were influenced by different CK applications and also by the developmental stage of embryogenic culture. Phytohormone analysis was followed by transcriptome analysis of the selected embryogenic line, sampled in the proliferation phase, after the application of CKs. Differentially expressed genes were identified and annotated using both Norway spruce and *Arabidopsis thaliana* databases, and gene ontology analysis was performed. The response of embryogenic cultures to aromatic CKs differs at both the expression and phytohormone levels; our approach allowed us to identify the affected processes.

Keywords: *somatic embryogenesis, topolin-type cytokinins, phytohormone analysis, RNASeq*

Acknowledgement: The work was supported by the project LUC24054 of the Czech Ministry of Education, Youth and Sports

Poster presentation**Initiation of Georgian grapevine (*Vitis vinifera* L.) a Georgian grapevine variety under different sterilization techniques****Maia Kukhaleishvili, Iveta Megrelishvili, Tamar Chipashvili***Georgian Technical University, Biotechnology Center, Kostava st. 77, 0160, Tbilisi, Georgia*maia.kukh@gmail.com**Abstract**

In vitro propagation of grapes involves the use of actively growing shoots, dormant buds, or meristems from disease-free mother plants. The aim of the study was to develop the best sterilization protocol for *in vitro* grapevine culture. The grape variety “Mujuretuli” was used for the study. Samples were collected from 2-3-year-old plants growing in a greenhouse. A 20-cm-long stem with active buds was collected and treated with a fungicide mixture of 1% Captan and 1% Ridomil Gold and stored in the refrigerator until further use. Samples were placed in a phytotron (temperature 25-27°C, humidity 75-80%, lighting 3000-3500 lux, photoperiod 16/8 h) for germination (before the development of green mass). Two sterilization options were used for comparison: treatments with sodium hypochlorite and mercuric chloride. The samples were first washed thoroughly with tap water and soap to remove all soil, followed by a treatment with a fungicide mixture of 1% Captan and 1% Ridomil Gold for 20–30 min. Under a laminar-flow hood, samples were treated: a) 1% mercuric chloride during 10 min for green tissue b) 1.5% mercuric chloride during 15 min for hard tissue, c) 1.5% active chlorine 10 min for green tissue, d) 2% active chlorine 15 min for hard tissue. Each procedure was followed by a rinse with 70% ethanol for 5 minutes. Finally, the samples were rinsed three times with sterile distilled water. Murashige and Skoog (MS) basal medium (3% sucrose, 7 g/l agar, pH 5.8) was used for initiation of *in vitro* grape plants. The best antiseptic treatment for sterilization was determined. Under treatment with a combined mixture of 1.0% Ridomil Gold, followed by treatment with 2% active chlorine for 15 min followed by 70% ethanol for 10 minutes, a 32% survival rate was observed in hard tissue (stems without green tissue). All the plants with the sprouted green mass died.

Keywords: *grapevine, aseptic culture, micropropagation, fungicide, in vitro regeneration*

Poster presentation**Targeting ABA to overcome adventitious rooting recalcitrance in chestnut and oak microshoots**

**Jesús M. Vielba¹, Nieves Vidal¹, Ricardo Castro-Camba^{1,2}, Saleta Rico¹,
María José Cernadas¹, Anxela Aldrey¹, Conchi Sánchez¹**

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

²Research and Innovation Center, E. Mach Foundation (FEM), 38098, San Michele all'Adige, Trento, Italy

conchi@mbg.csic.es

Abstract

Adventitious root (AR) formation is a critical step in the clonal propagation of woody plants. Many trees, including chestnut (*Castanea sativa*) and oak (*Quercus robur*), exhibit a marked decline in rooting competence as donor plants mature. This age-related recalcitrance constitutes a major bottleneck for breeding programs and commercial forestry. While auxin is the principal hormone regulating AR induction, other hormones such as abscisic acid (ABA) modulate the process, with high ABA concentrations inhibiting root primordia formation. This study evaluated whether a pre-treatment with an inhibitor of ABA biosynthesis (ABA-I), applied prior to the auxin (indole-3-butyric acid, IBA) inductive treatment, could enhance adventitious rooting in microshoots derived from mature chestnut and oak trees. Microshoots were pre-treated with ABA-I before exposure to auxin treatment consisting of 25 μ M IBA for 3-5 days. ABA-I pre-treatment improved rooting rates in both species compared to IBA alone, and the quality of the rooted plantlets was notably enhanced: ABA-I strongly promoted shoot regrowth and effectively prevented shoot apical necrosis. These findings suggest that endogenous ABA acts as a negative regulator of AR formation in adult tissues and that reducing its levels prior to auxin induction creates a more favourable hormonal balance for both root initiation and subsequent shoot recovery, offering a promising strategy to overcome rooting recalcitrance in mature trees.

Keywords: ABA inhibitors, auxin, hormonal balance, overcoming recalcitrance, woody plants

Acknowledgements: This research was partially funded by the Spanish Ministry of Science, Innovation and Universities (PID2024-162781-OB100).

Poster presentation**Advancing in the establishment of a micropropagation protocol for the endemic Mediterranean seagrass *Posidonia oceanica***

**Ricardo Castro-Camba^{1,2}, Laura Guerrero-Meseguer¹, Inés Castejón¹,
María Rubio¹, Jorge Terrados¹, Carlos Encina³**

¹Department of Marine Ecology, IMEDEA-UIB-CSIC, C/ Miquel Marquès, 21, 07190, Esporles Illes Balears, Spain

²Vegetal Production Department, Mision Biologica de Galicia Avenida de Vigo s/n, 15780, Santiago de Compostela, Galiza, Spain

³Institute of Subtropical and Mediterranean Horticulture "La Mayora", CSIC-UMA. Algarrobo-Costa, 29750 Málaga, Spain

ricardo.castro@csic.es

Abstract

Posidonia oceanica is a seagrass endemic of the Mediterranean Sea that forms extensive marine meadows, providing significant ecological benefits. However, its distribution has been declining during last decades. The achieving of a micropropagation protocol could enhance restoration efforts, providing a large number of individuals with the desired genetic background for transplantation. However, micropropagation techniques successfully applied to marine seagrasses are not reported. There are few studies with limited efficiency. The establishment of successful protocols with conventional culture media is difficult since they are designed for terrestrial environments making them unsuitable for marine conditions (pH $\approx$ 8 and salinity 38 ‰). Moreover, *P. oceanica* presents specific challenges: it is difficult to obtain plant material, the species has a very slow growth rate (between 0.1 and 6 cm/year). Initial trials which were conducted to assess the effects osmotic pressure through salinity modification on explant survivorship. After them, experiments were carried out testing different auxins (naphthalene-acetic acid and indole-butyric acid) and cytokins (meta-topolin, kinetin and benzylaminopurine) at different concentrations dissolved in Artificial Seawater (ASW) supplemented with 30 g/L of sucrose and 5 g/L of agar. These experiments aim to establish a basic micropropagation protocol as a starting point for further optimization.

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

Poster presentation**Improving *in vitro* shoot multiplication of temperate fruit species using novel CKX inhibitors****Tatjana Andelić¹, Mirjana Mićević¹, Jaroslav Nisler², Tatjana Vujović¹**¹*Fruit Research Institute, Čačak, Republic of Serbia*²*Institute of Experimental Botany of the Czech Academy of Sciences, Czech Republic*tvujovic@institut-cacak.org**Abstract**

Cytokinin oxidase/dehydrogenase inhibitors (CKX inhibitors) are compounds that reduce cytokinin degradation, thereby enhancing cytokinin activity in plants. In this study, the effects of two diphenylurea derived CKX inhibitors were evaluated: 1-(3,5-dichlorophenyl)-3-(3-(trifluoromethoxy)phenyl)urea (compound 52) and 2-(3-(3,5-dichlorophenyl)ureido)-4-methoxybenzamide (compound 77). Benzyladenine (BA) was applied at three concentrations (0, 1, and 4.4 μM), while compounds 52 and 77 were used at concentrations of 0, 100 nM, and 1 μM . Three fruit tree species were included in the experiment: plum (*Prunus domestica* L.) cv. 'Crvena Ranka', raspberry (*Rubus idaeus* L.) cv. 'Willamette', and sour cherry (*Prunus cerasus* L.) cv. 'Oblačinska'. The main multiplication parameters assessed were multiplication index, length of axial and lateral shoots, and the number of leaves on both shoot types. In addition, fresh and dry weights of callus, axial shoots, and lateral shoots were determined. The highest values of multiplication index were recorded in plum and raspberry on medium supplemented with 4.4 μM BA and 100 nM compound 52. In contrast, the highest multiplication index in sour cherry was achieved on medium containing 4.4 μM BA and 100 nM compound 77, indicating that the effectiveness of CKX inhibitors depends on the plant species.

Keywords: cytokinin oxidase/dehydrogenase, micropropagation, plum, raspberry, sour cherry

Poster presentation**Initial establishment of *in vitro* cultures of sweet chestnut (*Castanea sativa* Mill.) and dog rose (*Rosa canina* L.)****Vanja Daničić¹, Svjetlana Zeljković²**¹University of Banja Luka, Faculty of Forestry, University City, Bulevar vojvode Petra Bojovića 1A Banja Luka, Bosnia and Herzegovina²University of Banja Luka, Faculty of Agriculture, University City, Bulevar vojvode Petra Bojovića 1A Banja Luka, Bosnia and Herzegovinavanja.danicic@sf.unibl.org**Abstract**

Sweet chestnut (*Castanea sativa* Mill.) is a woody species of high ecological and economic importance, while dog rose (*Rosa canina* L.) is important for its medicinal and ornamental uses. Conventional propagation of both species is often limited by low multiplication rates and plant health issues, making *in vitro* culture a suitable method for producing healthy and uniform plant material. The aim of this study was to establish *in vitro* cultures of *C. sativa* and *R. canina* using a standard surface sterilization protocol and to evaluate explant establishment on different nutrient media. Explants of *C. sativa* and *R. canina* were obtained from branch segments collected in February. After induction of axillary shoot development under controlled conditions, isolated buds of *C. sativa* were cultured on Woody Plant Medium (WPM) and Murashige and Skoog (MS) media supplemented with 0.5 mg L⁻¹ BAP and 0.1 mg L⁻¹ IBA, while *R. canina* explants were established on MS medium under the same culture conditions. Cultures were maintained at 21 ± 2 °C under a 16 h photoperiod. Successful establishment of sterile cultures was achieved for both species. For *C. sativa*, MS medium showed better results than WPM, and no phenol production was observed during cultivation on MS medium. *R. canina* explants also showed satisfactory establishment and shoot development under the applied culture conditions. These findings provide a basis for further micropropagation and *ex situ* conservation of selected genotypes of both species.

Keywords: *Castanea sativa*, *Rosa canina*, *in vitro* culture, micropropagation, explant establishment

Poster presentation

WGCNA identification of gene modules and transcription factors relevant for adventitious rooting in chestnut

Jesús M. Vielba¹, Ricardo Castro-Camba^{1,2}, Nieves Vidal¹, Saleta Rico¹, Conchi Sánchez¹

¹*Department of Plant Production, Misión Biológica de Galicia (CSIC), Avda de Vigo s/n, 15705 Santiago de Compostela, Spain*

²*Research and Innovation Center, E. Mach Foundation (FEM), San Michele all'Adige, 38098, Trento, Italy*

jmvielba@mbg.csic.es

Abstract

Vegetative propagation of recalcitrant woody species is a challenging task that limits biotechnological exploitation of economically relevant trees. In the case of chestnut, a severe decline in its ability to form adventitious rooting is imposed during maturation. In previous reports, both juvenile-like and mature microshoots were subject to different hormonal treatments to gain deeper insights into the molecular processes driving to the formation of adventitious roots while allowing to increase our understanding on failed treatments. In the present work, RNA-seq libraries from those treatments were used to develop a Weighted Gene Co-expression Network Analysis, in order to identify gene modules correlated with the formation of adventitious roots. Hub genes within relevant modules were then used for promoter region analysis, in order to identify transcription factor families that may play a role in the process by controlling the expression of the genes within those modules. bZIP and bHLH families in juvenile-like microshoots, and WRKY and SBP families in mature microshoots showed to be particularly relevant for this developmental process. Specific transcription factors, like *CsbHLH30* and *CsbZIP44*, seem to play key roles in the rooting process, while the activity of *CsWRKY75* and *CsSBP1* is related to recalcitrant responses in mature shoots.

Keywords: *adventitious rooting, chestnut, recalcitrance, transcription factors, WGCNA*

Acknowledgements: This research was partially funded by the Spanish Ministry of Science, Innovation and Universities (PID2024-162781-OB100).

Poster presentation

Impact of vacuum infiltration on the efficiency of temporary *Agrobacterium* transformation in different ornamental sunflower explant types

Şükran Yıldız¹, Ashkan Hodaiei², Sara Yasemin³, Dilek Tekdal¹, Stefaan P.O. Werbrouck²

¹*Department of Biotechnology, Faculty of Science, Mersin University, Mersin, Türkiye*

²*Department of Plants & Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium*

³*Department of Horticulture, Faculty of Agriculture, Siirt University, Siirt, Türkiye*

yldzs Kran@gmail.com

Abstract

Efficient and reproducible genetic transformation remains a major limitation for applying genome-editing technologies in sunflower (*Helianthus annuus* L.). This study compared transient *Agrobacterium*-mediated transformation in three ornamental sunflower explant types—root, leaf, and cotyledon—using two inoculation methods: vacuum-assisted infiltration (10 min) and non-vacuum infiltration (30 min). After inoculation, explants were co-cultured for 16 h, and transient T-DNA expression was evaluated by luminescence imaging using the luciferase reporter gene. No luciferase signal was detected in root and cotyledon explants under either method. Leaf explants, however, showed clear, reproducible luciferase expression in both treatments, with vacuum infiltration producing significantly stronger luminescence than non-vacuum infiltration despite the shorter inoculation time. These results indicate that leaf tissue is the most responsive explant type for transient *Agrobacterium*-mediated transformation in ornamental sunflower, and that vacuum infiltration enhances transient reporter-gene expression under the conditions tested.

Keywords: *Vacuum infiltration, luciferase reporter gene, ornamental plants*

Poster presentation

Small motif, big function: A 19 amino acid signal links chloroplast targeting to viral replication

**Yajiao Wang¹, Richita Saikia², Andreas Maurice Grant³, Dianqiu Lv⁴,
Xifeng Wang⁵, Robert C. Wilson⁶, Huanbin Zhou⁵, Carl Spetz²**

¹*Plant Protection Institute, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang, China*

²*NIBIO, Norwegian Institute of Bioeconomy Research, Division of Biotechnology and Plant Health, Ås, Norway*

³*Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway*

⁴*College of Agronomy and Biotechnology, Southwest University, Chongqing 400715, China*

⁵*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China*

⁶*Department of Biotechnology, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, Hamar, Norway*

carl.spetz@nibio.no

Abstract

Positive strand RNA viruses remodel host membranes to establish replication sites, but the viral signals that determine organelle selection remain poorly understood. We investigated how Poinsettia mosaic virus targets chloroplasts and whether this localization is linked to genome replication. Live cell imaging with CRISPR dCas13BT3 showed that viral negative strand RNA accumulated predominantly at the chloroplast periphery, whereas positive strand RNA had a broader chloroplast associated and cytoplasmic distribution. Fluorescent fusion assays identified a 19 amino acid region of the coat protein that was sufficient to target a heterologous reporter to the chloroplast periphery. Deletion of this region or substitution of conserved residues disrupted chloroplast localization. When the same mutations were introduced into infectious viral clones, negative strand RNA accumulation was strongly reduced, linking motif dependent targeting to replication. Structural modelling further revealed similarity between the viral motif and conserved regions of the membrane insertion proteins Alb3, YidC and Oxa1. These findings identify a minimal chloroplast binding signal that connects viral protein targeting with the establishment of a replication competent complex at chloroplast membranes.

Keywords: *Poinsettia mosaic virus, chloroplast targeting, viral replication, coat protein, live cell imaging*

Poster presentation

From explant stress to regeneration competence in recalcitrant woody species: physiological responses and emerging epigenetic perspectives

Paula Oros, Corina Cătană

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

paula.oros@usamvcluj.ro

Abstract

Recalcitrant woody species remain difficult to establish *in vitro*, particularly when explants are collected from mature or field-grown donor plants. Culture establishment is frequently limited by phenolic oxidation, endogenous contamination and low regeneration rate. This study examines whether early explant stress and the potential role of stress memory can influence the acquisition of regeneration competence during *in vitro* initiation. Preliminary observations were obtained from meristems, dormant buds and nodal segments of *Juglans regia*, *Corylus avellana* and *Malus domestica* cv. Jonathan collected at different phenological stages. Plant material was cultured on Murashige & Skoog (MS) and Driver and Kuniyuki Walnut medium (DKW) basal medium, supplemented with different treatments designed to reduce endogenous contamination and oxidative stress, including Plant Preservative Mixture (PPMTM), polyvinylpyrrolidone (PVP) and activated charcoal. Phenolic oxidation, contamination, survival, and regeneration rate were evaluated. Preliminary results indicated that the regeneration rate reached values of up to 62% for *Juglans regia*, 95% for *Corylus avellana*, and 93.33% for *Malus domestica* cv. Jonathan. Based on these observations, we propose a preliminary framework in which recalcitrance is interpreted as the result of an imbalance between explant stress and regeneration competence. The study integrates emerging epigenetic perspectives, suggesting that differences between responsive and non-responsive explants may reflect physiological and developmental states influenced by stress memory. Future molecular validation will be necessary to test the involvement of these mechanisms. This approach may contribute to the development of more predictive protocols for the clonal propagation of valuable woody genotypes.

Keywords: recalcitrant woody species, *in vitro* initiation, explant stress, regeneration competence

Poster presentation**WB3: Possibilities for using somatic embryogenesis for scale production of Scots pine (*Pinus sylvestris* L.)****Sara Raggi, Malin Abrahamsson, Magnus Hertzberg***SweTree Technologies AB, Skogsmarksgränd 7, SE-903 36 Umeå, Sweden**Sara.Raggi@Swetree.com***Abstract**

SweTree technologies has been developing an automation solution for the large-scale production of conifers based on somatic embryogenesis (SE). A pilot facility has been built to test and further refine our automation solutions in preparation for the first large-scale production facility for Norway spruce. We have therefore expanded our focus to include other economically important conifer species. Scots pine is one of the most challenging species among pine to regenerate via SE, due to low initiation frequencies. Moreover, embryogenic cell lines exhibit high proliferation rates, as well as a long maturation period with low yield. Consequently, large-scale production of Scots pine will require more resources and effort compared to Norway spruce. Over two consecutive years, we have performed initiation using zygotic embryos from diverse genetic materials. We successfully initiated cultures from all tested mother trees and evaluated the ability of these cell lines to produce somatic embryos. Our work has focused on the most challenging steps, particularly proliferation and maturation, with the aim of increasing the yield of high-quality embryos that can be successfully established and converted into plantlets. We will present results from protocol optimizations, as well as initial tests conducted using the SweTree pilot facility, with the goal of automating the selection and planting processes for large-scale production of Scots pine.

Keywords: *Scots pine, somatic embryo, initiation, embryogenesis, pilot facility*

Working Group 2

Diagnosis, sanitation, and conservation

Diagnosis of viruses, viroids, and bacteria, sanitation protocols for clean stock production, mid- and long-term storage of clean stocks, and germplasm.

Keynote speaker

Cryopreservation and *in vitro* culture of meristems for the conservation of forest genetic resources *ex situ*

Paweł Chmielarz¹, João Paulo Rodrigues Martins¹, Juan Manuel Ley-López¹, Mikołaj Krzysztof Wawrzyniak¹, Marcin Michalak², Małgorzata Palucka³, Urszula Wasileńczyk³

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

²*Faculty of Agriculture and Forestry, Department of Genetics and Plant Pathophysiology, University of Warmia and Mazury in Olsztyn, M. Oczapowskiego 1A, 10-721 Olsztyn, Poland*

³*Kostrzyca Forest Gene Bank, State Forests, Miłków 300, 58-535 Miłków, Poland*

pach@man.poznan.pl

Abstract

Genetic resources of plant species producing desiccation-tolerant *orthodox* seeds, can be stored *ex situ* in gene banks using classical methods (at -18°C). Such seeds can also be successfully cryopreserved, as a backup for traditional storage, in a species-specific safe range of seed moisture content. Genetic resources of *recalcitrant* seeds, sensitive to desiccation and storage, as well as plants of elite genotypes or breeding lines can be cryopreserved in liquid nitrogen (LN, -196°C). The most common starting materials for cryopreservation of “recalcitrant plants” (different from embryogenic tissue or somatic embryos) are meristems of zygotic embryos or buds. The cryopreservation procedure involves *in vitro* culture to regenerate meristems into plantlets after thawing from liquid nitrogen. Vitrification or encapsulation-dehydration protocols are used, and the success of such cryopreservation depends on several factors such as species, genotype, preculture sugar conditions, dehydration time and physical state of the tissue before freezing. As our investigations showed, embryonic axes and meristems of different oak species show different physiological reactions of the tissue to desiccation and liquid-nitrogen temperatures. Critical water content was determined for desiccated embryonic axes using *in vitro* culture and unfrozen water was measured using Differential Scanning Calorimetry. Successful cryopreservation of plumules was described for *Quercus robur* and *Quercus petraea* after vitrification of plumules with alginate beads, desiccation on cryo-plates, and direct cooling in liquid nitrogen. Cryopreservation of *Q. robur* and *Q. petraea* is applied in forestry practice in the Kostrzyca Forest Gene Bank in Poland. For monumental, 800-year-old oaks (*Quercus robur*), *in vitro* culture alone was successfully applied to clone them (while preserving the original genotypes of the shoots and roots). Seven-year-old *in vitro* clones—two-metre-high trees—were planted near a mother tree for future generations. They are growing very well.

Keywords: micropropagation, cryopreservation, gene banks, seeds, oaks

Oral presentation

Tiny allies and hidden threats: Bacteria in woody plant micropropagation

Stefaan P.O. Werbrouck

Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, Ghent, Belgium

Stefaan.werbrouck@ugent.be

Abstract

Reliable micropropagation of woody plants depends not only on optimised culture media and regeneration protocols, but also on the effective management of bacteria that accompany plant material into culture. Bacteria in plant tissue culture are often regarded solely as contaminants, yet their roles are more complex: some cause visible losses, tissue necrosis, and failed propagation, whereas others persist as latent endophytes that may contribute to plant vigor, stress tolerance, and developmental stability. Particular attention is given to endogenous bacteria, including intracellular cytofacts, which challenge the assumption that fully axenic plant material is always attainable. In woody plant cloning, where recalcitrance, long production cycles, clean-stock maintenance, and commercial scalability are major concerns, understanding this hidden microbiome is essential. A shift is therefore needed from indiscriminate sterilization toward evidence-based microbial governance: detecting harmful taxa early, preventing outbreaks, applying targeted sanitation when required, and preserving plant physiological quality. Integrating molecular diagnostics, mother-plant indexing, improved asepsis, antibiotics, PPM™, and nanoparticle-based approaches can strengthen the reliability, safety, and sustainability of micropropagation pipelines for elite woody genotypes.

Keywords: *bacterial endophytes, cytofacts, microbial contamination, clean stocks, microbial governance, sanitation, tissue culture*

Oral presentation

Transgrafting: a biotechnological solution for plant virus control

Nuria Alburquerque, Lidia Faize, Francisco Javier Alfosea Simon, Marina Martín-Valmaseda, Elena Yelo, Lorenzo Burgos

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

nalbur@cebas.csic.es

Abstract

Several *Prunus* species are highly affected by Plum pox virus (PPV), which causes sharka disease. Transgrafting is a biotechnological technique included among the New Genomics Techniques that combines the use of a genetically modified (GM) rootstock capable of producing and transmitting systemic defense signals to a non-transgenic (non-GM) graft via the vascular system. The main objective of this work is to obtain pathogen-resistant *Prunus* plants through transgrafting. In previous projects, we obtained transgenic European plum trees resistant to sharka by silencing viral sequences of PPV. These plants, used as GM-rootstocks, transferred sharka resistance to non-GM PPV-infected apricot grafts under greenhouse conditions, resulting in 92% PPV-free plants. Additionally, *in vitro* plants of two GM lines resistant to PPV and susceptible plants (non-GM controls) were generated, acclimatised and grafted with apricot, peach and Japanese plum scions for field planting. The physiological state, agronomic performance and disease incidence were observed in these composite plants. Although the results are promising, with no negative influence of transgenic rootstocks on plant health and no disease symptoms observed in the grafts, further evaluations are necessary. The use of NGTs, such as transgrafting, could be an effective alternative for eradicating viruses from *Prunus* plants definitively, obtaining trees resistant to the pathogen and allowing them to recover from future reinfections.

Keywords: *virus elimination, plum pox virus, new genomic techniques, Prunus*

Acknowledgements: This work was partially supported by the PID2022-137499OB-I00 project funded by MCIN/AEI/10.13039/501100011033/FEDER

Oral presentation

From virome to clean planting stock: Cost-effective NGS-guided sanitation for *in vitro* propagation of heritage grapevine

Sebastián Gómez Talquenca¹, Humberto Debat², Raul Cuello³, Diego Zavallo³, Rocio Torres¹, Sebastian Asurmendi³

¹*Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (EEA-Mendoza-INTA), Mendoza, Argentina*

²*Instituto de Patología Vegetal, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria (IPAVE-CIAP-INTA), Córdoba, Argentina*

³*Instituto de Agrobiotecnología y Biología Molecular (IABIMO), CICVyA. Instituto Nacional de Tecnología Agropecuaria (INTA), Consejo Nacional de investigaciones Científicas y Técnicas (CONICET), Los Reseros y Nicolas Repetto, Hurlingham, Buenos Aires, Argentina*

gomez.talquenca@inta.gob.ar

Abstract

Grapevine (*Vitis vinifera*) hosts an expanding virome: over 100 viral and viroid species are described, and the count keeps rising as NGS becomes routine. Heritage varieties propagated vegetatively for centuries are especially prone to their accumulation, yet conventional sanitation relies on targeted assays that detect only known viruses and miss emerging ones. Broad NGS screening is therefore increasingly necessary, provided its cost and accessibility improve. NGS-based metatranscriptomics enables full virome characterization prior to sanitation. We applied it to plant pools and individual accessions from two germplasm sets: candidate clones of Syrah and Bonarda, and a collection of Criolla varieties preserved at INTA Mendoza, Argentina. Sequencing at two depths (50 and 150 million reads) assessed detection sensitivity and cost efficiency. Criolla varieties showed markedly higher virome complexity, with single plants harboring up to 17 viral and viroid species, including pathogens absent from current Argentine certification schemes. Data also revealed mismatches between viral genomes and primer binding sites used in post-sanitation diagnostics, guiding more accurate detection tools for cleaned material. Pooling proved a cost-effective route to comprehensive virome inventories that prioritize sanitation and validates stock cleaned by meristem culture, which can then enter certified schemes and be multiplied at scale by standard *in vitro* micropropagation.

Keywords: *grapevine virome, next generation sequencing, sanitation, clean stock certification, criolla varieties*

Oral presentation

Establishing a Cryobank for Your Tree Species

Bart Panis^{1,2}

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

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

b.panis@cgiar.org

Abstract

Cryopreservation is widely regarded as the most reliable long-term method for conserving genetic resources of plants that cannot be effectively preserved through conventional seed storage. It is particularly valuable for clonally propagated species, sterile plants, species producing recalcitrant (non-storable) seeds, and plants that do not breed true from seed. Over the past decades, a wide range of cryopreservation protocols has been developed for tree genetic resources, tailored to different tissue types. These include slow-freezing techniques for embryogenic cultures, droplet vitrification of *in vitro* shoot-tips and meristems, and cryopreservation of dormant buds collected from field-grown trees. Establishing and maintaining a plant cryobank, however, involves a number of technical, logistical, and financial challenges. These include selecting and acquiring appropriate equipment, developing and validating species-specific protocols, implementing rigorous safety and quality-control measures, ensuring a reliable supply of liquid nitrogen, managing endogenous microbial contamination, and securing sustainable long-term funding. In addition, cryobank managers must address issues related to documentation, traceability, and risk management to guarantee the long-term security of the collection. This presentation will discuss these challenges in detail and illustrate practical solutions through examples and case studies from operational plant cryobanks.

Keywords: *genetic resources, cryopreservation, collections*

Oral presentation***In vitro* conservation and propagation of endangered Mediterranean thymes for sustainable genetic resource valorisation**

Daniela Duarte¹, Bernardo Onofre¹, Jorge Canhoto¹, Paula Castro¹, Sandra Correia¹

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

daniela.d@sapo.pt

Abstract

Thymes are valued aromatic and medicinal plants due to their rich bioactive compounds. However, the biotechnological potential and conservation needs of lesser-known and endangered woody endemic thymes remain largely unexplored. This study aimed to establish *in vitro* conservation and propagation protocols for three Iberian endemic species included on the IUCN Red List: *Thymus carnosus*, *Thymus camphoratus* and *Thymus albicans*. It also explored callus induction as a biotechnological tool to obtain biomass for potential phytochemical production. *T. camphoratus* cultures initiated from shoots achieved an establishment success of 18.6%, and exhibited the highest growth performance, with a multiplication rate of 3.97 and 100% phytomer production. *T. albicans* and *T. carnosus* established from seedlings, showed success rates of 53.3% and 13.33%, respectively. Callus induction was higher under light conditions for *T. albicans* (81.7%) and *T. carnosus* (94.4%). Moreover, MS media with 2,4-D and cytokinin outperformed NAA-supplemented media. Proliferation assays for *T. albicans* callus revealed the highest fresh weight increase (+2.73 g) in MS medium with 1 mg/L 2,4-D and 0.5 mg/L KIN. These findings provide efficient *in vitro* conservation strategies for endangered endemic thymes while demonstrating their potential as sources of biotechnologically relevant biomass. This dual approach offers a framework to integrate conservation and phytochemical production in underexplored Mediterranean species.

Keywords: Mediterranean thymes, *in vitro* conservation, biomass production, plant tissue culture

Oral presentation

Understanding the antiviral effects of silver nanoparticles through omics tools in apricot plants cultured *in vitro*

Francisco Javier Alfosea-Simón¹, Alejandro Castro-Cegri², Leilei Zhang², Luigi Lucini^{2,3}, Lorenzo Burgos¹, Nuria Alburquerque¹

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

²Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Piacenza, Italy

³Institute of Bioimaging and Biological Complex Systems (IBSBC), National Research Council (CNR), Milan, Italy

fjalfosea@cebas.csic.es

Abstract

Silver nanoparticles (AgNPs) have shown great potential in plant *in vitro* culture, particularly for producing *Plum pox virus* (PPV)-free apricot plants (*Prunus armeniaca* L.). However, the mechanisms underlying their antiviral effects remain poorly understood. To investigate their mode of action, an integrative omics approach was applied using the commercial AgNP formulation Argovit™-C (Ag-C) in apricot shoots grown in Plantform™ Temporary Immersion Systems. The experimental design included two groups: healthy (HAP) and PPV-infected apricot plants (IAP). Each group received three treatments: control, coating, and 50 mg L⁻¹ Ag-C, resulting in a total of six conditions. The coating treatment, which acts as an additional control, contained the same stabilizers present in Ag-C (protein hydrolysate and polyvinylpyrrolidone) but without silver. Samples were analyzed by non-targeted metabolomics and lipidomics using UHPLC/QTOF-MS with different analytical methods, followed by data processing and multivariate analysis to identify key metabolites and affected pathways. Results showed overall omics changes associated with PPV infection and AgNP treatment. AgNPs and/or the coating appeared to reduce virus-induced secondary metabolism, especially affecting isoprenoids and lignans, and influenced amino acids, carbohydrates, lipids, and fatty acids. These findings provide new insights into AgNP effects and support improved nanotechnology-based strategies for plant sanitation.

Keywords: silver nanoparticles, metabolomics, lipidomics, *Prunus armeniaca*, antiviral

Acknowledgements: This work was partially supported by the PID2022-137499OB-I00 project funded by MCIN/AEI/10.13039/501100011033/FEDER

Oral presentation**How long is too long? Preservation of *Pinus radiata* embryogenic cultures at -80°C and evaluation of alternative freezing methods**

**Katherine P. Sandoval, Ander Castander-Olarieta, Paloma Moncaleán,
Itziar A. Montalbán**

¹NEIKER, Campus Agroalimentario de Arkaute, s/n, N 104 - km355, Arkaute, 01192, Álava, Spain

imontalban@neiker.eus

Abstract

Long-term culture of pine embryonal masses leads to decreased maturation capacity and increased genetic/phenotypic variation. Cryopreservation at -80°C is an economical strategy to maintain embryogenic potential and meet the requirements needed for the implementation of multi-varietal forestry. Nonetheless, the effectiveness of this approach after several years has not been tested before. In this study, we thawed radiata pine embryogenic masses preserved at -80°C up to 6 years, and evaluated the effect of eliminating the DMSO cryoprotectant solution, applying new freezing methods (vitrification) and post-thawing treatments, and tested the effect of sodium butyrate on the maturation of regenerated material. Results demonstrated that this storage protocol in an ultra-low freezer is viable along the whole tested period, showing fluctuating values potentially associated with the genotype and the morphology of the embryogenic masses, rather than with the methodology. The presence of the cryoprotectant DMSO is not a determining factor for the success of the process but improves both regeneration and maturation rates. Vitrification is not an effective alternative over the slow cooling method for storage at -80°C, and none of the modifications tested during post-thawing and maturation improved the results from the actual protocol. These findings corroborate the usefulness of this low-cost technique for the breeding programs of an economically important species like radiata pine.

Keywords: dimethyl sulfoxide, long-term storage, slow cooling, somatic embryogenesis, ultra-low temperature freezing

Acknowledgements: PID2020-112627RB-C32 funded by MICIU/AEI/10 and PID2024-156422OB-C32 funded by MICIU/AEI/10. COST Action CA21157 “European Network for Innovative Woody Plant Cloning” www.copytree.eu, funded by COST (European Cooperation in Science and Technology) www.cost.eu. Basque Government (Spain) through Euskobasoa 2050 (BERPIZ 24-00080) and Ikertalent programmes.

Oral presentation

Assessing Cryotherapy as a Sanitation Tool for Hop Stunt Viroid in Apricot

Marina Martín-Valmaseda¹, Zhibo Hamborg², Lydia Faize¹, Elena Yelo¹, Lorenzo Burgos¹, Nuria Alburquerque¹

¹*Group of Fruit Tree Biotechnology, Department of Plant Breeding, CEBAS-CSIC, Campus de Espinardo, 25, 30100 Murcia, Spain*

²*Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research, Ås, Norway*

mmartin@cebas.csic.es

Abstract

Hop stunt viroid (HSVd) causes severe fruit deformations in apricot, leading to important economic losses. Viroids are often difficult to eliminate by conventional meristem culture, due to their ability to invade the meristematic dome. This study evaluates cryotherapy as a method to eliminate HSVd from infected *in vitro* apricot shoot tips and combines histological analysis and HSVd in situ hybridization to better understand the eradication mechanism. Apical shoot tips were excised from HSVd-infected *in vitro* apricot plants (cultivar ‘Canino’). A droplet-vitrification protocol using Plant Vitrification Solution 3 (PVS3) was applied to the shoot tips before immersion in liquid nitrogen. Regeneration rates were evaluated after each step of the protocol. Pathogen presence in regenerated shoots was analyzed by RT-PCR. Both HSVd-free and -infected shoot tips were fixed, embedded in paraffin, and sectioned (10 µm) for histological analysis and HSVd in situ hybridization to determine the spatial distribution of the viroid. Cryotherapy successfully eliminated HSVd in 34.3% of the regenerated plants. In contrast, all plants derived from meristem culture controls remained infected, highlighting the crucial role of cryotherapy in pathogen eradication. These results, supported by microscopy observations, indicate that while HSVd can be eliminated from apricot shoot tips, the efficiency remains low. Therefore, combining cryotherapy with other techniques could optimise pathogen elimination.

Keywords: *Droplet-vitrification, Prunus armeniaca, viroid-elimination*

Acknowledgements: This work was conducted through an international collaboration between CEBAS-CSIC (Spain) and NIBIO (Norway), established within the CopyTree network.

Flash talk presentation**New technological tools for improving chestnut stress tolerance**

**Vera Pavese¹, Lorenzo Antonio Marino¹, Paola Ruffa¹, Eric Mozzanini¹,
Davide Lucien Patono¹, Alessandra Sereno², Martina Ferrero¹, Anita
Fuda¹, Guglielmo Lione^{1,3}, Paolo Gonthier^{1,3}, Silvia Sabbadini⁴, Luca
Capriotti⁴, Bruno Mezzetti⁴, Roberto Botta¹, Daniela Torello Marinoni¹,
Andrea Moglia¹**

¹Department of Agricultural, Forest and Food Sciences (DiSAFA), University of Turin (UNITO), Largo Paolo Braccini, 2, 10095 Grugliasco (TO), Italy

²Department of Veterinary Sciences (DSV), University of Turin (UNITO), Largo Paolo Braccini, 2, 10095 Grugliasco (TO), Italy

³Centro Interdipartimentale per l'Innovazione in campo Agro-ambientale—AGROINNOVA, Università degli Studi di Torino, Largo Paolo Braccini 2, Grugliasco

⁴Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, Ancona, Italia

vera.pavese@unito.it

Abstract

The sweet chestnut (*Castanea sativa* Mill.) is a species of significant ecological and economic importance in Europe, currently facing major threats, including ink disease caused by the oomycete *Phytophthora cinnamomi*. This study investigates the potential of Spray-Induced Gene Silencing (SIGS) as an eco-friendly strategy to combat this disease. By employing double-stranded RNA (dsRNA) molecules to silence both pathogen effector genes and plant susceptibility genes, we aim to enhance the chestnut tree's tolerance to *P. cinnamomi*. Specifically, we targeted the *DICER-LIKE* (*DCL*) gene of the pathogen, a key regulator of its pathogenicity. *In vitro* synthesized dsRNA molecules were applied to *P. cinnamomi* colonies, resulting in a significant 30% reduction in mycelial growth compared to water-treated controls. These findings suggest that dsRNA spray could serve as an effective biocontrol strategy against *P. cinnamomi*. In parallel, we explored an alternative approach using light-emitting diodes (LEDs) to promote chestnut growth under stress conditions. Four LED treatments with distinct light spectra were tested, with *Red:Blue:Far-red* and *Red:Blue:Green:Far-red* proving the most effective in enhancing plant performance. These treatments significantly increased the multiplication index, improved photosynthetic efficiency, stimulated root development, and boosted secondary metabolite production. This LED-based strategy offers a promising method for strengthening plant resilience, providing an innovative approach to improving chestnut growth and stress tolerance.

Keywords: *Castanea sativa*, dsRNA, *Phytophthora cinnamomi*, LED, micropropagation

Flash talk presentation

From poplar transcriptomics to CRISPR validation: Functional characterisation of a Key Gene for metal-toxicity tolerance in trees

Kubilay Yildirim, Şükriye Coşkun

*Ondokuz Mayıs University, Department of Molecular Biology and Genetics,
Samsun, TURKIYE*

kubilay.yildirim@omu.edu.tr

Abstract

Metal- and heavy-metal-contaminated soils, although unsuitable for conventional agriculture, can become strategic sites for afforestation through the development of tree species adapted to these toxic environments. In our study, several poplar species were screened for metal toxicity tolerance, and *Populus alba* (P. alba) and *Populus nigra* (P. nigra) exhibited contrasting responses to boron (B) toxicity. The former showed greater tolerance, accumulating more than threefold less B in its tissues than the latter. Transcriptomic comparison between them revealed a striking expression pattern of a Heavy Metal Associated Isoprenylated Plant Protein (HIPP): its expression increased 100-fold in the higher B-accumulating P. nigra under metal stress, while it was downregulated 10-fold in P. alba. A homolog of the HIPP gene in tomato was targeted using a dual-gRNA CRISPR/Cas9 vector system, and HIPP mutant tomato plants were generated through Agrobacterium-mediated transformation and regeneration. Under toxic levels of B and Cd, CRISPR-edited plants exhibited greater tolerance, with higher biomass production, delayed toxicity symptoms, and maintenance of higher chlorophyll levels. More importantly, B and Cd accumulation in mutant tomatoes was more than fourfold lower than in wild-type controls. These findings demonstrate that HIPP is a key regulator of metal-toxicity tolerance and a strong candidate for CRISPR-based improvement of tree species for sustainable afforestation of contaminated soils.

Keywords: *HIPP, Poplar, tomato, CRISPR, metal toxicity, tolerance*

Poster presentation

Enhanced viral detection in grapevine via exome depletion and next-generation sequencing (NGS)

Raúl Cuello¹, Diego Zavallo¹, Pablo Vera¹, Agustín Sattler², Andrea Puebla¹, Humberto Debat³, Sebastián Gómez Talquenca², Sebastian Asurmendi¹

¹*Instituto de Agrobiotecnología y Biología Molecular (IABIMO), CICVyA. Instituto Nacional de Tecnología Agropecuaria (INTA), Consejo Nacional de investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*

²*Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (EEA-Mendoza-INTA), Mendoza, Argentina*

³*Instituto de Patología Vegetal, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria (IPAVE-CIAP-INTA), Córdoba, Argentina*

gomez.talquenca@inta.gob.ar

Abstract

Grapevine (*Vitis vinifera* L.) viral infections threaten global viticulture. Traditional detection methods (PCR, ELISA) are limited to known pathogens, highlighting the need for comprehensive, unbiased approaches. Here, we present a cost-effective viral enrichment system adapted for next-generation sequencing (NGS) to detect and characterise grapevine viruses. Our strategy uses hybridization-based capture with biotin-labeled cDNA probes ("Chloro-Zero"), designed via transcriptomic analysis to selectively deplete highly abundant host transcripts (plastid and ribosomal RNAs) while preserving viral RNA. Evaluating different RNA ratios, we observed consistent host RNA reduction and moderate viral sequence enrichment. NGS showed improved recovery of low-abundance viral transcripts with coverage comparable to commercial kits, but at significantly lower costs. Despite some variability in depletion efficiency, this scalable, adaptable protocol demonstrates strong potential for virome profiling. It facilitates the detection of emerging threats, supporting better certification programs and sustainable management. Ongoing improvements will further enhance this robust platform for broader plant virology applications.

Keywords: *grapevine virome, viral RNA enrichment, chloro-Zero probes, hybridization-capture techniques*

This work has been published in Annals of Applied Biology (DOI: 10.1111/aab.70131).

Poster presentation

Plant biotechnology in support of the Albanian certification system for seedlings of autochthonous fruit tree cultivars: From scientific research to market

Efigjeni Kongjika¹, Valbona Sota^{1,2}, Magdalena Cara³, Jordan Merkuri⁴, Ariola Bacu², Raimonda Sevo⁵, Elektra Papakosta⁶, Maurizio Lambardi⁷, Carla Benelli⁷

¹Research Center “Biotechnology & Genetics”, Academy of Sciences, Tirana, Albania

²Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

³Plant Protection Department, Tirana Agricultural University, Albania

⁴Research and Didactic Center, Durrës, Albania

⁵Institute of Plant Genetic Resources, Tirana Agricultural University, Albania

⁶Center of Transfer of Agricultural Technologies, Vlorë, Albania

⁷Institute of BioEconomy, CNR, Florence, Italy

kongjikaef@yahoo.com

Abstract

Albania's autochthonous fruit tree germplasm includes ancient cultivars threatened by genetic erosion, requiring genetic and phytosanitary certification to support nursery production. The local plum cultivar *Prunus domestica* 'Tropojane' is valued for high yield and superior organoleptic traits, based on IPBGR descriptors, but is affected by viral infections, particularly Plum pox virus (PPV). This study combines plant biotechnology, molecular characterisation and phytosanitary testing to produce true-to-type and virus free material for certification. Selected material was sanitized through meristem culture and micropropagated in temporary immersion bioreactors. Genetic fidelity was assessed using RAPD markers, while phytosanitary status was evaluated by ELISA, PCR and qPCR. Before sanitation, PPV incidence ranged from 35.5% to 45.6%. Optimal nutrient medium was MS with 1.5 mg/L BAP and 0.1 mg/L NAA for proliferation, and half-strength MS with 1 mg/L IBA for rooting. A Plantform bioreactor operated with 1 min immersion every 8 h improved growth based on relative growth rate. Molecular, morphometric and agronomic analyses confirmed genetic purity and fidelity of regenerated plants. PPV was not detected in *in vitro* plants produced through sanitation, allowing the establishment of “basic” material in a greenhouse repository before market transfer. The study supports including “*in vitro* culture-derived production” in phytosanitary certificates issued to nurseries, in line with FAO guidance.

Keywords: meristem culture, bioreactors, RAPD, ELISA, PCR

Poster presentation

The Sycamore Maple Clone Collection – when Minis grow up

Anne-Mareen Eisold, Diana Morgenroth, Volker Schneck

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

a.eisold@thuenen.de

Abstract

Sycamore maple (*Acer pseudoplatanus* L.) is a tree species native to Central Europe and Western Asia with valuable wood properties, such as wavy grain, and it is considered an ornamental species in urban greens. Although sycamore maple supplies excellent wood and ecological properties in the forests, it is not yet focused on intensive breeding programs in Germany. Our work focuses (i) on the conservation of trees with valuable wood properties, (ii) on the investigation of tree-pathogen interactions, and (iii) on physiological stress tests. Therefore, we established a tissue culture collection of clones from sycamore maple. During the propagation process, several parameters have been evaluated, such as the performance under different light conditions, the propagation ratio, and root development. After the acclimatisation in the greenhouse, the plants were grown in pots for several months before finally being planted in the field for long-term assessments of the clonal growth performance. First data regarding shoot growth and susceptibility to leaf pathogenic fungi causing mildew-like symptoms reveal strong differences between the investigated maple clones. Therefore, we assume a high selection and breeding potential for sycamore maple. Future investigations will be addressed to physiological responses to drought stress and putative correlations to susceptibility to pathogens, which are core aspects for breeding purposes.

Keywords: *breeding programs, forest germplasm, in vitro techniques, clonal growth performance*

Poster presentation

Tissue culture techniques in the diagnosis of viral tree diseases

Anne-Mareen E. Eisold¹, Marius Rehanek², Ben Bubner¹

¹*Thünen Institute of Forest Genetics, Eberswalder Chaussee 3a, D-15377
Waldsiedersdorf, Germany*

²*Division Phytomedicine, Thae-Institute of Agricultural and Horticultural Sciences,
Humboldt-Universität zu Berlin, Lentzeallee 55/57, D-14195 Berlin, Germany*

a.eisold@thuenen.de

Abstract

Plant-pathogenic viruses occur worldwide and infect woody plants. This includes forest trees and shrubs, where viruses cause both economic and ecological damage. Unlike those of other pathogens, the effects of viruses on trees at both the physiological and ecological levels have been studied little. Trees are long-lived organisms that exist in their habitat for decades and are exposed to a wide variety of factors. In order to investigate the impact of viral diseases on the physiology of host plants, appropriate experiments need to be conducted. Tissue culture techniques are used to enable the handling of tree species on an experimental scale for such studies. To test the seed-borne transmission of viruses in *Ulmus laevis* PALL., seeds are sown under sterile conditions *in vitro* to produce clonal lines, and the material produced in this way is tested for the presence of viruses using molecular methods. The generated virus-infected clones will subsequently be available for further experiments, such as cryopreservation for virus clearance, stress tests in the greenhouse, or as a virus reservoir available for mechanical or vector transmissibility studies. This represents a new method for investigating viral diseases in trees. It enables the execution of various experimental settings under controlled laboratory conditions within a manageable time frame and is therefore an important and useful addition to existing standard protocols.

Keywords: *virus infections, in vitro culture, virus detection, seed transmissibility*

Poster presentation**CKX20 as effective booster for shoots formation in wild service tree (*Sorbus torminalis* L.) explants**

Eva Pokorná¹, Pavlína Máchová¹, Helena Cvrčková¹, Jaroslav Niesler², Jozef Lacek³, Peter I. Dobrev³, Daniel Haisel⁴, Petr Soudek⁴

¹Department of Forest Tree Species Biology and Breeding, Forestry and Game Management Research Institute, Strnady 136, 252 02 Jíloviště, Czech Republic

²Isotope Laboratory, Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 165 02 Prague 6, Czech Republic

³Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 165 02 Prague 6, Czech Republic

⁴Laboratory of Plant Biotechnologies, Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 165 02 Prague 6, Czech Republic

pokorna@vulhm.cz

Abstract

The wild service tree (*Sorbus torminalis* (L.) Crantz) is a valuable but rare forest tree in the Czech Republic. Outstanding genotypes are often represented by old trees that are difficult to propagate, making *in vitro* techniques important for their conservation. We previously optimized a micropropagation protocol and established valuable genotypes in the National Bank of Forest Tree Explants. However, some genotypes show low shoot proliferation, requiring further optimization. We evaluated the effects of the isoprenoid cytokinin isopentenyladenine (iP) alone or combined with the inhibitor of cytokinin oxidase/dehydrogenase, CKX20 tested at two concentrations (1 and 5 μ M). In the first subculture, 5 μ M iP and 5 μ M iP + CKX20 increased the multiplication coefficient by 1.5-fold compared with the control (explants routinely cultured on BAP). However, this effect was not maintained in subsequent subcultures, where lateral shoot formation ceased. Interestingly, 1 μ M iP and 1 μ M iP + CKX20 promoted the accumulation of photosynthetic pigments compared with the higher concentration. Preliminary phytohormone analyses revealed increased levels of DHZ, MeS-iPR and MeS-iP, and *cis*- and *trans*Z. Our results indicate that replacing BAP with isoprenoid cytokinin combined with the CKX20 can transiently stimulate shoot formation, suggesting a "restarting" effect in long-term BAP-maintained cultures.

Keywords: *Sorbus torminalis*, explants, CKX20 treatment, shoot formation

Acknowledgement: This work was supported by the Ministry of Agriculture of the Czech Republic (MZE-RO0123).

Poster presentation

Endophyte-assisted cold hardening and cryopreservation of apple microshoots *in vitro*

Rytis Rugienius, Jurgita Vinskienė, Inga Tamošiūnė, Olha Posunko, Šarūnė – Morkūnaitė Haimi, Perttu Juhani Haimi

Department of Orchard Plant Genetics and Biotechnology, Lithuanian Research Centre for Agriculture and Forestry, Kauno str. 30, Babtai, Kaunas distr. LT54333 Lithuania

rytis.rugienius@lammc.lt

Abstract

In unstable climatic conditions, it is important to preserve genetic diversity and genetic resources for breeding and production of orchard trees. One of the most effective and safest storage methods is cryopreservation at liquid nitrogen temperature. However, not all plant genotypes can be successfully preserved due to different reactions to hardening, cold, oxidative stress, or dehydration. The potential of endophytic microorganisms for improving *in vitro* cold hardening and recovery after cryopreservation has not been sufficiently studied, although bacterial endophytes that increase plant growth *in vitro* have been identified. The aim of this study was to evaluate the impact of additional pure endophytic microorganism isolates on the survival and regeneration of microshoots of apple (*Malus x domestica* Borkh.) after cryopreservation *in vitro*. The results differed depending on cold hardening duration (0, 2, or 4 weeks). The survival of non-hardened microshoots inoculated by endophytes increased from 21.0 to 23.0% and regeneration from 9.7 to 18.0%, compared to uninoculated microshoots. The survival and regeneration of inoculated microshoots hardened for 4 weeks at 4 °C increased up to 48.3% and 27.1%, respectively. Thus, with a sufficient duration of cold hardening, the beneficial effect of endophytes on the survival and regrowth of microshoots of apple trees is reliable and significant. The search for novel endophytic microorganisms with the potential to enhance cold hardening and improve cryopreservation efficiency of orchard plant shoots *in vitro* is ongoing.

Keywords: *cold acclimation, endophytic microorganisms, cryopreservation, Malus domestica, regrowth*

Poster presentation

Safeguarding Albanian grapevine germplasm: phytosanitary assessment of Shesh i Bardhë and Shesh i Zi

Adriatik Çakalli¹, Doriana Bode², Magdalena Cara^{3,4}, Klevis Hoxhallari^{3,4}, Jordan Merkuri⁴

¹*Institute of Plant Genetic Resources, Tirana Agricultural University, Albania*

²*Faculty of Agriculture and Environment, Agricultural University of Tirana, AL*

³*Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, AL*

⁴*NanoAlb/NanoBalkan, Academy of Sciences of Albania*

acakalli@ubt.edu.al

Abstract

Shesh i Bardhë and Shesh i Zi are autochthonous cultivars of *Vitis vinifera* L. that represent valuable Albanian viticultural resources. Their conservation depends on the availability of true-to-type and phytosanitarily reliable propagation material. During 2024–2025, a comparative field and laboratory assessment was conducted to support the safeguarding of these cultivars and inclusion in a national certification pathway. Candidate vines were selected on the basis of varietal conformity, vigour, productivity, and the absence of virus-like symptoms. Symptomatic and asymptomatic plants were georeferenced, labelled and sampled to ensure traceability. Serological and molecular screening targeted the principal grapevine viruses relevant to certification, including GFLV, GFKV, GLRaV-1, GLRaV-3 and GVA, using ELISA and RT-PCR. Selected amplification products were purified and subjected to bidirectional Sanger sequencing, followed by comparison with reference sequences in GenBank. The combined approach enabled comparison of the sanitary profiles of the two cultivars and supported the exclusion of infected vines from propagation. Plants showing favourable agronomic traits and negative diagnostic results were retained as candidate mother vines. Integrating field selection, targeted virus detection and sequence confirmation provides a practical basis for conserving Shesh i Bardhë and Shesh i Zi and sustainably producing traceable, virus-tested planting material for Albanian viticulture.

Keywords: *Vitis vinifera* L., Shesh i Bardhë, Shesh i Zi, grapevine germplasm, phytosanitary assessment, grapevine viruses, Sanger sequencing, certification

Poster presentation

Cryotherapy as a tool for virus elimination in selected blueberry genotypes

Barbora Tunklová¹, Jana Fráňová², Miloš Faltus¹, Jaroslava Příbylová²,
Igor Koloniuk², Olena Bobrova^{1,3}, Lesia Golosna¹, Alois Bilavčík¹

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

²Institute of Plant Molecular Biology, Biology Centre, CAS, Ceske Budejovice, Czech
Republic

³Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine

barbora.tunklova@carc.cz

Abstract

Cryotherapy, based on the selective survival of meristematic cells following exposure to ultra-low temperatures, represents a promising approach for the production of virus-free planting material. Virus infections represent a major constraint to the maintenance of healthy blueberry (*Vaccinium corymbosum* L.) germplasm, reducing plant health, productivity, and the value of genetic resources. The aim of this study was to evaluate the effectiveness of cryotherapy for virus elimination in selected blueberry genotypes and to assess post-cryotherapy recovery. Shoot tips were excised from *in vitro* cultures, pre-cultured in sucrose solutions, treated with a loading solution, and exposed to the cryoprotective solution PVS3 for different durations. Samples were subsequently immersed in liquid nitrogen, thawed, and regrown on Anderson medium under controlled culture conditions. Regrown plants were tested for blueberry latent virus (BBLV) and tobacco necrosis virus A (TNV-A) using molecular diagnostic methods. Recovery after cryotherapy differed markedly among genotypes, confirming a strong genotype-dependent response. The highest recovery rates were achieved in genotypes BCH7 and 16/0/11, while satisfactory recovery was also observed in genotype REKA. RT-qPCR confirmed BBLV eradication in a proportion of regrown plants. Complete elimination of BBLV was achieved in genotype DK7, whereas virus-free plants were also obtained in genotype 16/0/11 following cryotherapy. TNV-A was not detected in any regrown plants. The molecular detection results are preliminary and require confirmation by repeated testing after a longer post-cryotherapy period. The results confirm that cryotherapy can serve as an effective method for obtaining virus-free blueberry plants while simultaneously facilitating the long-term conservation of valuable blueberry germplasm. The combination of cryotherapy and molecular diagnostics provides an effective tool for producing healthy planting material and supporting gene bank management and breeding programs.

Keywords: *Vaccinium corymbosum*, blueberry latent virus, tobacco necrosis virus A, PVS3, cryopreservation, germplasm conservation

Acknowledgement: This work was supported by the Technology Agency of the Czech Republic under the TREND Programme (Project No. FW10010417).

Poster presentation**Establishment of an endangered *Cydonia oblonga* collection in vitro**

**Alois Bilavčík¹, Barborá Tunklova¹, Olena Bobrova^{1,2}, Lesia Golosna¹,
Miloš Faltus¹**

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

²Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine

alois.bilavcik@carc.cz

Abstract

Fire blight caused by *Erwinia amylovora* (Burrill) Winslow *et al.* represents a serious threat to pear and quince genetic resources. Due to severe infection, a valuable field collection of quince (*Cydonia oblonga* Mill.) had to be removed. To preserve endangered genotypes, plant material was established *in vitro* with the long-term aim of cryopreservation. The establishment of healthy *in vitro* stock cultures represents the first essential step in this process. Actively growing shoots were obtained by forcing dormant branches to sprout in water supplemented with 8-hydroxyquinoline (200 mg L⁻¹). Newly developed shoots (2–5 cm long) were surface sterilized using sodium hypochlorite and introduced into *in vitro* culture. Three basal media, namely Murashige and Skoog (MS), Quoirin and Lepoivre (QL), and Driver and Kuniyuki Walnut (DKW), were evaluated. Among the tested media, DKW supplemented with 2% (w/v) sucrose, 1% (w/v) sorbitol, 7 g L⁻¹ agar, 1 mg L⁻¹ meta-topolin, 0.2 mg L⁻¹ zeatin, and 0.12 mg L⁻¹ IBA showed the best results. A total of 43 quince genotypes have been successfully established and maintained *in vitro*. Cultures are maintained at 22°C under an 18/6 h photoperiod and are subcultured every 4–5 weeks. In parallel, regeneration protocols for shoot tips are being optimised as a prerequisite for future cryopreservation. The proposed approach demonstrates the feasibility of rescuing endangered quince genetic resources through *in vitro* culture establishment and provides a basis for their long-term conservation in the Czech National Cryobank.

Keywords: *Erwinia amylovora*, micropropagation, germplasm conservation

Acknowledgement: This work was partly supported by the Ministry of Agriculture of the Czech Republic (QL26010112).

Poster presentation

**Integrating pesticide registration and food safety surveillance:
An in silico framework for mode-of-action analysis and
prioritisation in Albania**

Magdalena Cara^{1,2}, Florida Gashi³, Anjeza Bekolli⁴, Klevis Hoxhallari^{1,2}

¹*Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, AL*

²*NanoBalkan, Academy of Sciences of Albania*

³*Department of Natural Resources Institute, Agriculture for Sustainable Development, University of Greenwich, UK*

⁴*Department of Mathematics and Informatics, Faculty of Economy and Agribusiness, Agricultural University of Tirana, AL*

magdacara@ubt.edu.al

Abstract

Pesticide prioritisation often relies on commercial plant protection product registers, although these inventories do not fully reflect the biochemical diversity of active substances or their occurrence in food safety alerts. This study proposes a reproducible in silico framework integrating the Albanian register of authorised plant protection products, updated in June 2026, with the Rapid Alert System for Food and Feed notifications concerning Albanian-origin products recorded during 2021–2026. Commercial formulations were disaggregated into unique active substances, harmonised by standard names and CAS identifiers, and classified according to IRAC, FRAC and HRAC mode-of-action systems. Biochemical diversity was quantified using the Shannon index and the Herfindahl–Hirschman index. A structured cross-referencing procedure linked authorised substances with RASFF hazard records and classified them as registered, transitional or non-listed. Registration frequency, mode of action and notification occurrence were then combined in a multi-criteria screening framework. The workflow supports identification of dominant and underrepresented biochemical groups, recognition of substances associated with food safety notifications, and resistance-aware prioritisation. It provides a transparent decision-support approach for regulatory monitoring and surveillance planning, while remaining distinct from a full toxicological risk assessment and can be adapted across comparable national datasets.

Keywords: *pesticide active substances, plant protection products, mode of action, in silico framework, RASFF, food safety surveillance, regulatory prioritisation, Albania*

Poster presentation**Phytosanitary selection and molecular testing of the Albanian grapevine cultivar Kallmet in support of plant material certification**

Magdalena Cara^{1,2}, Efigjeni Kongjika³, Valbona Sota^{3,4}, Klevis Hoxhallari^{1,2}, Orges Cara⁵, Elektra Papakosta⁶, Adriatik Cakalli⁷, Jordan Merkuri²

¹*Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, AL*

²*NanoAlb/NanoBalkan, Academy of Sciences of Albania*

³*Research Center of Biotechnology & Genetics, Academy of Sciences, Tirana, Albania*

⁴*Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania*

⁵*International Centre for Advanced Mediterranean Agronomic Studies, Valenzano, Italy*

⁶*Center of Transfer of Agricultural Technologies, Vlora, Albania*

⁷*Institute of Plant Genetic Resources, Tirana Agricultural University, Albania*

magdacara@ubt.edu.al

Abstract

The Albanian grapevine cultivar Kallmet is a valuable genetic resource, but its sanitary status may be compromised by graft-transmissible viruses. Within the VINECERT project, a vineyard in Bukmirë, Mirditë, also used as a source of propagation material, was surveyed to identify candidate vines for clonal and phytosanitary selection. Visual inspection focused on symptoms associated with grapevine leafroll disease, including premature reddening and downward rolling of leaves, delayed berry colouring and uneven bunch ripening. In the first plot, 9 of 21 vines showed suspicious symptoms, while an incidence of 8–10% was recorded in the second plot; no typical leafroll symptoms were observed in the third plot. Six asymptomatic Kallmet vines with favourable agronomic performance were labelled and documented as candidate mother plants. Samples from symptomatic and asymptomatic vines were tested by ELISA and RT-PCR for the main grapevine viruses included in the certification scheme. GLRaV-3 was the most prevalent virus detected. Representative amplicons were subjected to bidirectional Sanger sequencing and compared with homologous GenBank accessions to confirm viral identity. The combined use of field inspection, targeted diagnostics and Sanger sequencing provides a practical basis for excluding infected material and selecting true-to-type, virus-tested Kallmet clones. This approach supports the national certification pathway and the conservation of valuable Albanian grapevine germplasm.

Keywords: *grapevine, Kallmet, phytosanitary selection, grapevine viruses, RT-PCR, Sanger sequencing, certification*

Poster presentation**Medium-term conservation of olive synthetic seeds using different carbohydrate sources, light conditions and abscisic acid****Doaa S. Elazab^{1,2}, Claudia Ruta³, Maurizio Lambardi¹**¹Department of Horticulture, Faculty of Agriculture, Assiut University, Egypt²IBE-Institute of BioEconomy, National Research Council (CNR), Florence, Italy³Department of Soil, Plant and Food Sciences, University of Bari 'Aldo Moro', Bari, Italydoaa.elkassas@agr.au.edu.eg**Abstract**

The conservation of olive germplasm is essential for the preservation of valuable genetic resources and the support of breeding and propagation programs. Synthetic seed technology represents a promising approach for the storage of elite plant material while maintaining its regeneration potential. In this study, synthetic seeds were produced from *in vitro*-derived olive microshoots (uni-nodal segments) and stored under different conservation conditions. The effects of carbohydrate source (mannitol or sorbitol), light regime, and the application of abscisic acid were evaluated during medium-term storage at 23±1 °X. Synthetic seeds were maintained under low-light conditions (about 12 µmol m⁻² s⁻¹) or dark conditions and periodically assessed through recovery tests to determine their viability and regeneration capacity. Distinct responses were observed among treatments, indicating that storage conditions significantly influenced the ability of synthetic seeds to retain their regenerative potential over time. While some treatments successfully supported medium-term conservation (up to 9 months) and subsequent plant recovery, others reduced regeneration capacity after storage. The application of abscisic acid contributed to growth suppression during conservation, with treatment-dependent effects on recovery. Overall, the results highlight the importance of optimizing carbohydrate source, growth regulator application, and light conditions to improve the storage performance of olive synthetic seeds. This study provides useful information for the development of efficient conservation strategies for olive germplasm and supports a wider application of synthetic seed technology in woody species.

Keywords: *Olea europaea*, synthetic seeds, germplasm conservation, abscisic acid, light regime

Poster presentation

Cryopreservation as a tool for the long-term conservation of edited embryogenic cultures in European chestnut

M^aTeresa Martínez, Cristina Buceta, Elena Corredoira

Grupo de Biotecnología y Mejora Forestal, Misión Biológica de Galicia (MBG-CSIC),
Avd Vigo s/n, 15705, Santiago de Compostela, La Coruña, Spain

elenac@mbg.csic.es

Abstract

Cryopreservation is a highly valuable biotechnological tool for the long-term preservation of embryogenic cultures. Limitations associated with the maintenance of somatic embryos through repetitive embryogenesis, including the occurrence of somaclonal variation, the reduced capacity for further embryo production, losses due to contamination, and the high costs of labor and materials, highlight the need for efficient long-term conservation strategies. In addition, cryopreservation enables the storage of lines generated through biotechnological approaches such as genetic transformation and genome editing, allowing their cost-effective preservation while the modified traits are being evaluated. The aim of this study was to validate a previously developed cryopreservation protocol for somatic embryos of *Castanea sativa* and to assess its applicability to embryogenic edited lines. Somatic embryos in which the *PDS* (phytoene desaturase) gene and the susceptibility genes *PMR4* (Powdery Mildew Resistant 4) and *DMR6* (Downy Mildew Resistant 6) had been edited using the CRISPR/Cas9 system were cryopreserved using a vitrification-based procedure. Groups of two to three somatic embryos at the globular and early developmental stages were treated with PVS2 solution for 60 minutes at 0 °C prior to cryostorage in liquid nitrogen. Following thawing and six weeks of culture on proliferation medium, embryo recovery rates exceeding 50% were obtained for all evaluated lines. These results demonstrate that the vitrification-based cryopreservation procedure developed for European chestnut is an effective strategy for the long-term conservation of genome-edited embryogenic lines.

Keywords: *Castanea sativa*, CRISPR/Cas9, vitrification procedure, somatic embryogenesis

Funding: This work was partially funded by the Spanish Ministry of Science, Innovation and Universities (MICIN, Spain) through project PID2024-156422OB-C33.

Poster presentation

Cryopreservation and micropropagation of *Prunus padus* L.

Essi Risu^{1,2}, Leena Yrjänä³, Tuija Aronen¹, Sakari Välimäki^{1,4}

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

²University of Eastern Finland, The Department of Environmental and Biological Sciences, Yliopistokatu 7, FI-80101 Joensuu, Finland

³Natural Resources Institute Finland (Luke), Latokartanonkaari 9, FI-00790 Helsinki, Finland

⁴Currently: Lauka Biotech Ltd, Laitaatsillantie 3, FI-57170 Savonlinna, Finland

tuija.aronen@luke.fi

Abstract

Bird cherry (*Prunus padus* L.) is a small deciduous tree native to Europe and northern Asia, valued as an ornamental species for its abundant and fragrant spring blossoms. The aim of this work was to develop cryopreservation for a special collection of *P. padus* to ensure its existence as a part of national program for conservation of forest genetic resources. To our knowledge, these are the first reported results on micropropagation and cryopreservation of *P. padus* using dormant winter buds as explants. Buds from 11 genotypes were used for testing tissue culture media and cryopreservation procedure. Of the tested media, DKW medium supplemented with 0.5 mg/l BA was found to be suitable for *P. padus* micropropagation, with 96% of the explants exhibiting shoot growth. Multiplication of the cultures was generally efficient, with plenty of adventitious and axillary shoots. For cryopreservation, the buds were lightly dehydrated to 45% moisture and pretreated with slow cooling into –38 °C using a programmable freezer before plunging them into liquid nitrogen. The cryopreservation lowered the initiation success from 96 to 55%, which is still acceptable for the purposes of genetic resources conservation. 42% of the shoots rooted when exposed to IBA induction treatment, and the rooted shoots were successfully transferred to *ex vitro*. The developed method is applied for conservation of *P. padus* genetic resources in Finland.

Keywords: Forest genetic resources, tissue culture, bird cherry

Poster presentation**Improvement of *in vitro* propagation of ‘Gisela 5’ (*Prunus cerasus* × *P. canescens*) cherry rootstock using the ElecTIS bioreactor****Elektra Papakosta¹, Efigjeni Kongjika², Magdalena Cara³**¹Centre of Agricultural Technology Transfer (ATTC) Vlore, Shmangjin, Albania²Research Center of Biotechnology & Genetics, Academy of Sciences of Albania³Agricultural University of Tirana, Faculty of Agriculture and Environment,
Department of Plant Protectionelektrapapakosta@gmail.com**Abstract**

‘Gisela 5’ (*Prunus cerasus* × *P. canescens*) is a widely used cherry rootstock commonly propagated *in vitro* to ensure uniform and healthy plant material. This study aimed to improve its micropropagation by comparing conventional agar-based culture with liquid culture in an ElecTIS temporary immersion bioreactor. Explants (1.0–1.5 cm) were sterilized and cultured on Murashige and Skoog medium supplemented with different concentrations of 6-benzylaminopurine (BAP), combined with 0.1 mg L⁻¹ GA₃ and 0.01 mg L⁻¹ NAA. After three subcultures, the highest multiplication rate (80%) and mean shoot number (3.19 per explant) were obtained with 1.0 mg L⁻¹ BAP. During rooting, 1.5 mg L⁻¹ IBA produced the highest mean root number (2.55) and a rooting rate of 75%. The optimal multiplication medium was then tested in the ElecTIS bioreactor under three immersion regimes. An immersion frequency of 8 min every 8 h produced the highest relative growth rate (RGR, 3.07), exceeding those recorded on solid and semi-solid media (2.31 and 1.88, respectively). These results indicate that ElecTIS improves biomass production and represents an efficient system for the *in vitro* propagation of ‘Gisela 5’.

Keywords: bioreactor, cherry rootstock, micropropagation, temporary immersion system

Poster presentation**Identification of promising grape varieties under Lithuanian agroclimatic conditions and the introduction of healthy propagation material****Birutė Frercks, Ingrida Mažeikienė**

Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture,
Kauno St. 30, Babtai, LT-54333 Kaunas dist., Lithuania

birute.frercks@lammc.lt

Abstract

Changing climate conditions are making viticulture more popular in Lithuania, increasing the need for locally adapted grapevine varieties and certified healthy propagation material. Therefore, the objective of this study was to identify promising Lithuanian grapevine genotypes, assess their genetic origin and phytosanitary status, and prepare initial propagation material for the future registration and long-term conservation of varieties. Three promising grape seedlings selected by A. Gailiūnas—"Juodupė," "Monika," and "Varduva" - were chosen for their adaptability to Lithuanian growing conditions and their potential value to the national wine sector. For the genetic analysis 11 microsatellite markers (SSR) recommended by the International Organisation of Vine and Wine (OIV) were used. According to the molecular profiles all three genotypes have unique allele combinations and can be clearly distinguished from one another. SSR analysis revealed their complex interspecific hybrid origin, dominated by *Vitis amurensis* and *V. labrusca* × *V. riparia*, with *V. vinifera* representing a smaller proportion. This genetic structure is consistent with the breeding objectives focused on winter hardiness, disease resistance, and adaptation to northern viticultural conditions. Phytosanitary monitoring of Lithuanian vineyards revealed both common epiphytic fungi and economically important grapevine pathogens, including *Erysiphe necator* and *Plasmopara viticola*. The three selected grapevine varieties have been introduced into *in vitro* propagation and prepared for conservation under *in vitro* and cryogenic conditions. These results provide a scientific basis for maintaining genetically identified, healthy initial propagation material and support the future registration of these Lithuanian grapevine genotypes as cultivars adapted to local agroclimatic conditions.

Keywords: viticulture, molecular markers, *Erysiphe necator*, *Plasmopara viticola*

Poster presentation**Development of a cryopreservation protocol for dormant buds of Georgian hazelnut (*Corylus avellana* L.) cultivars****Mariam Gaidamashvili**¹*Dept. of Biology, Faculty of Exact and Natural Sciences, Iv. Javakhishvili Tbilisi State University, 1, Chavchavadze Ave., 0179 Tbilisi, Georgia*mariam.gaidamashvili@tsu.ge**Abstract**

The European hazelnut (*Corylus avellana* L.) is a major nut crop of high nutritional and economic value worldwide. Georgia ranks among the leading global hazelnut producers and possesses a rich diversity of locally adapted cultivars. However, the long-term sustainability of hazelnut production is increasingly threatened by climate change and other environmental stressors. The conservation of valuable genetic resources has therefore become a priority for maintaining cultivar diversity and supporting future breeding efforts. To prevent the loss of autochthonous hazelnut cultivars through genetic erosion and other hazards, a cryopreservation protocol was developed and evaluated for dormant buds of *C. avellana* cvs. ‘Anakliuri’, ‘Gavazuri’, and ‘Ata-Baba’. Dormant twigs were collected from field-grown plants during mid-winter. Nodal segments containing a single bud were dehydrated at 4°C until their moisture content reached 20–30% of fresh weight. The segments were subsequently pre-frozen at –20°C for 24 h and then immersed in liquid nitrogen for long-term storage. Bud viability following prefreezing and cryostorage was assessed by evaluating necrosis in the bud base, primordia, and vascular tissues, followed by 2,3,5-triphenyl tetrazolium chloride (TTC) staining. Post-thaw recovery was evaluated using an *ex vitro* forcing technique based on bud swelling, shoot emergence, and sustained shoot elongation as an alternative to grafting. The cryoresponsiveness of these autochthonous Georgian hazelnut cultivars was evaluated for the first time, providing new insights into the physiological responses of woody species to cryogenic preservation. This study represents a pioneering effort to establish a dedicated cryogenic conservation system for hazelnut genetic resources in Georgia and provides a foundation for the development of a national hazelnut cryobank.

Keywords: *Corylus avellana*, cryopreservation, dormant buds, genetic resources, conservation.

Poster presentation**High-resolution DNA methylation profiling and transcriptomic analysis of a wheat-barley 7H addition line****Norbert Hidvégi, István Molnár, Andrea Gulyás***HUN-REN Centre for Agricultural Research, Agricultural Institute, Martonvásár, Hungary**norbert.hidvegi@atk.hun-ren.hu***Abstract**

Interspecific hybridisation is a crucial mechanism for expanding genetic diversity, yet the introgression of divergent genomes typically triggers intense epigenetic reprogramming to harmonise the coexistence of donor and recipient chromatin. This study investigates the genome-wide methylomic consequences and preliminary transcriptional dynamics of maintaining a barley chromosome within a wheat nuclear environment. We analysed a stable wheat-barley 7H chromosome addition line and its parent cultivars (winter wheat ‘Mv9kr1’, winter barley ‘Igri’) using Reduced Representation Bisulfite Sequencing (RRBS). Our high-resolution data captures DNA methylation profiles across CpG, CHG, and CHH contexts. The results demonstrate the stable inheritance of parental CpG methylation landscapes within the 7H addition line, whilst simultaneously capturing the highly dynamic and stochastic nature of CHH methylation. To complement this established epigenetic landscape, whole-transcriptome sequencing of the same genotypes is currently underway using the Illumina NextSeq 2000 platform. Preliminary findings from this ongoing project phase point towards active transcriptional reprogramming in response to the alien chromosome. Ultimately, integrating these new transcriptomic profiles with our robust epigenetic dataset will help map context-specific regulatory networks and provide deeper insights into the mechanisms driving phenotype formation in wide crosses.

Keywords: *epigenetics, bisulfite sequencing, transcriptomics, interspecific hybridisation, Triticeae*

Poster presentation**Occurrence of *Plum Pox Virus* (PPV) in *Prunus* species in Azerbaijan and its effects on oxidative stress and antioxidant responses****Nargiz Sultanova**

Department of Food Engineering and Biotechnology, Azerbaijan State Oil and Industry University, Baku, Azerbaijan

sultanova.nargiz@asoiu.edu.az

Abstract

Sharka disease, caused by *Plum pox virus* (PPV), is one of the most destructive viral diseases of stone fruits worldwide. This study investigated PPV occurrence in Azerbaijan and evaluated physiological and oxidative stress responses in naturally infected *Prunus* species. Symptomatic leaf samples collected from orchards in the Quba district were analyzed using immunochromatographic test strips, DAS-ELISA, and RT-PCR, confirming PPV infection in plum (*Prunus domestica*), apricot (*Prunus armeniaca*), and peach (*Prunus persica*). Infection significantly increased hydrogen peroxide (H_2O_2 ; 3.20–3.90 to 6.80–10.60 $\mu\text{mol g}^{-1}$ FW) and malondialdehyde (MDA; 4.5–8.2 to 8.0–15.2 nmol g^{-1} FW), indicating enhanced oxidative stress. Activities of catalase (CAT) and ascorbate peroxidase (APX), together with the non-enzymatic antioxidants ascorbic acid (ASA) and glutathione (GSH), increased in infected plants, demonstrating activation of antioxidant defense mechanisms. PPV infection also reduced total chlorophyll (17.3–30.8%), chlorophyll stability index, maximum quantum efficiency of photosystem II (Fv/Fm; 0.716–0.728 to 0.408–0.561), and soluble protein content. These findings demonstrate that PPV infection disrupts cellular redox homeostasis and photosynthetic performance in *Prunus* species and provide integrated physiological and biochemical evidence supporting improved monitoring and management of sharka disease in stone fruit orchards.

Keywords: *Plum pox virus*, *Prunus* species, Sharka disease, oxidative stress, catalase, ascorbate peroxidase, chlorophyll degradation, DAS-ELISA

Poster presentation**Cadmium effects on ion balance, leaf structure, and antioxidant regulation in micropropagated Paulownia plantlets**

Viktor Husak¹, Ulyana Kilchytska¹, Yurii Vakiv¹, Viktoriia Nykolysyn¹, Yuliia Savchuk¹, Viktoriia Zadarko¹, Volodymyr Lushchak^{1,2}

¹*Department of Biochemistry and Biotechnology, Vasyl Stefanyk Carpathian National University, 57 Shevchenko Str, Ivano-Frankivsk, 76018, Ukraine*

²*Research and Development University, 13a Shota Rustaveli Str, Ivano-Frankivsk, 76018, Ukraine*

volodymyr.lushchak@cnu.edu.ua

Abstract

Cadmium contamination is a persistent environmental challenge, and fast-growing woody species such as Paulownia are promising candidates for phytoremediation. This study investigated the effects of cadmium ions at concentrations 0.5, 1.0, 2.5, and 5.0 mg/L on the biochemical responses of microclonally propagated Paulownia Pao Tong Z07 plantlets, a superhybrid of *P. tomentosa* × *P. fortunei* × *P. kawakamii*, which were cultivated *in vitro*. X-ray fluorescence analysis demonstrated that cadmium exposure was associated with reductions in the mass fractions of manganese, potassium, and phosphorus across different plant parts, while chlorine showed a marked increase. Correlation analysis indicated strong negative relationships among cadmium, sulfur, aluminum, potassium, calcium, and phosphorus, consistent with disruption of ion transport and stress-related sulfur utilization. Cadmium treatment also decreased chlorophyll and carotenoid contents in leaves. Histological observations confirmed dose-dependent impairment of leaf tissues, including parenchymal deformation and local cell wall disruption at 2.5 and 5 mg/L. Antioxidant responses were selective: ascorbate peroxidase activity remained unchanged, whereas superoxide dismutase activity increased at higher cadmium concentrations, and guaiacol peroxidase activity increased at the lowest cadmium concentration. Non-enzymatic redox markers were also affected: low-molecular-mass and high-molecular-mass thiol contents remained unchanged; lipid peroxidation products were lower at high cadmium levels; and total polyphenol and flavonoid levels were higher at moderate cadmium concentrations. Overall, the results demonstrate that cadmium induces coordinated changes in mineral composition, photosynthetic pigments, and redox-related metabolism in Paulownia plantlets grown *in vitro*.

Keywords: *Paulownia, cadmium, mineral profile, oxidative stress, antioxidant enzymes, histology*

Poster presentation

Biotechnological approaches of the OMIBREED project for sustaining olive genetic resources: *In vitro* conservation by slow growth storage

Anna De Carlo, Waed Tarraf

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

waedtarraf@cnr.it

Abstract

The conservation of plant biodiversity, particularly for species with high cultural and economic value such as *Olea europaea* L., has become a global priority. In this context, the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF) has funded the project “Characterisation and Valorisation of Agrobiodiversity through Multiomic and Next-Generation Breeding Approaches for Resistance against *Xylella fastidiosa* - OMIBREED”. The project aims to identify genetic traits associated with resistance against *X. fastidiosa* and to promote the sustainable use and conservation of available olive genetic resources. This study reports the results of *in vitro* conservation of selected olive genotypes of interest within the project, using the Slow Growth Storage (SGS) technique. For low-temperature conservation, healthy micropropagated shoots were transferred onto Rugini Olive Medium (OM) supplemented with different concentrations of zeatin and maintained under either light or dark conditions. The results identified optimal conditions for *in vitro* storage of olive shoots for up to 12 months, significantly reducing shoot growth during cold storage and preserving their ability to resume normal multiplication after transfer to standard culture conditions. Overall, the OMIBREED project offers effective strategies for the clonal conservation of olive genetic resources, ensuring both the conservation of genetic diversity and high phytosanitary quality.

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

Poster presentation**Comparative evaluation of aluminum cryo-plate methods for the cryopreservation of autochthonous plum (*Prunus domestica* L.) genotypes in Albania****Yllka Lala¹, Tatjana Vujović², Efigjeni Kongjika³, Valbona Sota^{1,3}**¹*Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania*²*Fruit Growing Institute, Čačak, Serbia*³*Research Center of Biotechnology and Genetics, Academy of Sciences of Albania, Tirana, Albania**yllkabami@gmail.com***Abstract**

This study aimed to optimise and evaluate vitrification (V cryo-plate) and dehydration (D cryo-plate) techniques for the cryopreservation of two autochthonous plum (*Prunus domestica* L.) genotypes, ‘Tropojane’ and ‘Vlonjate’. Shoot tips excised from *in vitro*-grown plantlets were precultured for 3 days at 23 °C in darkness on Murashige and Skoog (MS) medium supplemented with 0.3 M sucrose. Subsequently, explants were transferred for 5 days to MS medium containing 0.5 M sucrose under dark conditions at 4 °C. After preculture, shoot tips were placed in calcium alginate within the wells of the aluminum cryo-plate, and polymerization was achieved by the addition of 0.1 M calcium chloride solution. In the D cryo-plate protocol, dehydration was performed by air-drying the cryo-plates under laminar flow at 23 °C for 2, 2.5, and 3 hours. In the V cryo-plate method, dehydration was achieved using plant vitrification solution 2 (PVS2), applied for 20, 30, and 40 minutes. Cryo-plates were directly immersed in liquid nitrogen (−196 °C). Rewarming was carried out rapidly, and explants were transferred to recovery medium. The results indicated a genotype-dependent response to the applied cryo-plate methods. The highest efficiency was obtained with the D cryo-plate method at 2.5 hours in the ‘Vlonjate’ genotype, reaching 52.5% survival and 45% regeneration. Under the same conditions, ‘Tropojane’ achieved 37.5% regeneration. In the V cryo-plate method (30 minutes), ‘Tropojane’ exhibited higher regeneration (35%) compared to ‘Vlonjate’ (27.5%). The D cryo-plate method demonstrated superior performance across both genotypes, indicating its suitability for long-term germplasm conservation. These findings highlight genotype-specific responses and emphasize the importance of protocol optimisation to improve cryopreservation efficiency.

Keywords: plum genotypes, cryogenic storage, germplasm conservation, D cryo-plate, V cryo-plate

Poster presentation**Innovative cryopreservation of dormant buds of apple and pear to safeguard genetic resources and commercial cultivars (DormaCryo)****Zhibo Hamborg¹, Linn Borgen Nilsen², Lill-Iren Hansen³, Dag-Ragnar Blystad¹**¹*Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research, Ås, Norway*²*Norwegian Genetic Resource Centre, Norwegian Institute of Bioeconomy Research, Ås, Norway*³*Sagaplant AS, Akkerhaugen, Norway.*Zhibo.hamborg@nibio.no**Abstract**

Cryopreservation of dormant winter buds is an efficient, genetically stable, and environmentally sustainable approach for the long-term conservation of fruit tree germplasm. This method is particularly important for apple and pear cultivars, which cannot be preserved through conventional seed storage. Utilizing naturally cold-hardened buds stored in liquid nitrogen (−196 °C) ensures secure preservation of both commercial and genetically valuable resources. The DormaCryo project (2025–2028) aims to develop and implement robust cryopreservation protocols tailored to Norwegian apple and pear cultivars, with a focus on maintaining genetic integrity, plant health, and commercial viability. The project integrates cryotechnology with comprehensive phytosanitary testing, including detection of viruses, viroids, and phytoplasmas, to ensure the use of disease-free material. Post-thaw regeneration protocols will be optimised to improve recovery rates and reduce the time required to produce viable planting material. Cryobank capacity will be expanded to support large-scale conservation. Environmental and economic performance of cryopreservation will also be assessed relative to alternative conservation methods. The project will deliver validated cryopreservation and regeneration protocols adapted to Norwegian conditions, improved access to healthy planting material, and strengthened national capacity for long-term conservation of fruit genetic resources. By combining advanced cryopreservation techniques with reliable pathogen diagnostics, DormaCryo supports sustainable fruit production, enhances breeding programs, and aligns with national priorities for biodiversity conservation and climate-resilient agriculture.

Keywords: *apple, cryopreservation, cryotherapy, dormant bud, pear***Acknowledgements** This project has received funding from Forskningsmidlene for jordbruk og matindustri (FFL/JA-Contract No.355751).

Working Group 3

Scaling up and automation

Initiation, multiplication, preservation, germination, and conversion of somatic embryos of new species. Temporary immersion systems. Fully automatic micro-propagation systems combining robotics, laser cutting, machine learning, and artificial intelligence.

Keynote speaker

Artificial intelligence for large-scale and data-driven optimisation of micropropagation: Towards open-access AI platforms

Pedro Pablo Gallego¹, Tomás A. Arteta¹

*Agrobiotech for Health, Plant Biology and Soil Science Department, Biology Faculty,
University of Vigo, 36310, Vigo, Spain*

pgallego@uvigo.es

Abstract

For over a decade, our group has used neurofuzzy logic and artificial neural networks combined with genetic algorithms to mine historical datasets and optimise *in vitro* culture media for apricot, kiwifruit, pistachio, grapevine and medicinal Bryophyllum, generating genotype-specific protocols that outperformed manually developed media. This approach relied on commercial software whose developer has since discontinued the product, exposing the field's dependence on closed tools. In response, we co-developed ADAM, an open-access AI platform for Design, Analysis and Modelling that reproduces and extends this workflow: systematic design of experiments, automated data quality control, and model building across several algorithms coupled with evolutionary optimisation, within a free, browser-based interface. This talk uses that trajectory to look forward: scaling up and automating micropropagation will increasingly generate two complementary data streams requiring different AI strategies. High-dimensional, largely unlabelled digital phenotyping data, RGB, hyperspectral, multispectral and 3D imaging, demand unsupervised or self-supervised methods to extract meaningful features, while structured, labelled data on media composition and plant responses remain the domain of supervised optimisation, as implemented in ADAM. Integrating both data types within open, reproducible platforms is the key step toward automatable, large-scale micropropagation of elite genotypes.

Keywords: *design of experiments, digital phenotyping, evolutionary algorithms, machine learning, scaling up and automation*

Oral presentation

Machine learning tools reveal the effect of topolin-type cytokinins on the induction of Norway spruce embryogenic cultures

Marzieh Mohri¹, Pedro Pablo Gallego², Lucie Fischerová¹, Zuzana Vondráková¹, Kateřina Eliášová¹

¹Institute of Experimental Botany, Laboratory of Biologically Active Compounds, Rozvojová 263, Prague, 16500, Czech Republic

²Vigo University, Faculty of Biology, 36310 Vigo, Spain

mohri.m@ueb.cas.cz

Abstract

During the induction phase of Norway spruce (*Picea abies*) somatic embryogenesis (SE) the cytokinins as alternatives to *N*⁶-Benzylaminopurine (BAP) were applied. The machine learning algorithms were also applied to predict the most suitable induction media. The investigation was set up to evaluate four independent variables: a) Norway spruce genotypes; b) Cytokinins (BAP, kinetin, *meta*-Topolin, *meta*-Topolin Riboside, *meta*-Methoxy topolin) and their combinations with novel CKX inhibitor; c) Induction season (three consecutive years); and d) Induction progress (evaluated at two different time points). All these independent variables served as inputs for data analysis to identify the key factor(s). Two main parameters (outputs) were analyzed: callus induction (non-embryogenic) and embryogenic culture induction rate. We employed tree-based models, specifically CHAID and random forest, to build predictive classification models for treatment effects which were cross-validated against the generalized linear mixed model (GLMM). These models identified genotype as the most critical factor influencing induction success. The results demonstrated that kinetin hampers embryogenic culture induction, while *meta*-topolin riboside is an effective alternative to BAP, showing comparable callus induction (non-embryogenic) rates. These findings indicate that the machine learning models successfully characterised the factors required to optimise culture protocols.

Keywords: Machine learning, Norway spruce, somatic embryogenesis, cytokinins, *meta*-topolin riboside

Acknowledgement: The work was supported by the project LUC24054 of the Czech Ministry of Education, Youth and Sports.

Oral presentation**Biophysics meets plant morphogenesis - monitoring callus growth and somatic embryogenesis with electrical signals****André Caeiro, Francisco C. Cotta, Paulo R.F. Rocha, Jorge Canhoto***Centre for Functional Ecology, Laboratory Associate TERRA, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal**jorgecan@ci.uc.pt***Abstract**

Electrochemical impedance spectroscopy (EIS) provides real-time data on physical properties of biological systems through their frequency-dependent electrical response. It has been used to monitor root development, cell growth and whole physiological status and its potential for analysing *in vitro* morphogenesis and differentiation has increased. Here, we explore the application of EIS to monitor callus growth during indirect somatic embryogenesis in tamarillo. Leaves cultured in MS medium with picloram generate two contrasting callus: embryogenic (EC), with compact nodular structures and non-embryogenic (NEC), friable and hydrated. Previous studies using planar Au electrodes showed that low-frequency impedance correlates with NEC growth, although the morphology of EC limited the applicability of the system. To overcome this, a 3D porous electrode coated with the copolymer PEDOT:PSS was used, which improved sensitivity to biological changes occurring at the electrode–callus interface and enabled the successful monitorization of both *calli*. Distinct profiles were observed, particularly at low frequencies, where impedance changes correlated with growth kinetics, hydration and tissue organization. Equivalent circuit modelling further suggested that electrical parameters reflect the contrasting morphologies and physiological properties of EC and NEC. Overall, the results demonstrate the potential of EIS as a real-time and non-destructive tool for analysing somatic embryogenesis.

Keywords: *electrochemical impedance spectroscopy, embryogenic callus, porous electrode, Solanum betaceum, tamarillo*

Oral presentation

Somatic embryogenesis and image-based deep learning approaches in *Coffea arabica*

Ceren Unek¹, Sena Melis Tutar², Ipek Baz², Bahar Sogutalmaz Ozdemir¹

¹*Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Yeditepe University, 34755 Istanbul, Türkiye*

²*Department of Electrical and Electronics Engineering, Faculty of Engineering and Natural Sciences, Yeditepe University, 34755 Istanbul, Türkiye*

ceren.unek@yeditepe.edu.tr

Abstract

In this study, an indirect somatic embryogenesis system was established in *Coffea arabica* using leaf explants. Callus induction, embryogenic callus formation, somatic embryo development, plant regeneration, and subsequent plantlet rooting and development were successfully achieved. Callus induction reached 100%, while embryogenic callus formation reached 75% in media containing 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine. Rooting and plantlet development parameters were improved in indole-3-butyric acid-containing media compared to indole-3-acetic acid-containing media. Furthermore, image datasets obtained during somatic embryogenesis were used to develop deep learning-based segmentation approaches for coffee somatic embryogenic cell structures. The developed workflow combines classical image processing, unsupervised clustering, and convolutional neural network-based segmentation methods for automated biological image analysis. This study integrates plant tissue culture and image-based computational approaches to support scalable and reproducible propagation systems in coffee.

Keywords: *Coffea arabica*, somatic embryogenesis, deep learning, image segmentation, plant tissue culture

Oral presentation

Biochemical and infrared spectroscopic characterisation of *Pinus radiata* somatic embryos during maturation

Caroline Teyssier¹, Jamie Bridson², Nina Eichler², Mark West², Cathie Reeves², Ilena Isak², Celine Mercier², Jana Krajnakova²

¹INRAE, ONF, BioForA, UMR 0588, 45075 Orléans, France

²Bioeconomy Science Institute, Titokorangi Drive, Private Bag 3020, Rotorua 3046, New Zealand

jana.krajnakova@scionresearch.com

Abstract

Somatic embryogenesis in *Pinus radiata* is sufficiently advanced to enable large-scale production of plants from multiple cell lines. However, somatic embryo (SE) maturation remains a critical and highly sensitive developmental phase influenced by genotype as well as physiological status of the culture. The success of maturation directly affects SE composition, and subsequent germination performance, plantlet vigour and establishment potential. An understanding of biochemical composition throughout maturation, together with the ability to characterise it, is therefore essential for early selection of high-performing cell lines and optimisation of protocols. The objectives of this study were to characterise biochemical changes occurring during SE maturation using metabolite analyses combined with germination assessments, and to develop a rapid characterisation approach based on mid-infrared (MIR) spectroscopy. During maturation of SEs, a clear developmental transition was identified between weeks 6 and 8 across all 3 tested cell lines. This transition was associated with significant changes in primary metabolite contents and profiles, with increased dry weight. MIR calibration models were developed for quantification of biochemical parameters, and for discrimination among maturation stages. The results demonstrate that MIR spectrometry provides a rapid and effective approach for biochemical characterisation and developmental assessment of *P. radiata* SEs during maturation.

Keywords: *embryo quality, storage compound, germination, somatic embryogenesis*

Oral presentation

Application of somatic embryogenesis to increase the range of conifer tree species used for forestry in Finland

Saila Varis¹, Mikko Tikkinen^{1,2}, Sakari Välimäki^{1,2}, Tuija Aronen¹

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

²Currently Lauka Biotech Ltd., Laitaatsillantie 3, FI-57170 Savonlinna, Finland

saila.varis@luke.fi

Abstract

In Finland the interest to use Siberian larch (*Larix sibirica*) in forest regenerations has increased due to predicted pest and drought problems in Norway spruce, but due to uncertainty surrounding larch flowering and seed development there is not enough planting material. Applying somatic embryogenesis (SE) in larch was successful using the same method as for Norway spruce forest regeneration material. Initiation success varied between cone collection day and media used, being 19 % at the best. Larch embryo germination and seedling growth were particularly good. The results laid the foundation for the mass propagation method of larch and the registration of lines as forest reproductive material, which is carried out in the new project co-funded by EU through South-Savo Regional Council. In juniper (*Juniperus communis*) the aim was to produce plants having narrow shape and/or abundant berry production for ornamental and commercial use, and for that the suitable sources for explants were mapped. Unfortunately, only few cell lines producing embryos were obtained, and maturing embryos without secondary proliferation was challenging. However, following successful germination and acclimatization, to our knowledge, the first common junipers SE plants are growing at Luke.

Keywords: *Juniperus communis*, *Larix sibirica*, ornamental, tissue culture

Oral presentation

Characterisation of embryogenic lines of *Abies pinsapo* Boiss.

Laura Cabero-Moreno, Carolina Sánchez-Romero

*Dept. of Botany and Plant Physiology, Faculty of Science, University of Malaga,
Campus de Teatinos s/n, 29071 Malaga, Spain*

c.sanchez@uma.es

Abstract

The Spanish fir (*Abies pinsapo* Boiss.) is a threatened species, with a very restricted distribution area. Different strategies have been considered in order to improve the conservation of this species, including the application of biotechnological tools. Somatic embryogenesis is a very efficient *in vitro* culture technique, which can be utilized for the management and conservation of plant genetic resources. Initiation of embryogenic cultures is one of the most limiting phases in somatic embryogenesis protocols. In this work, different culture factors were tested in order to improve this process from mature zygotic embryos. The embryogenic lines obtained were characterised by analyzing their structural composition and morphological features. Variations in their proliferative and morphogenic capacity were also investigated. Additionally, the cryopreservation protocol optimised in a previous work was applied to the different embryogenic lines, thus investigating differences in their cryotolerance and response to the preconditioning treatment. This study represents an advance in the knowledge of somatic embryogenesis in *Abies pinsapo*, a technique recently applied to this species.

Keywords: *Abies pinsapo*, cryopreservation, induction, somatic embryogenesis

Oral presentation

**Mimicking drought in germination of somatic hybrid larch
(*Larix* × *eurolepis*) by inducing mild and sublethal osmotic stress
*in vitro***

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

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

emma.ehmke@hu-berlin.de

Abstract

Forestry needs to prepare for elevated drought events due to climate change. One solution can be planting of drought tolerant genotypes, multiplied via somatic embryogenesis. Tolerance of hybrid larch genotypes might be increased compared to parental species due to heterosis. However, testing genotypes in the field takes several years from initiation up to sufficiently aged trees. To test genotypes at an early stage of plant development, we applied mild and sublethal osmotic stress in germination to potentially link the reaction *in vitro* to drought stress tolerance *ex vitro*. Young plantlets were placed on control BM5, or media containing sorbitol as treatment. Emblings were photographed at the beginning and end of stress application and length of root and cotyledon were measured using Fiji. Most genotypes show no growth of measured organs during sublethal stress and slowed growth during mild stress. However, we were able to identify two groups of genotypes showing altered growth of organs during mild stress compared to control: “more root, less leaf” and “less root, more leaf”. Further experiments with 2 year old plants will investigate if these altered strategies can be linked to drought stress tolerance in the greenhouse. Early detection of drought stress tolerance would be an invaluable tool for the production of selected trees for forestry applications. Additionally, we plan to test whether stress *in vitro* could prepare plants for drought stress in later stages.

Keywords: *somatic embryogenesis, drought stress, early stage screening, potential climate change adaptation*

Oral presentation**Establishing an efficient *in vitro* micropropagation protocol for *Fraxinus excelsior* tolerant to ash dieback disease****Karuna Shrestha^{1,2}, Oliver Gailing², Dheeraj Singh Rathore¹**¹Teagasc, Forestry Development Department, Oak Park Research Centre, Carlow, R93 XE12, Ireland²Forest Genetics and Forest Tree Breeding, Buisgenweg-Institute, Georg-August University of Göttingen, Buisgenweg 2, 37077, Göttingenkaruna.shrestha@teagasc.ie**Abstract**

Ash dieback disease (ADB), caused by the fungus *Hymenoscyphus fraxineus*, poses a severe threat to *Fraxinus excelsior* (European ash) populations across Europe. The large-scale propagation of tolerant ash material remains a major bottleneck for restoration and conservation programmes targeting European ash. This study aims to establish an efficient *in vitro* micropropagation protocol for tolerant ash genotypes using indirect organogenesis. Mature zygotic embryos were collected from genotypes showing tolerance to ADB in the Irish ash gene bank. Murashige and Skoog medium supplemented with varying concentrations of cytokinins (n= 5) and auxins (n=6) was used to optimise culture initiation, shoot multiplication and plantlet establishment. Preliminary results indicate a specific cytokinin treatments improved shoot initiation and multiplication, with regeneration rates reaching approx. seven plantlets per explant. A 1:3 cytokinin-to-auxin ratio supported successful plantlet establishment, while gradual acclimatisation under *in vitro* and *ex vitro* conditions improved plantlet survival rates. Additional genotypes are being tested to assess the robustness of the protocol and support the development of a genotype-independent propagation protocol. This protocol will provide a promising platform for the large-scale propagation of tolerant ash material and support long-term conservation, restoration, and breeding efforts for ash affected by ash dieback and emerging threats such as emerald ash borer.

Keywords: ash dieback, micropropagation, indirect organogenesis

Oral presentation

Date palm artificial seeds: current results and perspectives

Lotfi Fki

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

lotfifki@yahoo.fr

Abstract

Date palm (*Phoenix dactylifera* L.) holds significant socioeconomic value, but its micropropagation is challenging. Artificial seeds offer a promising solution for large-scale commercial regeneration and advancing cryopreservation methods. This study presents an efficient protocol for generating thousands of somatic embryos from juvenile inflorescences. Artificial seeds were successfully developed using 3% (w/v) sodium alginate and 100 mM calcium chloride in MS medium. These concentrations maintained high germination rates, especially when moderate desiccation was applied. Furthermore, encapsulated proembryogenic masses resisted cryotreatments, achieving regeneration rates up to 70%. Despite these advancements, agricultural application remains limited by technical challenges, particularly contamination during soil transfer. To overcome this, integrating artificial intelligence such as computer vision and robotics, offers a pathway to standardize production, optimize formulations, and reduce contamination. This presentation discusses the potential of artificial seeds for date palm propagation and cryopreservation while proposing automation strategies to minimize contamination and facilitate adoption in modern agriculture.

Keywords: *date palm, somatic embryos, large scale micropropagation, artificial seeds, cryopreservation*

Oral presentation

Automation in tissue culture – transforming the transplanting and hardening process of tissue cultured plants

Michiel van Bennekom

Iribov BV, Middenweg 591b, 1704BH, Heerhugowaard, the Netherlands.

m.vanbennekom@iribov.com

Abstract

Large scale tissue culture is a labour-intensive process. Therefore, many productions take place in countries where labour costs are lower. However, hardening of this tissue cultured material often takes place at locations where labour is more expensive, or less available. As large-scale producer Iribov has taken the step to automate the transplanting part, by use of its TC tray system. The development consists of a tissue culture unit, equipped with a grid. The fixed positions of the rooted plants create the possibility for automation. With our automated transplanting process as example, we will provide an overview of the considerations that preceded this development, the challenges we have solved to come to the current process, and the next steps to further improve the product. Next to automation of the planting process Iribov has invested in its hardening facilities, by raising an indoor farm facility. With control over growing conditions like temperature, humidity, light spectrum, -intensity and duration plants are nursed under laboratory-like conditions, enabling a directed development of the plants. Besides control over plant development, the indoor farm facility has proven to be an energy and resources efficient production system. With the full control over its circumstances, pest risk and seasonal influences (cold, heat) do not play a role in hardening, ensuring limited losses of plants during the crucial period of transition from lab to forest or orchard ready plants.

Keywords: *automation, tissue culture, indoor farming, hardening, young plants*

Oral presentation

***In vitro* multiplication of *Quercus robur* in the RITA system**

Teresa Hazubska-Przybyl¹, Joao Paulo Rodrigues Martins¹, Mikołaj Krzysztof Wawrzyniak¹, Maciej Rajek¹, Agata Obarska¹, Paulina Pilarz¹, Bruce Christie², Nieves Vidal³, Pawel Chmielarz¹

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

²*Greenplant Co, Palmerston North 4410, New Zealand*

³*CSIC Misión Biológica de Galicia, Avda Vigo S-N, Santiago de Compostela 15705, Spain*

hazubska@man.poznan.pl

Abstract

Quercus robur is a forest species that is difficult to propagate conventionally, therefore *in vitro* micropropagation is used for large scale production. This study used a RITA temporary immersion system to evaluate the effects of two BAP concentrations (0 and 3.5 μ M) and three concentrations of meta-topolin (0, 1.75, and 3.5 μ M) on *Q. robur* shoot multiplication. The plant material was grown on Woody Plant Medium for sixty days. Growth and physiological responses were assessed through fresh weight, number of microshoots, pigment contents, and hydrogen peroxide (H_2O_2) levels. Results showed that BAP significantly increased microshoots fresh biomass and number, carotenoid content, and the carotenoid/total chlorophyll ratio, while meta-topolin mainly influenced fresh weight increase. Although total Chl *a+b* content was similar across treatments, the Chl *a/b* ratio decreased at low meta-topolin concentrations and in the absence of BAP. A significant interaction between BAP and meta-topolin was found for pigment ratios, indicating combined effects on photosynthetic pigment balance. The analysis of H_2O_2 content did not show significant differences depending on the applied BAP concentration. However, the addition of 1.75 μ M meta-topolin had a significant impact on mean H_2O_2 values, causing a nearly 50% decrease compared to the control. Generally, cytokinin type and concentration strongly influenced shoot proliferation and physiological status of *Q. robur* in the RITA system.

Keywords: oak, microshoots, chlorophyll content, H_2O_2

Oral presentation**Temporary immersion bioreactors as a promising approach for large-scale micropropagation and sustainable supply of high-value terpenes in *Myrtus communis*****Waed Tarraf¹, Carla Benelli¹, Francesca Ieri², Gabriele Cencetti², Anna De Carlo¹**¹*Institute of BioEconomy (IBE), National Research Council (CNR) of Italy, 50019 Sesto Fiorentino, Florence, Italy*²*Institute of Biosciences and Bioresources (IBBR), National Research Council of Italy, 50019 Sesto Fiorentino, Florence, Italy*waedtarraf@cnr.it**Abstract**

Myrtus communis is an aromatic medicinal species widely used in the pharmaceutical, food, and cosmetic industries. The growing demand for its bioactive compounds makes biotechnological techniques, such as *in vitro* propagation, an effective method for providing a sustainable and consistent supply of high-quality plant material under controlled conditions, independent of seasonal variations. In this study, the Temporary Immersion System (TIS) was evaluated as a tool for large-scale micropropagation and secondary metabolite production in myrtle. The *in vitro* culture in liquid medium using three TIS bioreactors (SETIS, Plantform, and ElecTIS) was compared with a conventional agar-based system. Biomass accumulation and biochemical parameters were measured to evaluate *in vitro* propagation efficiency, and terpene composition was characterised and quantified by GC–MS analysis for *in vitro* shoots grown on semisolid medium, in TIS bioreactors, and under *ex vitro* conditions. The results demonstrated that TIS improved plant growth, enhanced biomass production and supported efficient large-scale propagation, while significantly increasing terpenes accumulation compared to the semisolid medium. Hence, the use of TIS bioreactors represents a promising and economically feasible strategy for industrial-scale production of myrtle bioactive compounds, reducing pressure on natural populations, preserving genetic diversity, and minimising yield losses due to environmental and climatic factors.

Keywords: *myrtle, in vitro propagation, bioreactors, biomass accumulation, secondary metabolites*

STSM presentation

ADAM: Advanced Design and AI-Driven Modeling for plant tissue culture media optimisation

Hans Bethge^{1,2*}, Traud Winkelmann³, Tomás A. Arteta², Esmaeil Nezami⁴, Marco Pepe⁵, Mohsen Hesami⁵, Andrew Maxwell Phineas Jones⁵, Mariana Landin⁶, Pedro P. Gallego²

¹*Institute of Botany, Department of Phytophotonics, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419, Hannover, Germany*

²*Agrobiotech for Health, Plant Biology and Soil Science Department, Biology Faculty, Vigo University, Vigo, Spain*

³*Institute of Plant Genetics, Section Reproduction and Development, Leibniz University Hannover, Hannover, Germany*

⁴*Department of Plant Breeding, Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute (NSTRI), Karaj P.O. Box 31485-498, Iran*

⁵*Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada*

⁶*Pharmacology, Pharmacy, and Pharmaceutical Technology Department, I+D Farma (GI-1645), Faculty of Pharmacy, the iMATUS and the Health Research Institute (IDIS), University of Santiago de Compostela, E-15782 Santiago de Compostela, Spain*

traud.winkelmann@zier.uni-hannover.de; pgallego@uvigo.gal; hans.bethge@hot.uni-hannover.de

Abstract

Plant tissue culture media optimization has been hindered by trial-and-error experimentation and limited access to advanced experimental designs and computational methods, particularly in resource-constrained settings where specialized software and expertise are unavailable. While machine learning and evolutionary algorithms offer powerful techniques, 80% of published studies rely on licensed software, creating significant barriers to adoption. We introduce ADAM (Advanced Design and AI-Driven Modeling for Plant Tissue Culture Media Optimization), an open-access, web-based platform integrating the full ML-EA workflow—from experimental design through multi-objective optimization—requiring no programming expertise. ADAM integrates Design of Experiments, Data analysis, Model Building (nine ML algorithms), and Optimization (genetic algorithm, particle swarm, NSGA-II, SMS-EMOA) modules. Validation across plant tissue culture applications demonstrated predictive performance equivalent to or exceeding the original published models, identifying non-dominated solutions balancing competing objectives.. Beyond plant tissue culture, ADAM's modular architecture supports diverse biotechnological processes—including bioreactor optimization, secondary metabolite production, and acclimatization—accelerating the transition from empirical intuition to systematic, data-driven protocol development.

Keywords: *plant tissue culture, machine learning, predictive modelling, evolutionary algorithms, culture media optimization*

Flash talk presentation

***In vitro* somatic embryogenesis and phytochemical profiling of the endangered *Hyphaene thebaica* for conservation and sustainable utilization**

Doa'a Jamal Abu-Darwish

Plant Biodiversity Research Directorate, National Seed Bank (NSB), National Agricultural Research Center (NARC), Al-Balqa' 19381, Jordan.

doaa.abudarwish@yahoo.com

Abstract

Hyphaene thebaica (L.) Mart. is a recalcitrant desert palm of high medicinal value whose propagation is limited by poor germination and strong physiological constraints. This study presents an experimental *in vitro* regeneration approach aimed at inducing morphogenic responses under controlled culture conditions. Immature female inflorescences, and shoot tips were cultured on MS and B5 media supplemented with 2,4-D, IBA, BAP, and 2iP under sterile conditions. Developmental responses were assessed through callus induction, organogenic activity, root initiation, and culture viability. Callogenesis was successfully induced from both inflorescence and meristematic tissues, indicating differential regenerative competence among explant sources. Early root formation occurred in radicle-derived cultures. Phenolic exudation causing browning was a major limitation, partially reduced using activated charcoal and antioxidants, improving survival. In parallel, preliminary phytochemical profiling using GC-MS and total phenolic content analysis, along with antioxidant and antimicrobial assays, is proposed to evaluate biochemical variation in regenerated tissues. These results demonstrate inducible morphogenic plasticity in *H. thebaica* and provide the first experimental baseline for somatic embryogenesis-oriented regeneration, forming a foundation for conservation and bioactive compound exploration.

Keywords: *Hyphaene thebaica* (L.) Mart, somatic embryogenesis, *in vitro* regeneration, callus induction, phytochemical profiling

Flash talk presentation

In vitro* Callus Induction and Metabolic Profiling of *Melaleuca alternifolia

Muhammad Mudasir

*Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences,
Czech University of Life Sciences Prague, Kamýcká 129, Prague 6, 16500, Czech
Republic*

mudasirbabbar@gmail.com

Abstract

Melaleuca alternifolia is an economically important medicinal plant known for its therapeutic properties, but optimised protocols for producing its valuable secondary metabolites through *in vitro* techniques remain limited. In this study, we developed a callus culture protocol using leaf explants on Murashige and Skoog (MS) medium supplemented with thidiazuron (TDZ) and 2,4-dichlorophenoxyacetic acid (2,4-D), alone or in combination. Combining 2,4-D with TDZ significantly enhanced biomass accumulation, yielding a maximal dry biomass of 14.2 g/l for leaf explants. Furthermore, optimised callus cultures exhibited a substantial increase in phenolics (305.2 mg/l for leaf-associated callus) and flavonoids (55.8 mg/l). Antioxidant assays revealed high scavenging capacities of 88.5% at optimised growth regulator concentrations. These findings suggest that the established *in vitro* system provides a reliable, season-independent platform for the large-scale production of bioactive compounds from *M. alternifolia*. This study contributes to the biotechnological advancement of *M. alternifolia* by offering a sustainable method to enhance secondary metabolite yields for commercial or pharmaceutical applications.

Keywords: *Melaleuca alternifolia*, *in vitro* propagation, callus induction, growth regulators, secondary metabolites

Poster presentation

Influence of different LED light wavelengths and auxin dependence on rhizogenesis induction in *Prunus amygdalus* Batsch (Rosaceae)

Dawid Kocot¹, Antonina Gluch², Tércia Lopes³

¹*Dept. of Ornamental Plants and Garden Art, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Mickiewicza 21 Ave., 31-120 Krakow, Poland*

²*Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Mickiewicza 21 Ave., 31-120 Krakow, Poland*

³*Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal*

dawid.kocot@urk.edu.pl

Abstract

Root induction is a key stage in the micropropagation process, determining successful plant production. In this study, the effects of selected auxins and LED light quality on rhizogenesis induction in almond (*Prunus amygdalus*), an economically important species classified as difficult-to-root under *in vitro* conditions (recalcitrant species), were evaluated. IAA and IBA at a concentration of 1 mg/L were added to MS medium. The effects of different LED light wavelengths were analyzed: 100% blue light, 100% red light, a combination of 70% red and 30% blue light (RB), and far-red light (FR). The control consisted of shoots grown under fluorescent lamps and on MS medium without growth regulators. Explants were shoot segments approximately 2 cm long, containing at least two nodes and deprived of leaves. It was hypothesized that the addition of auxins would stimulate root induction, and that light quality would significantly modulate this process. The percentage of rooted explants, the number of roots per shoot, and root length were evaluated. The highest rhizogenesis efficiency was obtained on MS medium supplemented with IBA, regardless of the light treatment applied. In the remaining experimental combinations, significantly lower rooting efficiency and a less developed root system were recorded. The obtained results indicate that both the type of auxin used and light quality play an important role in regulating rhizogenesis in almond *in vitro*.

Keywords: *almond tree, in vitro rooting, light quality, recalcitrant species*

Poster presentation***In vitro* establishment of European Ash (*Fraxinus excelsior*) for a clonal trial on ash dieback resistance****Franka N. Thiesen, Ben Bubner**

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

franka.thiesen@thuenen.de

Abstract

Common ash (*Fraxinus excelsior*) has been an important forest tree, covering 2.37 % of Germany's forested area. Today, virtually all stands are affected by ash dieback, caused by the invasive fungal pathogen *Hymenoscyphus fraxineus*. During 2019-2021 a clonal seed orchard was established in Northeastern Germany. The Tressow seed orchard contains 126 genotypes of plus trees selected between 2016 and 2019 in the federal state of Mecklenburg-Western Pomerania. Infection pressure in the original stands of the plus trees started before 2002, the year the first records of ash dieback were made in the region. This early infection pressure may have promoted a high tolerance to the disease. The orchard was planted on agricultural soils far from other infected ash stands in order to prevent foreign pollen influx and to reduce infection pressure. At the Thuenen Institute, several ash genotypes have been established and maintained *in vitro*, providing a valuable collection for research and propagation. In 2026 a new establishment campaign was launched to introduce additional genotypes derived from the Tressow plus trees that had shown apparent tolerance to ash dieback. Particular emphasis was placed on the use of dormant winter buds as explants and on the development of protocols suitable for mature tree material. We present the methodology used for explant collection, surface disinfection, and culture establishment and we discuss the challenges associated with introducing mature ash material into tissue culture. The resulting cultures will provide material for clonal trials that allow further selection for ash dieback resistance among the genotypes of the Tressow seed orchard genotypes.

Keywords: *European ash, ash dieback, clonal propagation, plus trees, plant conservation*

Poster presentation

**Preserving historic trees for Pückler Park Branitz, Germany by
in vitro propagation**

Juliane Raschke¹, Aisha Mehar, Liis Viljak, Anika Dreilich, Christian Boehm², Juliane Ribbeck-Lampel², Andrea Rupps¹

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

²*Stiftung Fürst-Pückler-Museum Park und Schloss Branitz, Robinienweg 5, 03042 Cottbus*

raschkej@hu-berlin.de

Abstract

Garden monuments face numerous problems - long-term cultivation on the one hand, climate change on the other, leading to the demise of trees due to prolonged droughts, late frosts, and extreme heat. Branitz Park, a unique cultural landscape shaped by Fürst Pückler in 1845/46 experiences loss of several valuable and partly unique trees. A collaboration between the Foundation Pückler Museum - Branitz Park and Palace (SFPM) and the Späth-Arboretum of Humboldt-Universität zu Berlin plans to ensure the conservation of historically valuable and ideally resilient tree genotypes of both landscape parks. Aiming to maintain a variety of species, we apply various cultivation techniques to produce true-to-type trees - from classical horticultural approaches such as rooting cuttings, to applying tissue culture protocols for recalcitrant species: We are trying to establish *in vitro* cultures by establishing micro-cuttings, embryogenic callus or the initiation of somatic embryogenesis, depending on species and available tissue. Further to set up a living collection and preserve the trees for the future.

Keywords: *historically valuable trees, multiple species, conservation strategies, true-to-type*

Poster presentation**Influence of silicon on the micropropagation of peach rootstock
GF 677****Lilyana Nacheva¹, Diyana Aleksandrova¹, Ivaylo Tsvetkov²**¹Fruit Growing Institute, Agricultural Academy, 12 Ostromila Str., 4004, Plovdiv,
Bulgaria²Forest Research Institute – BAS, 132, Kliment Ohridski Blvd., Sofia 1756, Bulgariatsvet_i@yahoo.com**Abstract**

The rootstock GF 677 (*Prunus amygdalus* × *Prunus persica*) is the most commonly used rootstock for peach in Europe, propagated almost exclusively by tissue culture. It is tolerant to Fe deficiency and especially suited to soils with poor fertility, low water availability, and high CaCO₃ content. In recent years, many studies have shown that silicon (Si) has a positive role in improving various aspects of plant micropropagation, cryopreservation, and production of secondary metabolites. The study was aimed at improving the micropropagation of the GF 677 rootstock by applying silicon (Si) as an additional component of the nutrient medium. The plantlets were cultivated *in vitro* on Murashige and Skoog (MS) nutrient medium, supplemented with 5 µM 6-benzylaminopurine, 0.01 µM indole-3-butyric acid, 30 g/l sucrose, 6.5 g/l Phyto agar (Duchefa, The Netherlands). Silicon was added to the culture medium as calcium (CaSiO₃) or potassium (K₂SiO₃) silicate (1 or 2 mg/l). Control plants were cultivated on a silicon-free medium. Some biometric indicators, fresh and dry biomass were recorded after three subcultures of four weeks each. The results obtained showed that the addition of 1 mg/l potassium silicate to the culture medium stimulated the accumulation of biomass and development of new shoots, although the latter was not statistically proven. Use of silicon as a supplement of tissue culture media might have favorable effect(s) on micropropagation of the peach rootstock GF677, but broader set of sources and concentrations should be tested.

Keywords: *in vitro*, silicon, woody plants

Poster presentation

**WB3: A highly automated somatic embryogenesis based method
for plant production - latest developments**

Malin Abrahamsson, Magnus Hertzberg

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

Malin.Abrahamsson@SweTree.com

Abstract

SweTree Technologies is a research and technology development company within the area of forest biotechnology. We have for many years been developing an automation solution for large scale (tens of millions) production of conifer plants based on somatic embryogenesis. The last years we have been running a pilot facility to test and further develop our automation solutions, and currently we run the pilot with a production on 100 000 to 150 000 SE based micro plants per year. We will present our developed automation technologies and our latest results and plans.

Keywords: *conifer, embryogenesis, micro plants, pilot facility, somatic embryo*

Poster presentation**Integrating micropropagation into conservation strategies for endangered field elm (*Ulmus minor* Mill.) populations in Croatia**

**Sanja Bogunović¹, Doroteja Bitunjac¹, Mia Marušić¹, Ivana Sirovica¹,
Marko Bačurin²**

¹Croatian Forest Research Institute, Cvjetno naselje 41, 10450 Jastrebarsko, Croatia

²Faculty of Forestry and Wood Technology, Svetošimunska cesta 23, 10000 Zagreb, Croatia

sanjam@sumins.hr

Abstract

Field elm (*Ulmus minor* Mill.) has experienced a dramatic decline across Europe due to Dutch elm disease, resulting in severe population fragmentation and progressive loss of genetic diversity. In Croatia, remaining populations are mainly represented by isolated trees and small groups of individuals, including potentially disease-tolerant genotypes that may play an important role in future restoration efforts. The recently initiated ElmForFuture project aims to identify, characterise and conserve valuable field elm genetic resources across the Croatian distribution range. An important component of the project is the development of micropropagation protocols for selected genotypes as a complementary approach to traditional conservation measures. Axillary buds collected from vital trees will be introduced into *in vitro* culture and tested through different sterilization procedures, culture media formulations and plant growth regulator combinations to optimise culture establishment, shoot multiplication, elongation, rooting and acclimatisation. The development of reliable micropropagation methods will support *ex situ* conservation, facilitate the production of disease-free planting material and provide a basis for the establishment of clone archives and future resistance studies. Furthermore, the project integrates *in vitro* propagation with field surveys, genotyping and ecological characterization of remaining populations.

Keywords: *Ulmus minor*, micropropagation, genetic conservation, forest restoration, plant tissue culture

Poster presentation**Fifteen years of research on hybrid larch somatic embryogenesis:
Progress, pitfalls and perspectives for forestry**

**Andrea Rupps¹, Emma Ehmke¹, Madlen Walther¹, Juliane Raschke¹, Ute Tröber², Ricardo Joffe², Silvan Liebsch², Thomas Hoppe³, Birgit Reiche⁴,
Sophie Maiwald⁵, Anna Rudzinski⁵, Tony Heitkam⁵**

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

²Staatsbetrieb Sachsenforst, Dept. 42 Forest Genetics and Forest Plant Breeding,
Bonnewitzer Str. 42, 01796 Pirna OT Graupa, Germany

³Baumschulen Oberdorla GmbH, Burgstr. 57, 99986 Vogtei OT Oberdorla, Germany

⁴TU Dresden, Institut of Forst Botany and Forest Zoology, Pienner Str. 19, 01737
Tharandt, Germany

⁵RWTH Aachen University, Institute of Biology I, 52074 Aachen, Germany

andrea.rupps@hu-berlin.de

Abstract

Over the last 15 years, somatic embryogenesis (SE) has been investigated as a tool for the clonal propagation of hybrid larch (*Larix × eurolepis*). While SE offers unique opportunities for the mass propagation of superior genotypes, its transfer from laboratory protocols to operational forestry remains challenging. We present the current status of hybrid larch SE, demonstrate major bottlenecks as well as ways to reduce them and discuss its practical value and acceptance from both scientific and end-user perspectives. Research has focused on optimising critical steps of the SE process, with particular attention to culture stability during prolonged maintenance, leading to the implementation of parallel maturation and propagation strategies to reduce the risk of line weakening. To support future deployment and certification, molecular approaches were developed for clone characterisation and identification. Marker systems based on repetitive DNA elements were established and complemented by *in vitro* and *ex vitro* assessments of clonal performance. Although persisting constraints, we are able to provide a realistic pathway toward the use of elite hybrid larch clones for future forestry and meet the high interest from the forestry side.

Keywords: *genotype characterisation, genetic markers, field evaluation*

Acknowledgement: The research on *Larix × eurolepis* is currently supported by the German Federal Ministry of Food and Agriculture (BMLEH) through the project LarchForFlexibility (grant number 2222NR005A).

Poster presentation**Evaluation of CKX inhibitors in the presence of trans-zeatin during micropropagation of *Prunus domestica* L. cv “Tropojane”**

Brunilda Çuko¹, Ledina Shkëmbi¹, Stefaan P.O. Werbrouck², Maurizio Lambardi³, Jaroslav Nisler⁴, Efigjeni Kongjika⁵, Valbona Sota^{1,5}

¹Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

²Ghent University, Belgium

³IBE-Institute of BioEconomy, National Research Council, Italy

⁴Isotope Laboratory, Institute of the Experimental Botany of the Czech Academy of Sciences, Prague, Czechia

⁵Research Center of Biotechnology and Genetics, Academy of Sciences of Albania, Tirana, Albania

cuko.brunilda@gmail.com

Abstract

Cytokinin oxidase/dehydrogenase plays a crucial role in maintaining cytokinin homeostasis in *in vitro* plants. Four compounds identified as CKX inhibitors were studied for their effect on the micropropagation of *Prunus domestica* L. cv. Tropojane. Each compound (CKX19, CKX20, CKX52, and CKX77) was added separately at 1 µM to QL nutrient medium supplemented with 4.4 µM trans-zeatin (tZ) and 0.537 µM naphthaleneacetic acid (NAA). These tests were organized in parallel experiments using semi-solid nutrient medium and a TIS bioreactor “Plantform™” with liquid nutrient medium. After 28 days of culture, the relative growth rate, shoot number and shoot length were evaluated and compared with those of plants grown on nutrient media supplemented with different concentrations of tZ (0, 4.4, 6.7 and 8.9 µM) combined with 0.537 µM naphthaleneacetic acid (NAA) in each case. The obtained plants demonstrated that the presence of trans-zeatin in both liquid and semi-solid nutrient media promoted the apical dominance of plum shoots and callus formation on the shoot bases. Based on the evaluated parameters, it was found that there were no significant differences when different concentrations of tZ were used, nor among the plants cultivated in the presence of CKX inhibitors. This finding suggests that the exogenous addition of tZ up to 8.9 µM or the inhibition of cytokinin oxidase/dehydrogenase when using CKX19, CKX20, CKX52 or CKX77 does not improve the micropropagation of *Prunus domestica* L. cv. Tropojane. However, regardless of chemical composition of nutrient medium, plants grown in Plantform™ bioreactor showed better performance in all evaluated parameters compared with those grown on semi-solid nutrient medium.

Keyword: *Prunus domestica* L., CKX inhibitors, trans-zeatin, TIS bioreactor

Poster presentation**Adaptation of a temporary immersion bioreactor system for facilitating *ex vitro* transfer of micropropagated blueberry plants****Olena Bobrova^{1,2}, Barborá Tunklova¹, Miloš Faltus¹, Lesia Golosna¹, Alois Bilavčík¹**¹*Plant Physiology and Cryobiology, Czech Agrifood Research Center, Drnovska 507, 16100 Prague 6, Czech Republic*²*Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine*olena.bobrova@carc.cz**Abstract**

Micropropagation is a powerful but relatively expensive and labor-intensive technique that has changed little over recent decades. The development of liquid culture bioreactors and temporary immersion systems (TIS) offers new opportunities to improve the efficiency of *in vitro* propagation. Commercially available systems such as Plantform and SETIS are increasingly used for large-scale plant production. However, the transfer of plantlets from *in vitro* to *ex vitro* conditions remains one of the most critical and labor-intensive stages. The aim of this study was to evaluate a modified Plantform bioreactor system designed to simplify acclimatisation by incorporating propagation trays containing rooting substrates directly within the bioreactor. Highbush blueberry (*Vaccinium corymbosum* L.) cv. 'Reka' maintained on agar-solidified Woody Plant Medium (WPM) was selected as a model plant. A modified autoclavable ViTray 126 propagation tray served as an internal support system for rooting and stabilization of plantlets. To improve tray stability, restrict movement, and prevent small plant fragments or substrate particles from passing through the bottom openings, an autoclavable PTFE-coated fiberglass mesh was used. In addition to previously tested horticultural rockwool cubes (Grodan), peat-based propagation plugs (Preforma 126) were evaluated as support substrates. Multinodal cuttings (3–5 cm long) were transferred to Plantform bioreactors containing 500 mL of liquid WPM rooting medium supplemented with 10 g L⁻¹ sucrose and 0.5 mg L⁻¹ IBA. Cultures were maintained at 26 °C under 16 h light/8 h dark photoperiod and light intensity of 40–50 μmol m⁻² s⁻¹. After one month of cultivation, plantlets remained vigorous and free of visible contamination or physiological disorders. Both support substrates allowed root formation and new shoot development, and root morphology was comparable to that observed on agar-solidified media. Based on plantlet size, branching, and leaf development, the plants appeared suitable for subsequent *ex vitro* transfer. The proposed system enables simultaneous rooting and preparation for acclimatisation while reducing plant handling. It therefore represents a promising technical modification for simplifying *ex vitro* transfer in blueberry micropropagation systems.

Keywords: *Vaccinium corymbosum*, temporary immersion system, micropropagation, rooting

Acknowledgement: This work was supported by the Technology Agency of the Czech Republic and the Ministry of Industry and Trade as part of the TREND Programme (FW10010417).

Poster presentation

Multiplication of *Melaleuca alternifolia* shoots in temporary immersion: effect of genotype, cytokinin and bioreactor type

Bruce Christie¹, Anxela Aldrey², Conchi Sánchez², Jesús M. Vielba², M José Cernadas², Saleta Rico², Nieves Vidal²

¹*Greenplant Co, Palmerston North 4410, New Zealand*

²*Department of Plant Production, Misión Biológica de Galicia, Sede Santiago de Compostela. CSIC, Avda de Vigo s/n, 15705, Santiago de Compostela, Spain*

brucechristie101@gmail.com

Abstract

Melaleuca alternifolia (Maiden & Betche) Cheel is an Australian tree widely cultivated for its essential oil. This study evaluated the impact of the following variables on the multiplication of melaleuca shoots in liquid medium (LM): 1) the type of bioreactor (RITA® and Plantform™), 2) the type of cytokinin (N⁶-benzyladenine (BA) and meta-topolin (mT)), and 3) the genotype. We used ten melaleuca genotypes established in semisolid Murashige & Skoog (MS) medium four months after seed germination. Shoots were cultured in MS with 2.22 µM BA, 2.69 µM α-naphthaleneacetic acid (NAA), 3% sucrose and 0.7 % agar for four years before starting the experiments in LM. For temporary immersion, 15 mm shoot sections were grown in MS with 1.11 µM cytokinin, 2.69 µM NAA and 3% sucrose for five weeks. Shoots were immersed 6 times per day for two min; in Plantform™ additional aerations of 2 min were applied after every immersion. Results showed that all genotypes could be cultured in LM with high multiplication coefficients irrespective of the bioreactor and cytokinin type, although interactions between the three variables were detected. Roots formed spontaneously in the multiplication medium in bioreactors and the plantlets were successfully acclimatized. Our conclusions are that both RITA® and Plantform™ bioreactors are suitable for multiplication of this species. Next experiments will study the effect of genotype and culture treatments on the production of bioactive compounds.

Keywords: *meta-topolin, Plantform™, RITA®, tea tree*

Poster presentation**Effect of bioreactor type and explant density on multiplication and shoot quality of *Corylus avellana* L. cultured by temporary immersion**

Conchi Sánchez¹, Anxela Aldrey¹, Jesús M. Vielba¹, Saleta Rico¹, M José Cernadas¹, Bruce Christie², Nieves Vidal¹

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

²Greenplant Co, Palmerston North 4410, New Zealand

nieves@mbg.csic.es

Abstract

Hazelnut (*Corylus avellana* L.) is a species of significant economic value in Europe. Current hazelnut micropropagation protocols are genotype-dependent, being necessary to increase their applicability to local cultivars or wild material of particular interest. In previous research we developed a protocol for the multiplication of hazelnut in RITA[®] bioreactors, evaluating different concentrations of Fe-Na EDDHA to avoid chlorosis and different types and concentrations of cytokinins. In this study we applied the best results of that research, using Murashige and Skoog medium (MS) supplemented with 400 µM Fe-Na EDDHA, 0.049 µM indole-3-butyric acid (IBA), 0.29 µM gibberellic acid (GA₃), 8.87 µM of cytokinin and 3% sucrose. The variables evaluated were the genotype (2), the explant type (apical or basal sections), the bioreactor type (RITA[®] or PlantForm[™]), the cytokinin type (6-benzyladenine (BA) or meta-topolin (mT)) and the density of the explants in the bioreactors. The explants were immersed 6 times per day during 2 min, and in the case of PlantForm[™] additional aerations of 2 min were applied after every immersion. Vigorous shoots suitable for rooting were obtained in both bioreactors and genotypes. The use of mT increased the multiplication coefficient in both genotypes. We detected significant influences of the genotype and type of explant, as well as interactions between this variable and explant density.

Keywords: hazelnut, meta-topolin, Plantform[™], RITA[®]

Poster presentation**Temporary immersion system as a valid method to isolate extracellular vesicles from *in vitro*-cultivated hop (*Humulus lupulus* L.) plantlets**

Benedetta Chiancone^{1,2}, Angela Paterna¹, Valeria Gianguzzi³, Estella Rao¹, Samuele Raccosta¹, Paola Gargano⁴, Sabrina Picciotto⁴, Giorgia Adamo⁴, Antonella Bongiovanni⁴, Valeria Guarrasi¹, Mauro Manno¹

¹Department of Food and Drug, University of Parma, Viale Parco Area delle Scienze 27/A, 43124 Parma, Italy

²National Research Council of Italy (CNR), Cell-Tech Hub and Institute of Biophysics, Via Ugo La Malfa 153, 90146 Palermo, Italy

³Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, 4, 90128 Palermo, Italy

⁴National Research Council of Italy (CNR), Cell-Tech Hub and Institute for biomedical research and innovation, Via Ugo La Malfa 153, 90146 Palermo, Italy

benedetta.chiancone@unipr.it

Abstract

Plant-derived extracellular vesicles (EVs) are emerging as natural nanocarriers for the delivery of bioactive compounds in pharmaceutical, nutraceutical, cosmetic, and agricultural applications. Here, we report the isolation and characterisation of extracellular vesicles from the liquid culture medium of hop (*Humulus lupulus* L.) plantlets propagated *in vitro* using a Temporary Immersion System (TIS). Hop-derived EVs (Hop-EVs) were isolated directly from the culture medium through differential tangential flow filtration followed by chromatographic purification. Vesicles were characterised using Atomic Force Microscopy (AFM), Cryo-Transmission Electron Microscopy (Cryo-TEM), Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), zeta potential measurements, protein quantification (BCA assay), and immunoblotting. The analyses confirmed the presence of intact nanovesicles expressing the EV-associated markers TET8 and PEN1. Biocompatibility studies demonstrated that Hop-EVs were non-toxic toward both normal and tumor cell lines at the tested concentrations. In addition, antioxidant assays revealed their ability to reduce intracellular reactive oxygen species (ROS) levels under oxidative stress conditions. These findings demonstrate that TIS cultivation represents a sustainable and non-destructive platform for the recovery of plant-derived EVs directly from culture media. This approach provides a scalable source of bioactive nanovesicles and highlights the potential of Hop-EVs as innovative carriers for applications in green biotechnology, nutraceuticals, and cosmetics.

Keywords: extracellular vesicles, green biotechnology, hop, nanovesicles, temporary immersion system

Poster presentation**Comparing the potential of somatic embryogenesis and organogenesis in Rosaceae species****Simón Miranda, Maddalena Nesler, Mickael Malnoy**¹*Research and Innovation Centre, Fondazione Edmund Mach (FEM), via Mach 1, 38098 San Michele all' Adige (TN), Italy**simondavid.mirandachavez@fmach.it***Abstract**

Genetic engineering techniques such as transgenesis and cisgenesis have the potential to generate improved crops with valuable traits, which could be particularly beneficial for commercially relevant species from the Rosaceae family such as apple and cherry. The long juvenile phase, self-incompatibility, and heterozygous nature, render the traditional breeding inefficient for these species. However, many agronomically relevant genotypes remain recalcitrant to regeneration. Moreover, the scarce knowledge of the fate of somatic cells and their regeneration trajectory through somatic embryogenesis and/or organogenesis hinders the application of optimised regeneration protocols suitable for different cultivars. The objective of this study was to evaluate the regeneration potential of several apple and cherry cultivars when leaf explants were cultured in media aimed at inducing somatic embryogenesis and organogenesis. We found that 'Gala' and 'Golden Delicious' were the most responsive apple cultivars to organogenesis compared to other scion and rootstock cultivars, and identified the media combinations providing the highest shoot induction rates. Meanwhile, preliminary data in cherry suggest that scion cultivars proved to be recalcitrant to all media tested, while rootstocks such as 'Gisela' were more responsive to organogenesis, although to a limited extent. Current efforts are now focused to induce somatic embryogenesis, and preliminary results will be presented in the conference.

Keywords: *embryogenesis, organogenesis, apple, cherry*

Poster presentation

Effect of PSK and CAP6 peptides on somatic embryogenesis of *Cryptomeria japonica* (Sugi, Japanese cedar)

Tsuyoshi E. Maruyama¹, Jesús Jiménez^{1,2}, Momi Tsuruta¹, Saneyoshi Ueno¹, Yoshinari Moriguchi³

¹*Department of Forest Molecular Genetics and Biotechnology, Forestry and Forest Products Research Institute (FFPRI), Matsunosato 1, Tsukuba, Ibaraki, 305-8687 Japan*

²*Graduate School of Science and Technology, Niigata University, Ikarashi 8050, Niigata, 950-2181 Japan*

³*Department of Agriculture, Faculty of Agriculture, Niigata University, Ikarashi 8050, Niigata, 950-2181 Japan*

maruyama_tsuyoshi300@ffpri.go.jp

Abstract

Somatic embryogenesis (SE) is a key technology for the mass propagation of *Cryptomeria japonica* (sugi, Japanese cedar). Peptide signaling molecules have been reported to stimulate cell proliferation and regulate developmental processes in plants. Although the effects of PSK (a plant-derived phytosulfokine) and CAP6 (an insect-derived cysteine-rich secretory protein, antigen 5, and pathogenesis-related 1) on SE have been examined in previous studies, their roles have not yet been fully clarified. In this study, we compared the effects of PSK and CAP6 on embryogenic cell proliferation and somatic embryo maturation in sugi. Embryogenic cultures were treated with PSK or CAP6 during the proliferation or maturation phase. Biomass accumulation was quantified as fold increase in fresh weight and embryo maturation was evaluated based on the number of somatic embryos produced. Peptide supplementation in proliferation phase, enhanced biomass accumulation across lines and systems. In maturation phase, PSK treatment resulted in significantly higher embryo formation, whereas CAP6 had little effect on embryo maturation under the tested conditions. These results suggest that PSK is an effective promoter of somatic embryo formation across different sugi genotypes, highlighting its potential application as biostimulant for improving clonal propagation systems.

Keywords: *embryogenic cell, proliferation, phytosulfokine, maturation, cotyledonary embryo*

Poster presentation

Somatic embryogenesis potential in *Hyphaene thebaica* (L.) Mart. via *in vitro* callus induction from female inflorescences

Doa'a Jamal Abu-Darwish

Plant Biodiversity Research Directorate, National Seed Bank (NSB), National Agricultural Research Center (NARC), Al-Balqa' 19381, Jordan

Doaa.abudarwish@yahoo.com

Abstract

Somatic embryogenesis represents a key biotechnological approach for the clonal propagation and conservation of recalcitrant desert palms such as *Hyphaene thebaica* (L.) Mart. In this study, embryogenic potential was evaluated through the induction of *in vitro* callus as a prerequisite for somatic embryo formation. Callus cultures were initiated from different explant sources, including shoot tips, root segments, and male and female inflorescences, using Murashige and Skoog (MS) medium supplemented with plant growth regulators. The highest morphogenic response was observed in female inflorescence explants, which produced friable, fast-growing, creamy-yellow callus under dark conditions. The combined application of auxin (2,4-D) and cytokinin (2iP) was essential for inducing dedifferentiation, while hormone-free medium failed to initiate any callus response. Sucrose supplementation further enhanced tissue proliferation and callus quality, supporting sustained cell division. The formation of highly friable and actively proliferating callus suggests the presence of embryogenic cell clusters, indicating strong potential for somatic embryo induction under optimised culture conditions. These findings provide the first evidence of embryogenic competence in *H. thebaica in vitro* cultures and establish a foundation for future development of a complete somatic embryogenesis system for conservation and large-scale propagation.

Keywords: *somatic embryogenesis, embryogenic callus, Hyphaene thebaica, inflorescence culture, in vitro regeneration*

Poster presentation

Meta-topolin applied in proliferation increases the yield of Norway spruce somatic embryos

Hana Konrádová¹, Nikola Švorc Štěpánová¹, Lukáš Ležal¹, Monika Kofroňová¹, Václav Motyka², Karel Doležal³, Helena Lipavská¹

¹Department of Experimental Plant Biology, Faculty of Science, Charles University, Viničná 5, 128 43, Prague 2, Czech Republic

²Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 00 Praha 6 – Lysolaje, Prague, Czech Republic

³Laboratory of Growth Regulators, Faculty of Sciences of Palacký University and Institute of Experimental Botany, Czech Academy of Sciences, Šlechtitelů 27, 783 71, Olomouc, Czech Republic

hana.konradova@natur.cuni.cz

Abstract

Replacing the standard 6-benzyladenine (BAP) with *meta*-topolin (*mT*) during the proliferation phase of four distinct Norway spruce embryogenic lines positively influenced their subsequent maturation, when cytokinin is no longer applied. Although, both, BAP and *mT* are aromatic cytokinins, and differ only in the presence of a hydroxyl group in *mT*, the application of *mT* resulted in an increased yield of good-quality mature embryos. Furthermore, in selected line C we have examined the consequences of BAP vs. *mT* use that underlay the modified culture behaviour, persisting until late maturation. Our results showed modifications of hormonal balance in BAP, resp. *mT* variants including not only members of the cytokinin group but also other phytohormones (abscisic acid, indole acetic acid, salicylic acid). Additionally, the activities of the most significant antioxidant systems were monitored in this context, involving compounds with prominent protective and signalling roles (ascorbate, glutathione, sugars) and antioxidant enzymes. The results indicate the creation of a memory imprint of *mT* in the proliferation phase that is preserved and passed onto subsequent phases, directing further development. It can be hypothesized that *mT* facilitates a superior realization of the line's embryonic potential, a feat that BAP is unable to achieve.

Keywords: antioxidants, cytokinins, glutathione, *Picea abies*, redox status

Poster presentation

Outcome of COPYTREE training school: Utilising the Plantform™ temporary immersion system at IBISS

Sladana Jevremović¹, Tatjana Vujović², Snežana Zdravković-Korać¹

¹Dept. of Plant Physiology, Institute for Biological Research Sinisa Stankovic – National Institute of Republic of Serbia, IBISS, University of Belgrade, Bulevar Despota Stefana 142, 11108, Belgrade, Serbia

²Fruit Research Institute, Kralja Petra 1, 32000, Čačak, Serbia

sladja@ibiss.bg.ac.rs

Abstract

This study aimed to summarise the current use of the Plantform™ temporary immersion bioreactor system (TIS) at IBISS following the completion of the COPYTREE training school in 2023. Plant material used in this study included shoots of chrysanthemum, blackberry and plum, as well as somatic embryos of horse chestnut. We evaluated the effects of cultivation in the Plantform™ TIS for shoot multiplication of chrysanthemum, while for blackberry and plum shoots rooting ability using rockwool cubes was assessed. In addition, growth of horse chestnut somatic embryos was evaluated in the same system. Cultures in the Plantform™ TIS system were aerated for 1 minute every 4 hours, immersed for 3 minutes every 8 hours and grown under standard conditions. The shoot multiplication rate of chrysanthemum was significantly higher in the bioreactor culture than in solid agar medium. Furthermore, the regenerated shoots grew significantly better during the rooting phase than shoots grown continuously on solid culture medium. Blackberry growth and rooting were much better in the Plantform™ TIS with rockwool cubes compared to plum shoots. Horse chestnut somatic embryos continued to grow in the Plantform™ TIS. According to our results, the COPYTREE training school had a significant impact in establishing the system and evaluating the feasibility of the Plantform™ TIS bioreactor culture for selected ornamental and fruit tree species at IBISS.

Keywords: *bioreactors, chrysanthemum, blackberry, plum, horse chestnut*

Poster presentation

RING Test study: The effect of three CKX inhibitors on somatic embryo germination of *Aesculus hippocastanum*

Sladana Jevremović¹, Snežana Zdravković-Korać¹, Jaroslav Nisler²

¹Dept. of Plant Physiology, Institute for Biological Research Sinisa Stankovic – National Institute of Republic of Serbia, IBISS, University of Belgrade, Bulevar Despota Stefana 142, 11108, Belgrade, Serbia

²Isotope Laboratory, Institute of Experimental Botany, The Czech Academy of Sciences, Vídeňská 1083, CZ-142 20 Prague, Czech Republic

sladja@ibiss.bg.ac.rs

Abstract

The aim of our study was to examine the effects of three cytokinin oxidase/dehydrogenase (CKX) inhibitors (No. 20, No 52 and No. 77) on somatic embryo multiplication and germination of horse chestnut (*Aesculus hippocastanum*). Somatic embryos were grown in liquid MS medium supplemented with 2,4-D (0.01 mg/L) and kinetin (1.0 mg/L) in the dark before the experiments. In this study, isolated somatic embryos were further grown on solid MS medium without plant growth regulators (CKX free) or with CKX inhibitors (1 µM) under light conditions. Multiplication and greening of somatic embryos, as well as parameters of somatic embryo germination such as development of epicotyls, roots and whole plantlets, were assessed after six weeks of culture. Horse chestnut somatic embryos cultured on solid medium multiplied (32.21–45.8%) and CKX inhibitors did not influence multiplication or development of epicotyls. All CKX inhibitors showed some effect on somatic embryo germination and conversion to plantlets. CKX inhibitor No. 20 showed a statistically significant effect on somatic embryo germination, with 12.45% of somatic embryos developing into whole plantlets, compared to CKX free medium where no whole plantlets developed. In addition, twice as many green somatic embryos were recorded when grown on medium with CKX inhibitor No. 77. compared to CKX free medium. Our results highlight the potential of CKX inhibitors in the germination of horse chestnut somatic embryos.

Keywords: cytokinin oxidase/dehydrogenase, somatic embryo germination, horse chestnut

Poster presentation**Salt tolerance of *Artemisia maritima* L. under *in vitro* conditions:
a comparison of agar-solidified and TIS cultivation systems****Liva Purmale-Trasune^{1,2}, Ksenija Nikitina², Gederts Ievinsh²**¹*Bulduri Biotechnology Center, Bulduri Technical School, 6 Viestura Str., LV–2010
Jurmala, Latvia*²*Department of Ecology, Faculty of Medicine and Life Sciences, University of Latvia, 1
Jelgavas Str., LV–1004 Riga, Latvia*liva.purmale@bulduri.lv**Abstract**

Soil salinity is a significant abiotic factor limiting plant growth, highlighting the importance of halophyte research. *Artemisia maritima* L. (sea wormwood) is a coastal plant species with considerable potential as a source of bioactive compounds. To evaluate its salt tolerance under *in vitro* conditions, MS medium supplemented with 0, 1.0, 2.5, and 4.0 g/L Na⁺ was used. For cultivation, both agarized and liquid mediums were tested. As no information was available on optimal cultivation regimes for *A. maritima* in Plantform TIS, three immersion/aeration regimes were tested: (I) immersion for 3 min every 3 h followed by 5 min of aeration; (II) immersion for 10 min every 12 h with 10 min of aeration every 2 h; (III) immersion for 15 min every 24 h with 5 min of aeration every 2 h. After four weeks, the experiment was terminated, and plant fresh weight, dry weight, water content, Na⁺ and K⁺ concentrations, and electrical conductivity (EC) were determined. The highest fresh weight was obtained in the TIS 24 h regime at 1 g/L Na⁺, whereas growth decreased in all treatments at 4 g/L Na⁺. Tissue water content was generally higher in TIS systems compared to agarised culture mediums. Increasing Na⁺ concentration in the medium resulted in higher Na⁺ concentration and EC in plant material, while K⁺ concentration remained relatively stable, suggesting effective ion regulation. Low Na⁺ concentrations had no negative effect on *A. maritima* growth confirming its salt tolerance.

Keywords: sea wormwood, halophyte, salt stress, Plantform

Poster presentation

Influence of light regimes and plant growth regulators on *in vitro* shoot proliferation of *Arbutus unedo* L. toward scale-up

**Mariana Correia¹, João Martins¹, Mónica Zuzarte^{2,3}, Sandra Correia¹,
Jorge Canhoto¹**

¹*Centre for Functional Ecology, Department of Life Sciences, University of Coimbra,
Portugal*

²*Quality Plant, Coimbra, Portugal*

³*Faculty of Medicine, University of Coimbra, Portugal*

mjcorreia324@gmail.com

Abstract

Arbutus unedo L. is a perennial tree/shrub of ecological and economic importance, valued for traditional products and soil erosion prevention in Mediterranean regions. *In vitro* tissue culture techniques are used to mass-produce clones of high-quality plants of this species. This study evaluated the effects of different light conditions on the morphological development of *A. unedo* plantlets grown *in vitro*. Plantlets from different genotypes were established *in vitro* and subcultured every 5 weeks on fresh WPM medium. Shoots were exposed to three light treatments during the multiplication cycle: i) control plants maintained under a 16 h light/8 h dark photoperiod for 5 weeks; ii) plants kept in darkness for 2 weeks followed by 3 weeks under the same photoperiod; and iii) plants kept in darkness for 4 weeks followed by 1 week under the same photoperiod. Two media were tested: one supplemented with zeatin (1 mg/L) and another with coconut water (5%) plus 6-benzylaminopurine (0.1 mg/L). Morphological analysis showed that reduced light availability promoted shoot elongation and increased node number, although some etiolation occurred. Chlorophyll, total flavonoids, and total phenolic compounds were also assessed. These results suggest that moderate light manipulation may enhance shoot multiplication and elongation, although responses remain genotype dependent.

Keywords: *Arbutus unedo*, micropropagation, light availability, media composition, morphological development

Poster presentation

Micropropagation of *Quercus suber* shoots in RITA® and Plantform™ bioreactors

**Conchi Sánchez¹, Anxela Aldrey¹, Jesús M. Vielba¹, M José Cernadas¹,
Saleta Rico¹, Beatriz Cuenca², Bruce Christie³, Nieves Vidal¹**

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

²*TRAGSA. Vivero de Maceda. Ctra Maceda Baldrey Km 2. 32700 Maceda, Ourense, Spain*

³*Greenplant Co, Palmerston North 4410, New Zealand*

nieves@mbg.csic.es

Abstract

Quercus suber L. is a forest species used for cork production that is recalcitrant to vegetative propagation. In this study we present the multiplication and rooting of axillary shoots of cork oak using two genotypes obtained from a juvenile source (seed) and adult source (a centenarian tree). Multiplication in liquid medium was carried out by temporary immersion in RITA® and PlantForm™ bioreactors using Murashige and Skoog (MS) medium with half-strength nitrogen supplemented with 0.05 mg L⁻¹ 6-benzyladenine and 3% sucrose. The variables evaluated were: the type of explant (apical and basal sections attached to the callus), the use of an inert support (rockwool cubes), the type of bioreactor and the supplementation with activated charcoal (AC). For rooting, vigorous shoots longer than 20 mm were inoculated in jars with MS with half-strength macronutrients and 0.5 mg L⁻¹ of indole-3-butyric acid (IBA), 3% sucrose and 0.7 % agar. After 5 days shoots were transferred to the same medium without IBA, either in jars with agar or inserted in rockwool cubes located inside PlantForm™ bioreactors. In both cases the medium was supplemented with 0 or 1 g L⁻¹ AC. Propagation in bioreactors was achieved for both genotypes, with more proliferation from juvenile material. Basal explants produced more and longer shoots with less hyperhydricity than apical shoots. Rooting was high in both genotypes, but the acclimatisation results have to be improved before this protocol is ready for large-scale propagation of this species.

Keywords: *cork oak, hyperhydricity, rooting, temporary immersion*

Acknowledgements: This research was partially funded by the Spanish Ministry of Science, Innovation and Universities (PID2024-162781-OB100).

Poster presentation

A protocol for somatic embryogenesis induction in argan using zygotic embryos

Pablo Piñeiro¹, M^aTeresa Martínez¹, Beck Costas¹, Fátima Mosteiro¹, Sol Campañó¹, Cristina Buceta¹, Clara González², Javier Sampedro³, Elena Corredoira¹

¹Grupo de Biotecnología y Mejora Forestal, Misión Biológica de Galicia (MBG-CSIC),
Avd Vigo s/n, 15705, Santiago de Compostela, La Coruña, Spain

²Jardí Botànic de Barcelona, Dr. Font i Quer, s/n, Parc de Montjuïc, 08038 Barcelona,
Spain

³Departamento de Biología Funcional, Facultad de Biología, Rúa Lope Gómez de
Marzoa, s/n. Campus Sur, 15782 Santiago de Compostela, A Coruña, Spain

pablomanuel.pineiro@mbg.csic.es

Abstract

Somatic embryogenesis (SE) is a key tool in biotechnology, enabling elite genotype propagation, cryopreservation, and genetic engineering. The aim of the present work was to establish a protocol for SE induction in argan (*Argania spinosa* (L.) Skeels), since none are currently available despite the economic importance of this species. Fruits were collected from four adult trees between January and March 2025. Zygotic embryos were isolated and cultured with the embryonic axis separated from the cotyledons. In the first protocol induction medium was Murashige and Skoog (MS) supplemented with 1 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 mg L⁻¹ 6-benzylaminopurine (BA). After six weeks in darkness, explants were transferred to MS medium containing 0.1 mg L⁻¹ 2,4-D and 0.1 mg L⁻¹ BA. In the second protocol induction medium included 4 mg L⁻¹ indole-3-acetic acid (IAA) and 0.5 mg L⁻¹ BA and the second medium 0.1 mg L⁻¹ IAA and 0.1 mg L⁻¹ BA. In both protocols, after four weeks under photoperiod, explants were transferred to MS medium without plant growth regulators. SE was successfully induced under both treatments, although 2,4-D (2.44%) was more effective than IAA (1.12%). In both treatments, somatic embryos developed mainly during the induction medium and in the second medium. SE occurred exclusively in embryonic axes and was achieved in seeds from all four tested trees, although at different frequencies.

Keywords: 2,4-dichlorophenoxyacetic acid, *Argania spinosa*, genotype, somatic embryos, zygotic embryo

Funding: This work was partially funded by the Spanish Ministry of Science, Innovation and Universities (MICIN, Spain) through project PID2024-156422OB-C33, and by the intramural CSIC project 202540. Pablo Manuel Piñeiro Insua received an FPU fellowship (Formación de Profesorado Universitario, FPU23/00921) from the MICIN.

Poster presentation**Application of somatic embryogenesis to Norway spruce special forms****Saila Varis¹, Mikko Tikkinen^{1,2}, Sakari Välimäki^{1,2}, Teijo Nikkanen¹, Tuija Aronen¹**¹*Natural Resources Institute Finland (Luke), Vipusenkuja 5, FI-57200 Savonlinna, Finland*²*Currently Lauka Biotech Ltd., Laitaatsillantie 3, FI-57170 Savonlinna, Finland*saila.varis@luke.fi**Abstract**

Norway spruce is known to have largest phenotypic variation of the major tree species in Finland. Somatic embryogenesis (SE) pipeline developed for forest regeneration material can be utilized to capture other genetic traits, such as special growth habits or colored needles, to be used as ornamentals for landscaping or Christmas tree production. Altogether 984 zygotic embryo explants originating in 13 crossings or open-pollinated donor trees were tested, using pendulous, snake-formed, globular and colored-needled parent trees. For crossings involving yellow-needled parents, only a few explants were obtained, while in other cases dozens or hundreds of explants were used. Embryogenic tissue was initiated from 44 % of the explants, variation among the families being 26,7 – 100 %. Besides the initiation success, the embryo productivity varied between crossings from 1,4 to 142,0 embryos per gram of fresh weight (E/g FW), the mean productivity was 63,2 E / g FW. The best propagation success among Norway spruce special forms was achieved with pendula forms having purple needles in sprouting shoots, but also other forms could be propagated via SE. Colored needles could be observed already in young plants, the intensity of the color varying according to the season and from genotype to another. The genotypes expected to have special growth habits must be followed for longer periods, e.g. pendulous phenotypes demonstrate themselves properly only at the age of 5-10 years.

Keywords: *crossings, ornamental forms, Picea abies, tissue culture,*

Poster presentation**Nanoparticle-induced modulation of antioxidant potential and polyphenol biosynthesis in *in vitro* shoot cultures of *Brassica rapa* var. *japonica* (mizuna)**

Aleksandra Łukaszyk^{1,2}, Inga Kwiecień¹, Magdalena Kniaziewicz³, Eliza Blicharska³, Małgorzata Tatarczak-Michalewska³, Katarzyna Czarnek⁴, Agnieszka Szopa¹

¹Department of Medicinal Plant and Mushroom Biotechnology, Faculty of Pharmacy, Medical College, Jagiellonian University, Medyczna 9 St., 30-688 Kraków, Poland

²Doctoral School of Medical and Health Sciences, Jagiellonian University Medical College, Św. Łazarza 16 St., 30-530 Kraków, Poland

³Department of Pathobiochemistry and Interdisciplinary Applications of Ion Chromatography, Biomedical Sciences, Medical University of Lublin, Chodźki 1 St., 20-093 Lublin, Poland

⁴Institute of Medical Science, Faculty of Medical, The John Paul II Catholic University of Lublin, Konstantynów 1H St., 20-708 Lublin, Poland

a.szopa@uj.edu.pl

Abstract

Brassica rapa var. *japonica* (mizuna; Brassicaceae) is a valuable source of phytonutrients, including phenolic compounds and glucosinolates, known for antioxidant and anti-inflammatory properties. This study evaluated the effect of noble metal nanoparticles—gold, platinum, silver, and copper (AuNPs, PtNPs, AgNPs, CuNPs; ≤5 nm)—on antioxidant activity and polyphenolic profile in *in vitro* shoot cultures of *B. rapa* var. *japonica*. Agitated cultures were grown on MS medium without plant growth regulators and supplemented with NPs at 0 (control), 5, 10, and 15 ppm. Dry extracts were analyzed using ABTS, DPPH, FRAP, and CUPRAC assays, as well as total flavonoid (TFC) and total polyphenol content (TPC). Nanoparticle treatment induced metal- and dose-dependent effects. The highest antioxidant activity was observed for AuNPs at 5 ppm (ABTS: 0.036 mg Trolox/mg extract; DPPH and FRAP: 0.015 mg Trolox/mg extract). In the CUPRAC assay, the strongest effect was recorded for CuNPs at 10 ppm (0.013 mg Trolox/mg extract). AuNPs also significantly increased TFC and TPC, reaching maxima of 0.014 mg quercetin/mg extract and 0.015 mg gallic acid/mg extract, respectively. These results indicate that especially 5 ppm AuNPs effectively enhance antioxidant potential and stimulate biosynthesis of phenolic compounds in mizuna cultures, highlighting their potential in nanobiotechnological applications for functional foods and phytopharmaceuticals.

Keywords: *Brassica rapa* var. *japonica*, *in vitro* shoot cultures, noble metal nanoparticles, antioxidant activity, polyphenols

Funding: This research was funded by the National Science Center (Narodowe Centrum Nauki), grant number 2023/49/B/NZ7/02428

Poster presentation

**Phytochemical composition and antioxidant potential of
Schisandra rubriflora biomass derived from *in vitro* cultures**

**Grzegorz Kos¹, Angelika Warzecha¹, Kubica Pawel¹, Michał Dziurka²,
Agnieszka Szopa¹**

¹*Department of Medicinal Plant and Mushroom Biotechnology, Faculty of Pharmacy,
Medical College, Jagiellonian University, Medyczna 9 St., 30-688 Kraków, Poland*

²*Polish Academy of Sciences The Franciszek Górski Institute of Plant Physiology, ul.
Niezapominajek 21, 30-239 Kraków, Poland*

a.szopa@uj.edu.pl

Abstract

The study aimed to evaluate the accumulation of secondary metabolites and antioxidant activity of *Schisandra rubriflora* biomass obtained from *in vitro* cultures in comparison to plant material. The rationale of the study was related to the limited phytochemical and biotechnological data available for this species. Qualitative and quantitative analyses of dibenzocyclooctadiene lignans, phenolic acids, and flavonoids were performed using chromatographic methods in extracts obtained from fruits, leaves, shoots, and microshoot cultures. The highest contents of lignans were detected in fruits, while leaves and shoots were rich in phenolic acids and flavonoids. In plant material, the maximum levels reached approximately 1055 mg/100 g dry weight (DW) for lignans, 758 mg/100 g DW for phenolic acids, and 2819 mg/100 g DW for flavonoids. Biomass from *in vitro* cultures showed significant accumulation of metabolites, reaching about 221 mg/100 g DW for lignans, 188 mg/100 g DW for phenolic acids, and 397 mg/100 g DW for flavonoids. The antioxidant potential assessed by CUPRAC method was highest in leaf and shoot extracts, while *in vitro* biomass showed moderate activity. The obtained results indicate that *in vitro* cultures may serve as a valuable alternative source of bioactive compounds and a useful model for further biotechnological studies on this species.

Keywords: *Schisandra rubriflora*, *in vitro* cultures, lignans, phenolic compounds, antioxidant activity

Poster presentation**Machine-learning-assisted evaluation of genotype-specific drought responses in myrtle (*Myrtus communis* L.) under *in vitro* conditions**

Tolga İzgü^{1,5}, Ümit Bektaş¹, Musab A. Isak², Taner Bozkurt³, Dicle Dönmez⁴, Mehmet Tütüncü⁶, Özhan Simsek^{1,2}

¹Faculty of Agriculture, Department of Horticulture, Erciyes University, Kayseri, Türkiye

²Graduate School of Natural and Applied Sciences, Department of Agricultural Sciences and Technologies, Erciyes University, Kayseri, Türkiye

³Tekfen Agricultural Research, Production and Marketing Inc., Adana, Türkiye

⁴Biotechnology Research and Application Center, Çukurova University, Adana, Türkiye

⁵Institute of Bioeconomy, National Research Council of Italy, Florence, Italy

⁶Department of Horticulture, Ondokuz Mayıs University, Samsun, Türkiye

tolgaizgu01@gmail.com

Abstract

Myrtle (*Myrtus communis* L.) is an economically and ecologically valuable Mediterranean species widely used in traditional medicine, pharmaceutical products, and culinary applications. However, increasing drought severity associated with climate change may adversely affect its cultivation and conservation. This study evaluated the responses of selected black- and white-fruited myrtle genotypes to *in vitro* drought stress and investigated the potential of machine-learning algorithms to predict their micropropagation and rooting performance. Drought stress was induced by supplementing the culture medium with polyethylene glycol (PEG) at concentrations of 1%, 2%, 4%, and 6%. Genotype performance was assessed during the micropropagation, rooting, and acclimatisation stages. Gaussian process regression, support vector machine, Random Forest, and Extreme Gradient Boosting algorithms were used to model the relationships among genotype, PEG concentration, and *in vitro* growth responses. The results revealed substantial genotype-dependent variation in response to PEG-induced drought stress. In general, black-fruited genotypes maintained higher micropropagation performance than white-fruited genotypes under stress conditions, indicating greater tolerance to reduced water availability. The machine-learning models effectively characterised the nonlinear relationships between the experimental factors and plant responses and successfully predicted micropropagation and rooting efficiency. These findings demonstrate that combining *in vitro* drought screening with machine-learning approaches can provide an effective strategy for identifying drought-tolerant myrtle genotypes and optimising propagation conditions. The study also highlights the importance of genotype selection for improving the resilience and sustainable cultivation of myrtle under increasingly water-limited environmental conditions. Further physiological, biochemical, and molecular investigations are required to clarify the mechanisms underlying genotype-specific drought tolerance and to support future breeding and conservation programs.

Keywords: *Myrtus communis* L., *in vitro* drought stress, polyethylene glycol, genotype response, machine learning

Poster presentation

The effect of embryo orientation on the *in vitro* germination of coconut in semi-solid medium

Hannes Wilms¹, Bart Panis^{1,2}

¹Laboratory of Tropical Crop Improvement, Dept. of Biosystems, KU Leuven, Willem de Croylaan 42, 3001, Heverlee, Belgium

²Bioversity International, Belgian Office at KU Leuven, Willem de Croylaan 42, 3001, Heverlee, Belgium

Hannes.wilms@hotmail.be

Abstract

The coconut palm or “tree of life” is one of nature’s most useful plants and a major agricultural crop. However, the production of new coconut planting material is limited, whilst their demand is increasing. This limitation is due to coconut palms being mainly propagated via seeds and alternatives such as micropropagation methods are not yet widespread. Recently a novel propagation method has been developed, starting from zygotic embryos. This method allows for the micropropagation of hybrid disease-free elite palms, which could solve the worldwide scarcity. The first step in this production process is the *in vitro* germination of zygotic embryos. During the optimization of the *in vitro* initiation step we observed that the orientation of the embryo in the semi solid medium could have an effect on the growth. To test this, we placed embryos either vertically upright or horizontally in the medium and observed the rooting and shoot size of the embryo’s during the following months. Vertically placed embryos showed less shoot growth and less root development compared to horizontally placed ones. As the explants were reoriented during subculture after a month the long-term effect was limited. However, these results are a clear indicator that orientation plays an important role in germination.

Keywords: coconut, orientation, growth, solid medium

Working Group 4

Technological risk assessment, public acceptance, legislation, and commercialisation

Risk assessment of plant cloning, strategies to stimulate technology awareness and stakeholder acceptance, stimulating investment and commercialisation.

Keynote speaker

Strategies for improving the phytosanitary and genetic quality of micropropagated materials. The strategic role of Conservation Centers

Marco Pancaldi

Centro Attività Vivaistiche, Via Tebano 45, 48019 Faenza (RA), Italy

pancaldi@cavtebano.it

Abstract

In recent years, phytosanitary issues affecting fruit crops have worsened significantly, driven by the climate change and by the reduction in the number of authorized agrochemicals. Fungal and bacterial problems, in particular, have increased: fungi once considered mere saprophytes are now causing substantial damage to fruit crops. Nursery production plays a crucial role in this context and must strive to ensure the supply of healthy plants to growers. The first strategy is the production of certified plants, in Italy this involves the voluntary national certification QVI (Qualità Vivaistica Italia). This certification requires nursery operators to start with initial stock maintained in protected environments - specifically the screen houses of Conservation Centers as CAV - ensuring the material has been rigorously inspected / tested and is both disease-free and genetically true-to-type. A second key strategy is micropropagation, as material sourced from these screen houses is transferred to another protected environment, the *in vitro* laboratory. Limiting exposure to the external environment - restricting it solely to the acclimatization phase in shade houses - guarantees the delivery of healthy plants. Combining certification and micropropagation offers the highest level of phytosanitary assurance. In recent years, the number of micropropagation laboratories partnering with CAV has grown: eight micropropagation labs are actually associated, among them some of the biggest facilities in Europe with millions of fruit plants and rootstock sold worldwide every year. Moreover, we have also seen some of our field nursery members establishing their own micropropagation facilities, thus demonstrating that the Italian nursery sector recognizes the value and potential of micropropagation.

Keywords: *nursery certification, pre base material, diagnostic tools, screen houses*

Oral presentation

Commercialisation of new SE automation – origins of Lauka Biotech

**Sakari Välimäki¹, Elmar Bernhardt^{1,2}, Jonne Pohjankukka^{1,3}, Elina Havia²,
Saila Varis³, Jaakko Salminen¹, Tuija Aronen³, Mikko Tikkinen¹**

¹*Lauka Biotech Ltd., Laitaatsillantie 3, FI-57170 Savonlinna, Finland*

²*South-Eastern Finland University of Applied Sciences (Xamk), Electronics 3K Factory,
Laitaatsillantie 3, FI-57170 Savonlinna, Finland*

³*Natural Resources Institute Finland (Luke), Vipusenkuja 5, FI-57200 Savonlinna,
Finland*

sakari@laukabiotech.com

Abstract

Somatic embryogenesis (SE) is a highly scalable method of vegetative propagation. Luke has extensive experience in conifer SE, and together with Xamk developed robotics for automating the transfer and screening of somatic embryos into germination, which is a limiting step in the process. In 2023, Laukansaari Giants project was set up to prepare the commercialisation of this technology. In the project, the performance of the robotics was tested in two plant lots of over 50,000 and 100,000 Norway spruce SE germinants. The greenhouse viability of the robot handled emblings was evaluated and was found to be similar to embryos transferred into germination manually. Machine learning models were trained to improve the screening accuracy of the robots. In the Laukansaari Giants Project, different options for commercialising the technology were evaluated. In 2026, a new company, Lauka Biotech was founded as a spin out of Luke and Xamk in order to bring this technology into markets. Experiences related to this academia business -transition are discussed here. Using IP and expertise transferred from Luke and Xamk, the company will focus on further developing and marketing SE automation; first for germination and expanding to other production steps of SE in the future. The company aims to work with customers globally to improve their SE processes using Lauka Biotech automation for a wide variety of forestry and agriculture species.

Keywords: *robotics, tissue culture, artificial intelligence, convolutional neural networks*

Oral presentation

Business models and commercialization strategies for woody micropropagation: From lab to forest, orchard and market impact

Rober Ahmad Kreimech

Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, Ghent, Belgium

r.a.kreimech@ugent.be

Abstract

This contribution addresses the problem that advances in woody plant micropropagation (PMP) have not consistently translated into scalable market uptake or deployment in forests and orchards. It examines which commercialisation strategies enable micropropagated plants can move effectively “from lab to forest and orchard” and achieve sustained market and value-chain impact. The rationale is that technological capability alone is insufficient; outcomes depend on business models (BM), value-chain positions, and revenue streams. The study uses a comparative, framework-based approach. It characterises the roles of PMP laboratories along woody value chains and analyses BM archetypes. The Business Model Canvas maps Creator and Landlord BMs, while strategic tools (VRIO, Ansoff) explore alternatives. Case-inspired scenarios (Paulownia) illustrate platform approaches. Results show that conventional BMs focused on plantlet sales or lab services position labs as upstream suppliers with limited downstream influence. In contrast, hybrid BMs such as Contractor–Distributor and Lab-as-a-Service models better coordinate planting stock and services, supporting more resilient value chains.

Keywords: *plant micropropagation, business model innovation, commercialisation strategies, market impact*

Oral presentation

**Effect of Plantform on commercial micropropagation of
Paulownia fortunei and *Paulownia catalpifolia***

Ademi Fahri Pirhan¹, Basar Sevindik², Hasan Yıldırım¹

¹Ege University, Faculty of Science, Department of Biology, İzmir Türkiye

²İzmir Demokrasi University, Vocational School, Department of Park and Gardening
Plant, İzmir, Türkiye

basar.sevindik@iduu.edu.tr

Abstract

Paulownia fortunei and *P. catalpifolia* are among the most promising fast-growing woody species for timber production, and ornamental horticulture. The sustainable commercialization of these species depends on the availability of genetically uniform, pathogen-free, and traceable planting material produced under standardized propagation systems. In accordance with Turkish regulations, the production and marketing of clonal forest reproductive material require certified propagation systems that ensure genetic identity, phytosanitary quality, and full traceability throughout the production chain. Commercial micropropagation has become an essential biotechnology for supplying high-quality planting stock while supporting nursery standardization and certification. In Türkiye, the commercial production of *Paulownia* through tissue culture has considerable potential to reduce dependence on imported propagules, strengthen domestic nursery, improve production uniformity, and increase the competitiveness of the forestry and ornamental plant sectors. Plantform provides an efficient large-scale propagation approach by reducing labor input, improving multiplication efficiency and acclimatization performance, and lowering unit production costs. The integration of scalable bioreactor-based micropropagation into commercial nursery systems is expected to contribute to sustainable forest-based bioeconomy, certified clonal production, and the expansion of high-value *Paulownia* plantations in Türkiye.

Keywords: *Paulownia*, *Plantform*, *temporary immersion system*, *micropropagation*, *woody plants*

Poster presentation***In vitro* propagation of elite *Hevea brasiliensis*****Filip Vandenbussche¹, Lucas Vanhaelewyn^{1,2}**¹R&D Department, Deroose Plants NV, Weststraat 129A, 9940 Evergem, Belgium²Department of Agricultural Economics, Ghent University, Coupure Links 653, 9000 Ghent, BelgiumFilip.Vandenbussche@DeroosePlants.com**Abstract**

Hevea brasiliensis, the para rubber tree, is the principal source of natural rubber and remains a crop of major global importance. Approximately 10 million hectares are cultivated worldwide, with typical planting densities of 500–700 trees per hectare. Considering both new planting and cyclical replanting, the annual requirement for planting material is estimated at 400–600 million trees, most of which are produced by grafting selected scions onto seedling rootstocks. In *Hevea*, field performance and latex yield depend not only on the selected scion clone, but also on rootstock-related effects such as vigour, compatibility and disease resilience. Since the 1980s, Deroose Plants has accumulated broad expertise in the *in vitro* propagation of diverse plant species, including woody crops such as *Vaccinium*, *Rubus*, *Prunus*, *Rhododendron*, *Kalmia*, *Eucalyptus*, *Elaeis* and *Hevea brasiliensis*. Over the past two decades, Deroose has established and optimised a propagation platform for *Hevea* based on somatic embryogenesis from mature elite donor material, followed by controlled growth and acclimatisation phases. This platform currently supports the production of 11 own-rooted varieties suitable for direct field establishment, thereby addressing several limitations of conventional grafting. In parallel, protocols have been developed for the *in vitro* propagation of selected elite seedling-derived material for use as rootstocks. Together, these complementary approaches provide a robust framework for producing elite planting stock, including rootstocks, scions and own-rooted varieties, and support the supply of high-quality, uniform and pathogen-free material to rubber producers worldwide.

Keywords: micropropagation, somatic embryogenesis, rootstock, *Hevea*, woody plants

Poster presentation**Micropropagation of *Alocasia bambino* via tissue culture****Soner Yağ², Bora Onur Hallaç¹, Cansu Ece Sari¹, Yeşim Yalçın Mendi²**¹*Bolu Abant İzzet Baysal University, Postgraduate Education Seed Science and Technology, Bolu*²*Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana*ymendi@gmail.com**Abstract**

Alocasia bambino (Araceae) is a tropical ornamental plant cultivated widely throughout the world owing to its compact growth habit, decorative foliage, and high commercial value as an indoor ornamental. Propagation through conventional vegetative methods yields only a limited number of plantlets and can prove inadequate for producing disease-free, uniform plant material. Tissue culture techniques therefore represent an important alternative for obtaining large numbers of plants of standard quality within a short period. The objective of this study was to establish an efficient *in vitro* micropropagation protocol for *Alocasia bambino*. Plantlets 8-10 cm in length were used as the explant source. After being rinsed under running tap water for 30 min, the explants were surface sterilized for 5, 10, and 20 min in a solution containing 0.1% mercury(II) chloride and 1 mL L⁻¹ polysorbate 20. The highest sterilization success (60%) was achieved with the 5-min treatment. During the induction phase, explants were cultured on Murashige and Skoog (MS) basal medium supplemented with 30 g L⁻¹ sucrose and 6 g L⁻¹ agar. To evaluate the effect of cytokinin concentration on shoot induction, 6-benzylaminopurine (BAP) was incorporated into the medium at concentrations of 0.1, 0.5, and 1.0 mg L⁻¹. The highest induction frequency (90%) and the greatest shoot proliferation response, with an average of three shoots per explant, were achieved on medium containing 0.5 mg L⁻¹ BAP. For shoot multiplication, explants were cultured on MS medium supplemented with 0.1, 0.5, and 1.0 mg L⁻¹ BAP. The highest multiplication rate was obtained on medium containing 0.5 mg L⁻¹ BAP, yielding an average of 6.5 shoots per explant. For *in vitro* rooting, media supplemented with 0.1, 0.5, or 1.0 mg L⁻¹ α -naphthaleneacetic acid (NAA) were compared with a hormone-free control medium. A 100% rooting rate was recorded in all treatments, and no marked difference was observed among treatments with respect to root formation. In conclusion, MS medium supplemented with 0.5 mg L⁻¹ BAP was identified as the most suitable medium for shoot induction and multiplication in the micropropagation of *Alocasia bambino*. The protocol developed here is expected to contribute to the commercial-scale production of disease-free, uniform plants.

Keywords: *Alocasia bambino*, micropropagation, tissue culture, BAP, NAA, *in vitro* propagation

Poster presentation**Micropropagation of *Caladium* ‘Sunfire’ via tissue culture****Bora Onur Hallaç¹, Soner Yağ², Cansu Ece Sari¹, Yeşim Yalçın Mendi²***¹Bolu Abant İzzet Baysal University, Postgraduate Education Seed Science and Technology, Bolu**²Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana
ymendi@gmail.com***Abstract**

Caladium ‘Sunfire’ (Araceae) is a high-value cultivar favored in both indoor and outdoor ornamental cultivation for its decorative foliage, which combines bright red, pink, and green hues. Although it is traditionally propagated by tubers, its low multiplication coefficient and the risk of pathogen transmission limit commercial production. Plant tissue culture techniques are employed as an important production method in the ornamental plant sector, as they enable large numbers of genetically uniform, disease-free plants to be obtained within a short period. The objective of this study was to establish an efficient *in vitro* micropropagation protocol for the *Caladium* ‘Sunfire’ cultivar. Young shoots 6-8 cm in length, grown under greenhouse conditions, were used as the explant source. After being rinsed under running tap water for 40 min, the explants were sterilized for 5, 10, and 15 min in a solution containing 0.1% mercury(II) chloride and 1 mL L⁻¹ polysorbate 20. The most successful sterilization treatment was the 10-min application, which yielded 72% sterile explants. For the induction stage, explants were cultured on Murashige and Skoog (MS) basal medium supplemented with 30 g L⁻¹ sucrose and 7 g L⁻¹ agar. The medium was fortified with 0.25, 0.75, and 1.25 mg L⁻¹ 6-benzylaminopurine (BAP). The highest shoot induction frequency (94%) and the greatest shoot formation response, with an average of 4.2 shoots per explant, were obtained on medium containing 0.75 mg L⁻¹ BAP. For shoot multiplication, explants were cultured on MS medium supplemented with 0.25, 0.75, and 1.25 mg L⁻¹ BAP. The highest multiplication rate was achieved with 0.75 mg L⁻¹ BAP, producing an average of 8.1 axillary shoots per explant. For *in vitro* rooting, media supplemented with 0.25, 0.75, and 1.25 mg L⁻¹ α -naphthaleneacetic acid (NAA) were compared with a hormone-free control medium. The highest rooting percentage (98%) and the most vigorous root development were observed in the medium containing 0.25 mg L⁻¹ NAA, whereas the control treatment resulted in an 85% rooting rate. The results demonstrated that MS medium supplemented with 0.75 mg L⁻¹ BAP was the most suitable for shoot induction and multiplication during the micropropagation of *Caladium* ‘Sunfire’. The protocol developed in this study has considerable potential for the commercial-scale production of high-quality, disease-free planting material.

Keywords: *Caladium* ‘Sunfire’, micropropagation, tissue culture, BAP, NAA, ornamental plants

Working Group 5

Communication, dissemination, and technology transfer

Oral presentation

**Public attitude toward new technologies in FRM production –
case study Bosnia and Herzegovina**

Branislav Cvjetković

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

branislav.cvjetkovic@sf.unibl.org

Abstract

In the era of climate change, forestry faces increasing challenges in ensuring sufficient quantities of forest reproductive material. Current trends indicate the use of a wide range of forest tree species, mainly propagated from seeds. To assess the level of awareness among forestry practitioners, a survey involving 30 forestry engineers engaged in silvicultural activities in Bosnia and Herzegovina was conducted. The questionnaire included 26 questions related to reforestation needs, types of planting material, and familiarity with specific vegetative seedling production technologies. Approximately 25% of respondents were familiar with these technologies. About 41% stated they would use seedlings produced in this way, while 33.3% were uncertain. All respondents emphasized the need for additional education, primarily through professional seminars. Most participants (72.7%) identified direct contact – seminars – as the most effective form of knowledge transfer, while others mentioned the internet and professional literature. All respondents indicated willingness to use such planting material if its advantages were adequately presented. Innovative technologies in forest reproductive material production are expected to gain increasing importance under climate change conditions. Effective knowledge transfer to forestry practitioners is therefore essential for wider implementation of advanced production technologies in forestry practice.

Keywords: *forestry practitioners, new propagation technologies*

Oral presentation

Mapping the CopyTree ecosystem: A complexity science approach to knowledge sharing and digital impact in woody plant biotechnology

Buhara Yücesan

¹*Dept. of Seed Science and Technology, Faculty of Agriculture, Bolu Abant İzzet Baysal University, 14030, Bolu, Türkiye*

buhara@ibu.edu.tr

Abstract

This study presents a multi-dimensional network analysis of the CopyTree COST Action (CA21157) using *Gephi* to map the intellectual and digital landscape of woody plant biotechnology. By synthesizing data from previous conferences in Latvia (2024), Portugal (2025), and the final event in Albania (2026), the research evaluates knowledge dissemination within Working Group 5 through the lens of complexity science. Approximately 180 scientific contributions, including oral and poster presentations, are analyzed based on plant species, research objectives, and biotechnological methods. A novel "Digital Visibility and Impact Index" is applied to weight nodes, integrating academic citations with real-world professional resonance tracked via *Google*, *Google Scholar*, and social media platforms such as *X*, *ResearchGate*, and *LinkedIn*. The analysis identifies "strange attractors"—key technologies such as micropropagation, somatic embryogenesis, and temporary immersion systems, alongside pivotal techniques like gene transformation, sanitation, and novel PGR applications—that stabilize the network's knowledge flow in woody crops. Furthermore, by examining country-specific research trends, we demonstrate "fractal" self-similarity within the network, where individual national expertise reflects the collective goals of the action. This holistic mapping serves as a strategic tool for WP5, highlighting the transition of woody crop innovations from laboratory success to industrial and societal impact.

Keywords: *network analysis, complexity science, knowledge sharing, woody plants, digital impact.*

Poster presentation

Propagation pathways in radiata pine: A comparative analysis of production performance and biochemical profiles

Itziar A. Montalbán¹, Ander Castander-Olarieta¹, Javier Herrero¹, Iker Domínguez-Basterretxea¹, Caroline Teyssier², Paloma Moncaleán¹

¹NEIKER, Campus Agroalimentario de Arkaute, s/n, N 104 – km 355, Arkaute, 01192, Álava, Spain

²INRAE, ONF, BioForA, UMR 0588, 45075 Orléans, France

imontalban@neiker.eus

Abstract

Climate change is threatening the availability of seeds, creating new challenges for forestry breeding programs. Although somatic embryogenesis (SE) seems a promising alternative, its practical efficiency must be carefully assessed. For this purpose, we compared conventional seed germination and SE in terms of plantlet yield and manual labour. The latter (SE) demanded greater technical expertise and time investment, but it also produced a much larger number of acclimatized plants. The number of plants obtained with this technique was three times that obtained by traditional means. Seed germination provided a wider range of genotypes, whereas SE combined with cryopreservation offers the possibility of implementing multi-varietal forestry. To better understand the physiological differences between both systems, we also analysed carbohydrate and total protein contents, and dry weight in somatic and zygotic embryos. The results revealed marked physiological differences, while highlighting the key role of high sucrose levels in successful germination across both propagation systems. Overall, both propagation methods present clear strengths and limitations. Their suitability will ultimately depend on the goal and the value of the genetic material to be conserved and multiplied.

Keywords: *Pinus radiata*, seed, somatic embryogenesis

Acknowledgements: PID2020-112627RB-C32 funded by MICIU/AEI/10 and PID2024-156422OB-C32 funded by MICIU/AEI/10. COST Action CA21157 “European Network for Innovative Woody Plant Cloning” www.copytree.eu, funded by COST (European Cooperation in Science and Technology) www.cost.eu. Basque Government (Spain) through Euskobasoa 2050 (BERPIZ 24-00080) and Ikertalent programmes.

Poster presentation

Growth Promotion of Pepper (*Capsicum annuum* L.) by *Bacillus halotolerans* Sp405 Inoculation: Phenotypic and Whole-Genome Characterization

Ibtisem Khalifa¹, Radhouane Gdoura², Stefaan P.O. Werbrouck¹

¹Laboratory for Applied In vitro Plant Biotechnology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, B-9000, Ghent, Belgium

²Research Laboratory of Environmental Toxicology Microbiology and Health (LR17ES06), Faculty of Sciences, Sfax University, BP 1171, Sfax 3000, Tunisia

Ibtisem.khalifa@ugent.be

Abstract

Plant growth-promoting rhizobacteria (PGPR) represent sustainable alternatives to chemical inputs for improving crop productivity. This study evaluated the effect of *Bacillus halotolerans* Sp405 inoculation on the growth of pepper (*Capsicum annuum* L.) and investigated the mechanisms underlying its beneficial activity. The strain was characterised for plant growth-promoting traits, including indole-3-acetic acid production, phosphate solubilization, exopolysaccharide production, and lipopeptide biosynthesis. Genomic analyses revealed genes associated with plant growth promotion and stress adaptation. The genome encoded pathways related to indole-3-acetic acid (IAA) biosynthesis, phosphate solubilization, phytate degradation, nitrogen utilization, siderophore-mediated iron acquisition and biofilm formation. Phenotypic *in vitro* assays confirmed the key plant growth promoting traits. Pepper plants inoculated with *B. halotolerans* exhibited enhanced vegetative growth, with improved plant height, root development, biomass accumulation, and overall vigor compared with non-inoculated controls. In addition to promoting pepper growth, Sp405 exhibited broad-spectrum *in vitro* antagonistic activity against *Fusarium oxysporum*, *Fusarium verticillioides*, *Rhizoctonia solani*, *Phytophthora capsici*, and *Pythium ultimum*. Together with genomic evidence of plant growth-promoting and antimicrobial traits, these results identify *B. halotolerans* as a promising multifunctional bioinoculant for sustainable pepper production.

Keywords: *Bacillus halotolerans*, *Capsicum annuum* L., plant growth-promoting rhizobacteria, whole-genome sequencing, bioinoculant

Poster presentation

Strengthening communication and technology transfer for innovative woody plant cloning in Albania

Rozarta Nezaj¹, Efigjeni Kongjika², Valbona Sota^{2,3}

¹*Department of Food Research Center, Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania*

²*Research Center of Biotechnology & Genetics, Academy of Sciences of Albania*

³*Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania*

rozartanezaj312@gmail.com

Abstract

In vitro propagation technologies for woody plants are becoming increasingly important for forest restoration, conservation of elite genotypes, production of high-quality planting material, and adaptation to climate change. In Albania, however, their practical application in forestry remains limited because of technical, institutional, and communication barriers. Strengthening technology transfer and stakeholder engagement is therefore essential to bridge the gap between scientific research and practical implementation. Effective communication of scientific knowledge is also important for addressing public concerns and misconceptions related to vegetative propagation, particularly regarding potential biodiversity loss and ecological risks. Current evidence indicates that polyclonal forestry can maintain high levels of genetic diversity while improving productivity, resilience, and long-term sustainability. In addition, advances in molecular and epigenetic tools, including DNA methylation analyses, provide opportunities to assess somaclonal variation and support the quality assurance of micropropagated planting material. This poster highlights the role of communication, dissemination, and technology transfer in promoting the responsible adoption of innovative woody plant cloning technologies in Albania. Stronger collaboration among research institutions, universities, forest managers, policymakers, nurseries, and local stakeholders is needed to improve awareness, facilitate knowledge exchange, and support evidence-based decision-making. Aligning national practices with European recommendations and sharing knowledge through international scientific networks can accelerate the implementation of sustainable propagation technologies and contribute to the conservation and restoration of forest genetic resources. Improved communication strategies and effective technology transfer will be essential for increasing public confidence, supporting innovation, and strengthening the sustainable management of Albania's forests under changing environmental conditions.

Keywords: *woody plant cloning; in vitro propagation; technology transfer; communication; stakeholder engagement; forest genetic resources; Albania*

CIP Katalogimi në botim BK Tiranë Akademia e Shkencave të Shqipërisë From lab to forest and orchard: Final conference of CopyTree: COST Action CA21157: European Network for Innovative Woody Plant Cloning: Book of abstracts: September 14-16, 2026 / organized by Academy of Sciences of Albania, under the auspices Skënder Gjinushi. - Tiranë: Akademia e Shkencave e Shqipërisë, 2026. ... f. ; ... cm. ISBN 9789928873255 1.Bioteknologjia bujqësore 2.Konferenca 606 :63 (062)