BOOK OF ABSTRACTS

The IX International Scienctific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation (Physiological, Biochemical, Embryological, Genetic and Legal Aspects)

BIOTECH 2021 12-13 July 2021

Faculty of Science, Mahidol University, Bangkok, Thailand

Organized by Department of Plant Science, Faculty of Science, Mahidol University and International Society for Horticultural Science: ISHS In collaboration with Department of Agriculture, Ministry of Agriculture and Cooperatives



Book of Abstracts

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The IX International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation (Physiological, Biochemical, Embryological, Genetic and Legal Aspects) (Biotech 2021)

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- S. Rodpradit, Queen Sirikit Botanic Garden, Chiang Mai, Thailand



Welcome Address

The Dean, Faculty of Science, Mahidol University, The Head, Department of Plant Science, Chair Division Plant Genetic Resources and Biotechnology, ISHS, Former Chair Commission Quality and Postharvest, ISHS, distinguished participants, honourable guests, ladies, and gentlemen:

It has been a great pleasure for me and the Conference Committee members to be assigned by the President of Mahidol University to organize the IX International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation (Physiological, Biochemical, Embryological, Genetic and Legal Aspects) (Biotech 2021) with the support from the International Society for Horticultural Science, the Department of Plant Science, Faculty of Science, Mahidol University, and the Department of Agriculture. Our committee has been working very hard for the preparation and orderly progress to this important event. We also thank all the participants for making it possible.

The main objective of this symposium is to learn the recent advances in all aspects of Plant Biodiversity Conservation, as well as to create an interactive opportunity for people involved in research, education and industry development on a global scale.

Due to the COVID-19 pandemic, this conference had to be postponed and rearranged as a virtual conference. Even then 90 participants from 18 countries are still interested to participate.

I would like to extend my appreciation to the keynote speaker, invited speakers, oral presenters and poster presenters for their excellent cooperation. The research findings to be presented at this conference will enable participants to keep abreast of plant conservation and the research of this fast-moving field.

As the convener, I would like to assure all participants that we will try our best to make this conference as fruitful and enjoyable as possible.

Thank you very much.

Associate Professor Dr. Kanchit Thammasiri Convener of the Biotech 2021

> Department of Plant Science, Faculty of Science, Mahidol University

Welcome Address

The Dean of the Faculty of Science at Mahidol University, Convener of Biotech 2021, Chair of the Division of Plant Genetic Resources and Biotechnology, ISHS, distinguished participants, honorable guests, ladies and gentlemen:



On behalf of the Department of Plant Science, Faculty of Science, Mahidol University, it gives me great pleasure to warmly welcome distinguished guests, presenters, and participants to the IX International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation, or Biotech 2021.

Since biodiversity conservation is a global issue, it is important and useful to have many approaches to conserve plant diversity worldwide. One of the key requirements for the survival of all plant species in their natural habitat is genetic variation. Therefore, it is important to investigate plant genetic diversity and to preserve their germplasm. Accordingly, the use of different biotechnological techniques is a promising approach.

The goal of Biotech 2021 is to bring together a multifaceted group of researchers, scientists, students, and also individuals from various industry sectors from all over the world to present and share their innovative ideas, and to discuss the recent excellent achievements in the field of biotechnology for plant biodiversity conservation.

On this occasion, I would like to express my sincere appreciation to the keynote speaker, invited speakers, and all presenters and participants who have given their time to attend this conference. I would also like to express my sincere gratitude to Assoc. Prof. Dr. Kanchit Thammasiri and his team, who have been working very hard for over a year during the global COVID-19 pandemic to organize this conference with enthusiasm and dedication.

I hope that all participants enjoy the conference, expand their knowledge base, and have fruitful discussions.

Thank you very much,

Assistant Professor Dr. Unchera Viboonjun

Head, Department of Plant Science, Faculty of Science, Mahidol University

Opening Address

Professor Kanchit Thammasiri, the Convener of the Biotech 2021 conference, the Head of the Department of Plant Science, Mahidol University, the Chair Division Plant Genetic Resources and Biotechnology, the International Society for Horticultural Science, distinguished participants, ladies, and gentlemen.



It is my great honour to be presiding over the IX International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation with the support from the International Society for Horticultural Science (ISHS), the Department of Agriculture, as well as the governmental and private sectors.

With the current pandemic situation locally and abroad, it is certainly a challenging task to organize this virtual international conference. So, I would like to sincerely congratulate the organizing committee and the hard-working team for this wonderful event. The Faculty of Science places a strong emphasis on world-class research with commitments to scientific advancement with sustainable development for the society. Therefore, the main objective of the Biotech2021 which is to learn the recent advances in Plant Biodiversity Conservation, as well as to create an opportunity for sustainable development on a global scale, is relevant to the Faculty's objectives.

I would like to extend my appreciation to the keynote speakers, invited speakers, oral and presenters from 18 countries as well as the organizing committee for making this international symposium a success. I hope that we will meet in person and have collaborations in the near future. I extend my warmest greetings by wishing the success of the Biotech 2021, as well as continued good health and prosperity of all the participants of this event.

At this auspicious moment, I have the honour to declare the IX International Scientific and Practical Conference on Biotechnology open.

Associate Professor Dr. Palangpon Kongsaeree

Dean, Faculty of Science, Mahidol University



Welcome Address

Welcome to Biotech 2021, the IX International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation.

Since the last meeting of this series, held in Yalta in 2018, our way of life has changed drastically due to the covid-19 pandemic. According to several estimates, and beyond other negative impacts, covid-19 will be responsible for an increase of global food insecurity with a rise of over 200 million undernourished people only in 2020-21. In this context, Plant Biotechnology has certainly a determining role to increase not only food production but also to contribute to better manage and conserve plant biodiversity. All of us, working on plant biotechnology, are well aware of how biodiversity is crucial to plant breeding, to find new chemicals and to social and economic development.

Thailand is located in a highly populated area of the globe. The global population growth has been constantly increasing and new challenges will be faced in the years to come to nourish a population which, according to some projections, will reach 10 billions by 2050. Holding this congress in Bangkok is a unique opportunity to discuss the challenges posed by the constant loss of biodiversity, mostly caused by anthropogenic reasons, and how biotechnology can be used to mitigate this damage.

As representative of the International Society for Horticultural Science (ISHS) I would like to thank all the contributors to Biotech 2019. My special and warm thanks first go to the convener, Associate Professor Dr. Kanchit Thammasiri who, leading a large team, and in spite of all the difficulties, managed to organize this event in which scientists from all over the world will participate. A special thanks also to the National Advisory Committee of Biotech 2021 and to Mahidol University that hosts this symposium. Many thanks also to the International Advisory and Scientific Committees and to sponsors and supporters for their help in organizing the conference. Without this collective effort, having this conference at these particularly hard social and economic times would simply not be possible.

Given the program and the quality of the participants, Biotech 2021 will be certainly a great success!

Jorge Canhoto

Chair of the division of Plant Genetic Resources and Biotechnology (ISHS)

The IX International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation (Physiological, Biochemical, Embryological, Genetic and Legal Aspects)

(Biotech 2021)

12-13 July 2021 Faculty of Science, Mahidol University, Bangkok, Thailand

Monday 12 July 2021

Room 1 (L02),	Faculty	of Science,	Mahidol	University
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MC: Assist. Prof. Dr. Alyssa Stewart MC: Dr. Saroj Ruchisansakun

9:00-10:00 hr. Opening Ceremony

Welcome Address

by Associate Professor Dr. Kanchit Thammasiri, Biotech 2021 Convener

Welcome Address

by Assistant Professor Dr. Unchera Viboonjun, Head of Department of Plant Science, Faculty of Science, Mahidol University

• Opening Address

by Associate Professor Dr. Palangpon Kongsaeree, Dean, Faculty of Science, Mahidol University

• Welcome and ISHS Presentation

by Professor Dr. Jorge Canhoto, Chair, Division Plant Genetic Resources and Biotechnology, ISHS

- Presentation of souvenirs to conference sponsors
- Group Photographs

Keynote Presentation

10:00-10:30 hr. S1_K Prof. Pei Shengji

Vital roles for ethnobiology in new era of biodiversity conservation in China

Shengji Pei, Kanchit Thammasiri

Q & A session

10:30-10:45 hr. Break

Monday 12 July 2021		
Room 1 (L02), Faculty of Science, Mahidol University		
	MC: Assist. Prof. Dr. Alyssa Stewart MC: Dr. Saroj Ruchisansakun	
10:45 – 12:00 hr.	Invited Oral Presentations	
	S2_I3 Dr. Gayle Volk	
	Plant cryopreservation: Research, implementation, and outreach	
	Gayle Volk	
	Q & A session	
	S4_11 Prof. Dr. S.M. Khasim	
	In vitro multiplication of some selected banana cultivars (Musa spp.) from India and their genetic fidelity using ISSR markers	
	Shaik Mahammad Khasim, Saifuldeen Ahme Hasan	
	Q & A session	
12:00 – 13:00 hr.	Lunch Break	
Room	1 (L02), Faculty of Science, Mahidol University	
	MC: Assist. Prof. Dr. Alyssa Stewart	
13:00-15:00 hr.	S2_I1 Dr. Bart Panis	
	Cryopreservation to preserve vital tropical crops for future generations	
	Bart Panis, Hannes Wilms	
	Q & A session	
	S2_I2 Dr. Manuela Nagel	
	Molecular mechanisms during cryopreservation of potato and garlic shoot tips	
	<u>Manuela Nagel</u> , Kanchit Thammasiri, Marion Grube, Claudia Kopnick, Kamatchi Ulagappan	
	Q & A session	
	S4_I2 Prof. Dr. Jorge M. Canhoto	
	Somatic embryogenesis and other in vitro techniques towards tamarillo breeding and conservation	
	Sandra Correia, André Caeiro, Daniela Cordeiro, Miguel Rito, Ana Pedrosa, Tércia Lopes, <u>Jorge Canhoto</u>	
	Q & A session	

Room	m 1 (L02), Faculty of Science, Mahidol University	
	MC: Assist. Prof. Dr. Alyssa Stewart	
13:00-15:00 hr. (cont.)	S1_I1 Prof. Dr. Hugh W. Pritchard	
	Seed `omics´ and the conservation of plant biodiversity	
	Hugh W. Pritchard, Liang Lin, Hongying Chen, Anne Visscher, Dani Ballesteros	
	Q & A session	

Monday 12 July 2021

Room	1 2 (L03), Faculty of Science, Mahidol University
	MC: Dr. Saroj Ruchisansakun
	Session II: Cryopreservation (S2)
13:00-13:15 hr.	S2_1 Fruit harvesting times and various dehydration methods for cryopreservation of <i>Paphiopedilum bellatulum</i> (Rchb.f.) Stein seeds
	Supaporn Rodpradit, Prasit Wangpakapattanawong, Pheravut Wongsawad, Kanchit Thammasiri
13:15-13:30 hr.	S2_2 Cryopreservation of <i>Grammatophyllum speciosum</i> Blume seeds by D cryo-plate method
	Dechathon Thanasuttanithi, Kanchit Thammasiri
13:30-13:45 hr.	S2_3 Cryopreservation and assessment of genetic stability of cryopreserved <i>Fragaria</i> spp. using SSR markers
	Sandhya Gupta, Prashant Tewari, Madhvi Mishra, Rakesh Singh, DK Nerwal
	Session III: Breeding (S3)
13:45-14:00 hr.	S3_2 Genetic diversity of an endemic medicinal orchid, <i>Coelogyne</i> <i>nervosa</i> R. Rich. from Southern India using morphological and molecular markers
	J. Ramudu, S.M. Khasim
14.00 14.001	
14:00-14:30 hr.	Q & A session

Tuesday 13 July 2021		
Room 1 (L02), Faculty of Science, Mahidol University		
	MC: Assist. Prof. Dr. Alyssa Stewart	
	Session VI: Plant Molecular Research (S6)	
09:00-09:15 hr.	S6_1 DNA Barcoding and molecular systematics in genus <i>Coelogyne</i> Lindl. (Orchidaceae)J. Ramudu	
09:15-09:30 hr.	 S6_2 Plant GARDEN: a portal web site for accessing plant genome, DNA marker, and SNP information <u>Sachiko Isobe</u>, Hisako Ichihara, Hideki Hirakawa, Andrea Ghelfi, Mitsuyo Kohara, Manabu Yamada, Takuro Tamura, Akihiro Nakaya, Satoshi Tabata 	
09:30-09:45 hr.	 S6_3 Molecular phylogeny and DNA barcode regions efficacy for identification the variety of <i>Capsicum annuum</i> L. in Thailand <u>T. Luangsuphabool</u>, A. Wongpia, P. Sangkasa-ad, T.N. Nan, K. Pipithsangchan, K. Thammasiri 	
	Session VII: Plant Protection (S7)	
09:45-10:00 hr.	 S7_1 Simple preparation of the dried galangal (Alpinia galanga) in ethanol is effective to suppress <i>Curvularia</i> sp. which causes grain discoloration of rice (<i>Oryza sativa</i>) (var. Suphan Buri 2) <u>Kyaw Soe Win</u>, Rachsawan Mongkol, Mana Kanjanamaneesathian 	
10:00-10:15 hr.	S7_2 In vitro screening of crude extracts from plants against <i>Fusarium sacchari</i> and <i>Curvularia lunata</i> and testing the efficacy of a selected crude extract of <i>Alpinia galanga</i> against grain discoloration and other rice diseases in the fields <u>Thi Thi Win</u> , Mana Kanjanamaneesathian, Rachsawan Mongkol	
10:15-10:45 hr.	Q & A session	
10:45-11:00 hr.	Break	
	Session I: Plant Diversity Conservation (S1)	
11:00-11:15 hr.	S1_1 In vitro propagation and conservation of Artocarpus lakoocha Roxb.: An underutilized fruit of India Sandhya Gupta, Vivek Kumar Biraji	
11:15-11:30 hr.	 S1_2 Native Thai waterlily survey as an evidence to support Jongkolnee' waterlily (<i>Nymphaea siamensis</i>) existing in a new subgenus <u>Vichai Puripunyavanich</u>, Woranuch La-ongsri, Primlarp Chukiatman, Kanokporn Boonsirichai 	

Room	1 (L02), Faculty of Science, Mahidol University
(cont.)	MC: Assist. Prof. Dr. Alyssa Stewart
11:30-11:45 hr.	 S1_3 Agro-morphological and genetic diversity of Indian large cardamom cultivars (<i>Amomum subulatum</i> Roxb.) in the sub Himalayan tropics <u>Reshma Ranjanan</u>, Mary Mathew K, Remashree A.B.
11:45-12:00 hr.	 S1_4 Biotechnological applications for plant germplasm conservation at ICAR-National Bureau of Plant Genetic Resources, India's recent achievements <u>Anuradha Agrawal</u>, Sandhya Gupta, Neelam Sharma, Sangita Bansal, Vartika Srivastava, Era Vaidya Malhotra, Subhash Chander, Ravi Gowthami, Kuldeep Singh
12:00-13:00 hr.	Lunch Break
13:00-13:15 hr.	S1_5 The use of zygotic embryos for the efficient long-term conservation of a Georgian provenance of sweet chestnut: an overviewMariam Gaidamashvili
13:15-13:30 hr.	 S1_6 Safeguarding and use of plant genetic resources in DOA genebank. In case of "Aditayadhorn agricultural project in contemplation of Her Highness Princess Aditayadhornkitikhun at Surin has collaborated with DOA genebank - ex situ conservation of some field crops and horticultural crops in DOA genebank, Thailand, 2019-2020" <u>K. Pipithsangchan</u>, N. Boain, S. Dachakumpoo, S. Bubpato, J. Suksawat, A. Songserm, A. Wongpia, T. Luangsuphabool, C. Samphunphuang, D. Narkprasert, P.P. Chareonsap, K. Thammasiri
13:30-13:45 hr. 13:45-14:30 hr.	 S1_7 Comparative assessments of alkaloids and phenolic compounds in a Thai medicinal plant, <i>Erycibe elliptilimba</i>, and other species in the genus <u>Tripatchara Atiratana</u>, Paweena Traiperm, Phongsakorn Kochaiphat, Thanika Pathomwichaiwat, Unchera Viboonjun Q & A session
14:30-15:00 hr.	Break
15:00-16:00 hr.	Business Meeting and Closing Ceremony

Tuesday 13 July 2021		
Room 2 (L03), Faculty of Science, Mahidol University		
	MC: Dr. Saroj Ruchisansakun	
	Session IV: Micropropagation (S4)	
09:00-09:15 hr.	S4_1 Shoot multiplication of three <i>Hedychium</i> species via immersion temporary bioreactor <u>Supaporn Rodpradit</u> , Pimphaka Klaharn, Paweena Pumisutapon	
09:15-09:30 hr.	 S4_2 Preventing tissue necrosis with activated charcoal alternatives during shoot multiplication of coconut <u>Hannes Wilms</u>, Dries De Bièvre, Rony Swennen, Juhee Rhee, Bart Panis 	
09:30-09:45 hr.	 S4_3 The effect of plant growth regulators on the in vitro regeneration capacity in some horticultural crops and rare endangered plant species <u>Irina Mitrofanova</u>, Nina Lesnikova-Sedoshenko, Svetlana Chelombit, Irina Zhdanova, Natalya Ivanova, Olga Mitrofanova 	
09:45-10:00 hr.	 S4_4 In vitro establishment and multiplication of <i>Vaccinium</i> virgatum Ait. 'Delite' <u>Carolina Schuchovski</u>, Luiz Antonio Biasi 	
10:00-10:15 hr.	 S4_5 A comparative analysis of in vitro responses of anthurium under temporary immersion (RITA[®]) Teresita Amore, Jaclyn Nicole Uy O & A session 	
10.13-10. 4 5 m.		
10:45-11:00 hr.	Break	
11:00-11:15 hr.	 S4_6 Chemotyping and in vitro conservation of strawberry tree (<i>Arbutus unedo</i> L., Ericaceae) <u>João Martins</u>, Teresa Batista, Glória Pinto, Jorge Canhoto 	
11:15-11:30 hr.	S4_7 Prospect and challenges in Rhododendron micropropagation <u>Sin Hoong Tan</u> , Rusea Go	
	Session V: Physiology and Production (S5)	
11:30-11:45 hr.	 S5_2 Effects of irrigation levels on growth and chemical constituents in <i>Curcuma alismatifolia</i> <u>Chaiartid Inkham</u>, Jakaphun Julsrigival, Sunee Chansakaow, Panupon Hongpakdee, Kanokwan Panjama, Soraya Ruamrungsri 	

Room	n 2 (L03), Faculty of Science, Mahidol University
(cont.)	MC: Dr. Saroj Ruchisansakun
11:45-12:00 hr.	 S5_3 Chemical composition analysis of essential oils from black gingers (<i>Kaempferia parviflora</i>) by gas chromatography-mass spectrometry (GC-MS) <u>A. Wongpia</u>, C. Samphunphuang, K. Pipithsangchan, W. Somprasong, S. Boonpradub, T. Luangsuphabool, K. Thammasiri
12:00-13:00 hr.	Lunch Break
13:00-13:15 hr.	 S5_4 Assessment of IAA synthesis by endophytic bacteria in <i>Vanda</i> (Orchidaceae) <u>Wanwisa Inkaewpuangkham</u>, Chaiartid Inkham, Kanokwan Panjama, Yupa Chromkaew, Soraya Ruamrungsri
13:15-13:45 hr.	Q & A session

Biotech 2021

ORAL PRESENTATIONS

Biotech 2021

Session I: Plant Diversity Conservation (S1)

S1_K

Vital roles for ethnobiology in new era of biodiversity conservation in China

Shengji Pei, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China; <u>peishengji@mail.kib.ac.cn</u>

Kanchit Thammasiri, Department of Plant Science, Faculty of Science, Mahidol University, Thanon Rama VI, Ratchathewi, Bangkok 10400, Thailand; <u>kanchitthammasiri@gmail.com</u>

Biodiversity is the product of interactions of earth life and environment. Overtime, the dynamic changes of biodiversity in time and space pattern was resulted by natural environment, which is natural process of biodiversity change over billion years. However, since human being entered into industrial society, the change of nature process accelerated by human activities mainly caused by over-exploration of biological resources and habitat lost that created great social and economic wealth but lost of biodiversity and habitat. Today, the biodiversity extinction speed is even accelerated than any time in human history, earth biodiversity faces crisis and biodiversity extinction threaten to all mankind.

Since 1980's, ethnobiology as an interdisciplinary scientific research field has been introduced and developed in China, covering ethnobotany, ethnoecology, and ethnomedicine. The fundamental scientific principal of ethnobiology is the study of human interaction with plants, animals, fungus, ecosystems, and medical sourcing plants, with focus on traditional botanical, ecological, and medical knowledge of people in different cultures for application into sustainable development of socio-economic and environment today. The nature of ethnobiology reflexes a science of nature-people based subject and closely linked with practices of biodiversity resources maintenance. It is therefore, ethnobiological science has its vital roles for biodiversity conservation in China and the world.

In China, ethnobiology can make contribution to three major areas of biodiversity conservation: 1) Documentation of traditional knowledge in use of biodiversity species and ecosystems and its management practices; 2) Application of ethnobiological knowledge and traditional techniques into rural community reconstruction and conservation activities; and 3) Supporting to implementation of CBD and Nagoya Protocol on 'Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity'. This speech will emphasize on application of ethnobiological sciences into sustainable use of biodiversity resources in China for biodiversity conservation.

Keywords: biodiversity conservation, ethnobiology, eco-civilization, herbal medicine, traditional knowledge

S1_I1

Seed `omics' and the conservation of plant biodiversity

Hugh W. Pritchard, Royal Botanic Gardens, Kew, Wakehurst Place, West Sussex, United Kingdom; h.pritchard@kew.org

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The conservation of plant biodiversity through seed storage depends on a number of functional traits, such as desiccation tolerance, survival of low temperatures, and efficient germination (1). Primarily, low temperature tolerance is achievable when tissue water content is reduced enough that intracellular ice formation is avoided on cooling, but not decreased so much that the tissues suffer severe osmotic stress. Cooling per se may also generate additional physical stresses to the tissues and cell components. The seeds of many species have evolved to cope with the natural or imposed removal of a large component of cellular water, making them amenable to the first step in a seed banking process, drying, and able to survive transfer to sub-zero temperatures. Such a response is found in seeds of many crops. However, woody species with seeds that tend to be large (> 1 g in weight) have a greater tendency to be drying sensitive (i.e., are recalcitrant). Using lipidomics, we have explored amongst seeds with differing desiccation sensitivities how drying impacts phospholipid composition, as membrane injury is one of the first signs of dehydration stress (2). As recalcitrant seeds have a limited shelf-life, researchers have developed model systems to enable the detailed exploration of the underlying mechanisms of stress tolerance. These include somatic embryo production technology. Whilst this system is more usually applied to elite trees for forestry, we have developed it for species in Magnoliaceae, one of the most threatened family of plants globally. Using lipidomics, we have dissected the response of Magnolia embryogenic cells to each step in a conventional cryopreservation protocol using plant vitrification solution (3). In both of these studies, on seeds and embryogenic cells, lipid remodelling is crucial during the imposition of stress and subsequent release and recovery. A different form of stress is apparent in seeds when they struggle to germinate under environmental conditions that are sub-optimal. Until recently, investigating the underlying molecular mechanisms governing the 'decision' to germinate, or not, has been the exclusive territory of scientists working on model /crop species. However, two technology transformations have changed this situation. The large fall in 'omics' costs and sudden increase in the number of species for which whole genomes sequences are available has brought significant opportunities for mechanistic studies on wild species. In our work 'beyond Arabidopsis and crops,' we have used transcriptomics to explore the germination response of seeds of an endangered palm, Pseudophoenix eckmanii, referencing the response to the model/crop genome of oil palm (4). Through these examples, we hope to be able to demonstrate the exciting new avenues of research that are opening up using 'omics' to address the challenges associated with the conservation of wild plant biodiversity.

Keywords: seed biology, desiccation tolerance, recalcitrant seed, threatened species, conservation, lipidomics, transcriptomics

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In vitro propagation and conservation of *Artocarpus lakoocha* Roxb.: An underutilized fruit of India

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The study was conducted for in vitro propagation and conservation of Artocarpus lakoocha Roxb. (family Moraceae). For in vitro propagation, plant growth hormones, such as Benzylaminopurine (BAP), Meta-Topolin (MT), α-Naphthalene acetic acid (NAA), and Thidiazuran (TDZ) were used. In the first experiment, MS medium supplemented with different conc. of BAP (0, 0.2, 0.5, 1, 2, and 5 mg/l), MT (0, 0.5, 1, 2, and 5 mg/l) to find their effects on growth parameters. The results showed that BAP was better for shoot multiplication than MT. There was no significant difference observed on no. of nodes by various concentrations of BAP and MT. As compared to control, BAP and MT showed much improvement on all shoot parameters. For slow growth, in vitro conservation with different treatments, such as temperature, culture tube enclosure, and inclusion of mannitol in media were used. The better conservation was observed in the cultures with screw cap as enclosure compared to culture with cotton plug as enclosure. The cultures were conserved for 6 months. When the effect of temperature was observed for conservation, the cultures kept at 25°C showed better results among 5°C, 10°C, 22/5°C alternate temp., and 25°C. In another experiment for slow growth, 1 mg/l, 2 mg/l, 5 mg/l, and 10 mg/l mannitol were added to the MS medium. All the cultures were green and healthy with single shoot and less shoot height after six months of inoculation. Cultures are being maintained at the respective conditions to find how long cultures can survive for optimum conservation period.

Keywords: micropropagation, Monkey jack, tissue culture, slow growth conditions for conservation

Native Thai waterlily survey as an evidence to support `Jongkolnee´ waterlily (*Nymphaea siamensis*) existing in a new subgenus

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An exploration was conducted to investigate native waterlilies which might be related to 'Jongkolnee' in Thailand. 'Jongkolnee' waterlily was given a scientific name as Nymphaea siamensis. Some waterlilies were highly specific for their habitat, such as 'Bua Bae Kao' (Nymphaea sp.) and a native tropical night blooming waterlily growing in Phu Sa Dok Bua National Park in Yasothon province were found to be rare. Studies of Nymphea siamensis revealed their characters that suggested its possible placement in an entirely new subgenus. 'Nilubon' or 'Bua Bae Muang', found in Roi Et province, showed similar morphology to 'Bua Khap', another native waterlily. However, 'Bua Bae Muang' produced bulblets; while, 'Bua Khap' did not produce any bulblet. Morphological and genetic comparison between N. siamensis and other Nymphaea species, including imported waterlily; Nymphaea colorata, were conducted. 'Bua Bae Muang' showed the closest similarity to N. siamensis. They showed similar leaf, stem, and root morphology and 'Bua Bae Muang' also produced bulblets. However, its flowers were purple petals with complete reproductive organs; while, N. siamensis lacked reproductive organ. Random amplified polymorphic DNA comparison revealed that N. siamensis was most similar to 'Bua Bae Muang' and 'Bua Bae Kao'. N. siamensis has no sexual reproductive structures and is propagated only via bulblets. Under condition of subgenus taxa system in genus Nymphaea, species are categorized into subgenera by their ovary characters: apocarpous ovaries, or syncarpous ovaries. But N. siamensis has no ovaries; therefore, it could not be categorized into any existing subgenera.

Keywords: Thai waterlily, Jongkolnee, Nymphaea siamensis, subgenera

Agro-morphological and genetic diversity of Indian large cardamom cultivars (*Amomum subulatum* Roxb.) in the sub Himalayan tropics

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Large cardamom (Amonum subulatum Roxb.) or black cardamom, indigenous to moist deciduous and evergreen forests of sub-Himalayan tropics, is a highly priced aromatic spice belonging to the family Zingiberaceae. The dried ripe fruit is the spice of commerce and the seeds have similar properties as those of small cardamom (Elettaria cardamomum Maton). It is the most important cash crop of Sikkim state of India, and is cultivated in North Eastern states and neighboring nations of Nepal and Bhutan. Though tremendous variability exists among the natural cultivars, genetic diversity has not been explored much and documented. The primary objective of this study was to assess the morphological and genetic diversity among natural cultivars and released varieties of Amomum subulatum. Six morphologically distinct natural cultivars viz. Ramsey, Golsey, Sawney, Varlangey, Seremna, and Ramla and 2 released selections ICRI Sikkim 1 and ICRI Sikkim 2 were selected. Total of 23 morphological traits were considered for morphological characterization based on IPGRI descriptor for small cardamom, a close relative, as descriptors are not yet released for this crop. Genetic diversity was evaluated using Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR) markers. Cluster analysis was performed using Jaccard's coefficient, to construct a dendrogram with unweighted pair group method by arithmetic average (UPGMA) matrices. The results based on ISSR markers substantiated the abundant morphological diversity among the natural cultivars. The morphological and molecular marker studies would be a step forward in defining descriptors for large cardamom and for breeding and introgression of new alleles from unutilized germplasm accessions.

Keywords: large cardamom, amomum, ISSR, diversity

Biotechnological applications for plant germplasm conservation at ICAR-National Bureau of Plant Genetic Resources, India's recent achievements

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In India, NBPGR is a leading institute under the Indian Council of Agricultural Research (ICAR), that undertakes management of plant genetic resources (PGR) including exploration, collecting, exchange, quarantine, characterization, evaluation, conservation, documentation, and distribution. For germplasm conservation, an integrated genebank system comprising field, seed, in vitro and cryogenebanks has been developed since the 1980s, which presently has ~0.46 million accessions of agri-horticultural crop diversity. In 1986, use of biotechnology for conservation of PGR was initiated in the Tissue Culture and Cryopreservation Unit (TCCU) at ICAR-NBPGR, New Delhi, to conserve economic plants through in vitro and cryopreservation techniques, for which conventional methods of storage are unsuccessful or inadequate. The research programmes primarily aim at development of micropropagation, in vitro conservation and cryopreservation protocols. Genetic integrity of the germplasm conserved is also researched upon. Capacity building on in vitro and cryopreservation techniques for germplasm management is an important activity, through national and international training programs.

Currently, 1,910 accessions of mandated crops are maintained in vitro in the form of ~36,000 cultures and/or in vitro cryopreserved meristems/shoot tips in the In Vitro Active/Base Genebank. These accessions comprise horticultural crops like fruit, tuber, bulb, spices, plantation, industrial crops, medicinal, aromatic, and rare/endangered plants. For seeded species, 11,902 accessions are conserved as seeds, embryos, embryonic axes, budwood, or pollen in the cryogenebank. In addition, 2,194 genomic resources are also cryostored. In this paper, we will showcase recent research progress of new in vitro conservation and cryopreservation protocols developed, especially of less-researched species of Indian importance.

Keywords: in vitro genebank, cryogenebank, biotechnology, conservation, Indian PGR

The use of zygotic embryos for the efficient long-term conservation of a Georgian provenance of sweet chestnut: an overview

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The forest ecosystems are of considerable significance for the conservation of plant biodiversity. Sweet chestnut (Castanea sativa Mill.) belongs to the hardwood forest species within the genus Castanea. It is dominant of mountainous forests of Western Georgia occupying the majority of areas covered with forests. Because of low self-renewal and diseases, the large massifs of chestnut forests are threatened by extinction. Castanea sativa is included in the 'Red List' of Georgia under state Vulnerable (VU) and has been subjected to conservation measures in response to the Global Strategy for Plant Conservation (GPSC). Rapidly developing in vitro techniques opens new possibilities for the safe ex-situ conservation of woody plant material. Various tissue culture-derived explants, such as shoot tips, nodal explants, somatic embryos, embryogenic cell lines, and embryonic axes have been widely employed for conservation purposes to ensure the sustainability of the genetic pool of the genus *Castanea*. The present overview focuses on the successful utilizing zygotic embryos for the conservation of a Georgian provenance of sweet chestnut germplasm. The study summarizes the recent achievements in species protection in Georgia via long-term preservation of a Georgian provenance of sweet chestnut using embryonic axes (EA) as an explant. The present overview focuses on the successful utilizing zygotic embryos for the conservation of a Georgian provenance of sweet chestnut germplasm and summarizes the recent achievements via long-term preservation.

The efficacy of different techniques for cryopreservation of EAs, such as specimen dehydration-'one-step freezing', PVS2-vitrification, and encapsula-tion–vitrification are evaluated. The advantages of the utilization of zygotic embryos for safe and effective long-term conservation of threatened hardwoods concerning the post-cryopreservation recovery of explants are discussed.

Keywords: Castanea, cryopreservation, embryonic axes, encapsulation, vitrification

Safeguarding and use of plant genetic resources in DOA genebank. In case of "Aditayadhorn agricultural project in contemplation of Her Royal Highness Princess Aditayadhornkitikhun at Surin has collaborated with DOA genebank - *ex situ* conservation of some field crops and horticultural crops in DOA genebank, Thailand, 2019-2020"

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Aditayadhorn agricultural project in contemplation of Her Royal Highness Princess Aditayadhornkitikhun at Thenmee, Amphur Muang, Surin "Sa-rae Aditaya" has collaborated with the DOA genebank (Genebank Research Development Group, Biotechnology Research Development office, Department of Agriculture), Thailand including Surin Agricultural Research and Development center, to study, survey, and collection. Twelve plants, such as holy basil, chili, sweet basil, garden bird chili, turkey berry, eggplant, rice, green oak lettuce, and etc. were collected and planting in the area of Aditayadorn agricultural project. Seven plants were characterized at Sa-rae Aditaya project area excluding rice which characterized in DOA genebank at Pathumthani. Seed management in laboratory after harvesting and seed conservation were conducted in DOA genebank. DOA genebank has conserved seeds in medium-term storage (5°C) and in long-term storage (-10°C).

The plant conservation of this project emphasizes on collection, evaluation, and conservation of some field crops and horticultural crops in the area of Aditayadhorn agricultural project in contemplation of Her Royal Highness Princess Aditayadhornkitikhun to increase genetic diversity and to be the back-up collection site among one another to assure the sustainable conservation and utilization of the plant genetic resources through the farmers around the area of Sa-rae Aditaya, and to promote and utilize research study, as well as increase the awareness of the values in plant genetic resources. At last, no poverty and zero hunger will occur around that area in the near future.

Keywords: safeguarding, plant genetic resources, Aditayadhorn agricultural project, DOA genebank

Comparative assessments of alkaloids and phenolic compounds in a Thai medicinal plant, *Erycibe elliptilimba*, and other species in the genus

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Ervcibe elliptilimba Merr. & Chun is documented as a Thai medicinal plant that has calystegines (nortropane alkaloids) and scopoletin (coumarins). However, the frequent collection of this species' stems for medicinal use is affecting its wild populations, and it is at risk of becoming a threatened plant species. Other plant species in the genus Erycibe Roxb., which are more commonly found, and other plant organs (apart from stems) may provide useful alternatives, but it is first necessary to investigate and compare the chemical compounds of these alternative sources. Therefore, the aim of this research was to use histochemistry to screen for two chemical compounds (phenolics via Naturestoff reagent, and alkaloids via Dragendroff's reagent and NaNO₂) in the stems and leaves of four species in the genus Erycibe (E. albida, E. citriniflora, E. elliptilimba, and E. stapfiana). The results show that the stems of *E. elliptilimba* have similar phytochemical compounds as the stems of *E. citriniflora* and *E. albida*. Moreover, the leaves of all three alternative species have the same phenolic compounds as E. elliptilimba, but the alkaloid results revealed some differences among four species. The results from this research are beneficial for finding new Thai medicinal plant candidates, which can be further developed through examining chemical profiles and pharmacological tests.

Keywords: Convolvulaceae, coumarins, histochemistry, leaves, scopoletin, stems

Biotech 2021

Session II: Cryopreservation (S2)

S2_I1

Cryopreservation to preserve vital tropical crops for future generations

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During the last decade, cryopreservation of plant genetic resources has been the subject of many research initiatives, such as European projects, a Crop Trust project, a feasibility study, a special issue of a plant biotech journal, and scientific symposia. The topic was in 2019 moreover put on the agenda of the International Treaty on Plant Genetic Resources for Food and Agriculture. Cryopreservation is now more and more considered as a valid long-term alternative for field and in vitro collections. This became especially important in view of the current COVID pandemic were regular access to field and in vitro collections for respectively their maintenance and subculture is not so obvious anymore. The results is that the number of crops and accessions that are stored in liquid nitrogen is steadily increasing.

There are some typical problems linked to long term conservation of tropical germplasm. Often, they produce seeds that show a recalcitrant seed behavior; meaning that they cannot be dried and subsequently stored at low temperatures. Well-known examples are cacao, coconut, and avocados. Cryopreservation of totipotent plant parts like meristems or zygotic embryos could here be part of the solution. There are also many tropical crops including cassava, sweet potato, taro, yam, and banana that are solely clonally propagated to maintain their genetic make-up or because they are sterile. Often cryopreservation procedures of clonal materials, such as meristems involve a cold hardening phase. Unfortunately, most, if not all, tropical plants are not programmed to cold acclimate, so alternatives, such as osmotic or sugar hardening needs to be applied.

In this presentation, we will give an overview of the cryopreservation efforts and prospects of some vital tropical crops with an emphasis on sweet potato, coconuts, edible aroids, cassava, and bananas.

Keywords: cryopreservation, tropical crop

S2_I2

Molecular mechanisms during cryopreservation of potato and garlic shoot tips

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The federal ex situ collection of agricultural and horticultural plants at IPK Gatersleben houses more than 2,200 cryopreserved accessions of plant genetic resources, predominantly shoot tips of potato, mint, and Allium. PVS3 droplet vitrification is used in all species, and induces a range of abiotic stresses including mechanical wounding, osmotic, and cold stress. These stress factors lead to complex stress response mechanisms that affect the regenerative capacity of shoot tips. To understand the stress response mechanisms during the cryogenic procedure, we investigated potato and garlic shoot tips and applied physiological, biochemical, and transcriptome analysis. Similar biochemical processes were observed in shoot tips of both species. During the process of shoot excision, preculture and cryoprotection, sucrose concentration increased stepwise. In parallel, adenosine triphosphate (ATP) and the activity of antioxidant enzymes (APX, GR, CAT) decreased gradually. In agreement and compared to fresh shoot tips, the number differentially expressed genes (DEGs) reduced in garlic shoot tips from 608 DEGs during preculture to 273 DEGs during cryoprotection. As soon as shoot tips were rewarmed, rehydrated, and recovered, 1,293 DEGs were present leading to increased levels of DNA binding and transcription factor activities and finally, increased levels of ATP and antioxidative enzymes. In conclusions, although we only observe a small section of the overall molecular processes, this study increased our understanding of the fundamental processes and will help to enhance the regrowth potential after cryopreservation of potato and garlic shoot tips.

Keywords: PVS3 vitrification, transcriptome, ATP, antioxidative enzymes, regrowth

S2_I3

Plant cryopreservation: Research, implementation, and outreach

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Genebanks conserve vast collections of plant genetic resources that are maintained as seeds, plant tissue cultures, and actively growing plants in the field and greenhouse. These collections must be securely backed-up at secondary locations. It is expensive to duplicate collections of vegetatively propagated plants, such as those maintained in tissue culture, the field, and greenhouse. Cryopreservation technologies have been developed to place dormant buds or shoot tips into liquid nitrogen, which minimizes the long-term costs of safety duplication. Cryopreservation methods for Citrus and Vitis have been developed and then implemented at the USDA National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, Colorado. These are among many of the clonal collections in the USDA National Plant Germplasm system that are being backed-up at NLGRP. The NPGS is developing free online educational resources to demonstrate dormant bud and shoot tip cryopreservation methods for many crops. These eBook resources include text, images, and embedded videos that are available online and can be used for university, national, or international classes, as well as individual learning opportunities. One goal of this outreach effort is to provide content that will train scientists and students in techniques that can be used to safeguard plant genebank collections around the world.

Keywords: training, outreach, plant cryopreservation, vitrification

S2_1

Fruit harvesting times and various dehydration methods for cryopreservation of *Paphiopedilum bellatulum* (Rchb.f.) Stein seeds

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Paphiopedilum bellatulum (Rchb.f.) Stein is an attractive terrestrial lady's slipper orchid that is threatened with extinction, due to over-collection and loss of suitable habitats. Consequently, *ex-situ* conservation of this orchid is urgently needed. This study was done to develop an effective technique to cryopreserve P. bellatulum seeds for long-term germplasm preservation. The capsules were harvested at one month interval from 5 to 9 months after pollination (MAP) and then seeds were subjected to three techniques before preservation in liquid nitrogen: (1) desiccation, (2) desiccation and wrapping with aluminum foil, and (3) vitrification. After harvesting the capsules at one month interval from 5 to 9 MAP, seed moisture content (MC) continuously decreased from 28.80% at 5 MAP to 19.74% at 9 MAP. Meanwhile, the seeds harvested at prolonged timing inclined to have more seed germination percentage. Nine MAP seeds showed the highest seed germination at 37.08%. After cryopreservation, desiccation technique and modification with aluminium foil wrap technique did not show different results. The seeds harvested at 9 MAP showed the highest germination by desiccation for 5 hours at 30.58 and 28.75%, respectively. For the vitrification technique, the seeds were harvested at 9 MAP, then pretreated with loading solution for 15 min, followed by exposure to PVS2 solution for 60 min gave the highest germination rate at 30.96%. However, the three methods were not significantly different in the highest germination after cryo-storage. Cryopreservation of P. bellatulum using fully mature seed by desiccation technique is recommended as it is the most practicable and reduces the time and cost for ex-situ long-term storage. These results can be used to develop protocols for cryopreservation of Paphiopedilum and play an important role in global conservation strategies.

Keywords: lady's slipper orchid; long-term storage; seed maturity; desiccation; vitrification

S2_2

Cryopreservation of Grammatophyllum speciosum Blume seeds by D cryo-plate method

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Grammatophyllum speciosum Blume (also known as tiger orchid) is in the family Orchidaceae and native to Thailand. It is an epiphytic and occasionally a lithophytic orchid. Moreover, it can grow to a length of 2.5 meter. This orchid plant has high consumption; while, the abilities of cultivating this orchid is too low. In this study, we focus on the development of cryopreservation by D cryo-plate method for *G. speciosum* seeds. Encapsulation was compared between 3% (w/v) alginate solution (normal method) and 3% (w/v) alginate solution supplement with loading soliton (2.0 M glycerol + 0.4 M sucrose) (modified method), then polymerized with 100 mM of CaCl₂ for 20 minutes. The encapsulated seeds on D cryoplates were dehydrated by using 3 different methods which are a laminar air-flow cabinet, silica gel, and drying bead treatments with the same exposure times (0, 30, 60, 90, 120, 150, and 180 minutes). The moisture content from modified method with silica gel and drying bead decreased to 35% and 22% in 120 minutes, respectively. After cryopreservation, encapsulated seeds were cultured on ½ MS medium. The viability results from tetrazolium test revealed that, after 120 minutes of dehydration, silica gel gave high viability rate up to 87.22% and drying bead gave 58.25%.

Keywords: plant cryopreservation, Orchidaceae, Grammatophyllum speciosum Blume, D cryo-plate

S2_3

Cryopreservation and assessment of genetic stability of cryopreserved *Fragaria* spp. using SSR markers

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Genus *Fragaria* (Strawberry) belongs to Family Rosaceae. Strawberries are important commercial fruit crop grown in all temperate regions of the world. Various cryopreservation techniques are in use for long-term conservation of in vitro germplasm. Cryopreservation at ultra-low temperature of liquid nitrogen, exposes shoot tip explants to physical, chemical, and physiological stresses which may cause genetic instability. Therefore, it is important to monitor genetic stability of *in vitro* conserved and cryopreserved germplasm. In the present study, encapsulation-dehydration, vitrification, V-cryoplate, and D-cryoplate on *Fragaria x ananassa* cv. Earliglow; whereas, encapsulation-dehydration, vitrification, and V-cryoplate on *F. chiloensis* were used. The post-thaw regrowth percentage of cryopreserved shoot tips ranged from 2-15%. Plants generated through these techniques were subjected to genetic stability analysis by eight simple-sequence repeats (SSRs) markers. No differences were observed between *in vitro* mother plants and *in vitro* cryopreserved plants of *Fragaria* spp. Based on data generated from the above primers, it was concluded that no genetic variation was induced due to cryopreservation.

Keywords: cryopreservation, strawberry simple sequences markers, Encapsulationdehydration, vitrification, genetic stability
Session III: Breeding (S3)

S3_2

Genetic diversity of an endemic medicinal orchid, *Coelogyne nervosa* from Southern India using morphological and molecular markers

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Genetic diversity of *Coelogyne nervosa* A. Rich. was investigated by using SDS-PAGE, RAPD markers and morphological characters. *C. Nervosa* is growing as epiphyte as well as lithophytes in Eastern and Western Ghats of India. Leaf samples collected from these two reference sites were taken for RAPD and protein profile analysis. Vegetative parts such as leaves, pseudobulbs and roots were fixed in FAA. Free-hand and microtome sections were cut and stained with safranin-fastgreen. The objective of this study is to assess the genetic diversity of endemic orchid *C. nervosa* distributed in southern India. The six populations collected from these two geographical regions exhibited significant variation in their morphological and molecular characters. The stomata are tetracytic and hypostomatic in distribution. The maximum thickness of cuticle and midrib region in leaf and, extensive lignification in exodermis and endodermis of root were recorded in populations located in Western Ghats as compared to those of Eastern Ghats. It is interpreted to be associated with the conservation of water. RAPD and protein profile data showed the inter population diversity between these two reference sites. This can be attributed to the ecological and climatic conditions prevailing in the Eastern and Western Ghats of India.

Keywords: SDS-PAGE, RAPD analysis, anatomical features, C. nervosa

Biotech 2021

Session IV: Micropropagation (S4)

S4_I1

In vitro multiplication of some selected banana cultivars (*Musa* spp.) from India and their genetic fidelity using ISSR markers

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In present study, in vitro multiplication of three banana cultivars, viz., Grand Naine, Monthan and Red banana, and genetic fidelity of regenerated plantlets have been taken up. The objective of this investigation is to carry out micropropagation of banana on commercial scale using cost-effective cytokinin (BAP) and auxin (IAA) in order to supply the quality saplings to farmers at affordable price. Commercial standard medium and MS basal medium supplemented with BAP at different concentrations and IAA at fixed concentration have been used for micropropagation. To study the genetic fidelity of in vitro-derived plants, ISSR markers were employed. In micropropagation experiments, shoot proliferation in commercial standard medium was found to be better in 1.0 and 5 mg/L BAP for Grand Naine cultivar; whereby, production schedule could be determined accurately with quality shoots. However, for Monthan, 5 and 10 mg/L BAP, and for Red banana 10 and 5 mg/L BAP along with 0.2 mg/L IAA for each, appeared to be more suitable for obtaining the productive shoots. Genetic fidelity studies showed that Monthan is 'true-to-type' as it shows high MIC value of 64.51%; whereas, Grand Naine with 48.41%. In case of Red banana, a lot of variation was reported with PIC value of 78.43. This genetic variation could be due to somaclonal variation that had been occurred in the micropropagated plants.

Keywords: banana cultivars, in vitro multiplication, ISSR markers, genetic fidelity

S4_I2

Somatic embryogenesis and other in vitro techniques towards tamarillo breeding and conservation

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Tamarillo (Solanum betaceum Cav.) is a solanaceous tree that produces edible fruits. The species is originated from South America but is now cultivated in several regions of the world, such as New Zealand, Australia, California, and Southern Europe. The species has a fast growth rate and, in appropriate conditions, enters production usually in the second year. In the last years, several protocols for in vitro cloning of this species have been developed at our lab, such as proliferation of axillary buds, organogenesis, and somatic embryogenesis. Suspension cultures, opening the way for large-cell culture in bioreactors, were also established. Based on these techniques, protocols for cloning adult trees have been developed. Moreover, hybrids and tetraploid were obtained g to develop more productive genotypes. Embryogenic callus can be kept in culture periods of time although chromosomal changes may occur in calli maintained for more than two years. Thus, cryopreservation assays have been carried out to preserve embryogenic material. Somatic embryogenesis showed to be a powerful technique not only for tamarillo cloning but also to analyse some of the molecular mechanism involved on somatic embryo induction and development. In particular, proteomic studies indicated that metabolism related proteins, such as enolases and threonine synthases, as well as heat shock proteins were up-regulated in embryogenic calli; whereas, pathogenrelated proteins were common in non-embryogenic calli. Detailed studies showed that a member of the SpoU rRNA methylase family, named NEP-TC, is consistently expressed in non-embryogenic calli of different origins suggesting that it may have a role as a SE blocker. More recently several genes were identified as internal controls for detection of miRNAs during somatic embryogenesis and metabolomic studies are being carried out to find differences between embryogenic and non-embryogenic calli. Taken together these data show tamarillo as an interesting model to analyse somatic embryogenesis and other biotechnological tools in tree angiosperms.

Keywords: cloning, micropropagation, proteomics, Solanum betaceum, somatic embryogenesis

Shoot multiplication of three Hedychium species via immersion temporary bioreactor

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Hedychium is a terrestrial zingiberaceous species with underground rhizomes, as a source of raw materials for pharmaceutical and cosmetic products. Therefore, demand for raw plant material was increased, resulting in over collection from the wild and habitat loss. This study was done to develop an effective technique for mass production by *in vitro* shoot multiplication of three *Hedychium* species, i. e. *H. flavescens*, *H. boloveniorum*, and *H. stenopetalum*. The effect of the semi-solid and liquid media using immersion temporary bioreactor (TIB) were compared. For the TIB, the frequencies and immersion durations were evaluated. After culture for 8 weeks, the results showed three *Hedychium* species cultured in TIB gave higher average shoot multiplication than semi-solid system. *H. flavescens* explants immersed in liquid MS medium for 5 min in every 4 hours, gave the highest shoot multiplication at 8.33 shoots per explant. *H. boloveniorum* immersed for 10 min in every 6 hours gave the highest shoot multiplication at 8.67 shoots per explant, and *H. stenopetalum* immersed for 5 min in every 3 hours, gave the highest shoot multiplication by TIB for large scale production of uniform and pathogen free of *Hedychium* for field plantations.

Keywords: Zingiberaceae, ginger lily, micropropagation, TDZ, immersion frequency

Preventing tissue necrosis with activated charcoal alternatives during shoot multiplication of coconut

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The demand for coconut products, such as coconut-oil, -water and -milk, is rising worldwide. However, the coconut production is currently not able to keep up with the demand due to ageing plantations, pests, and diseases. High quantities of high producing, disease resistant, and drought tolerant coconut plantlets are therefore urgently needed. Our lab developed an innovative clonal micropropagation method, that enables mass production of desired varieties. With current in vitro methods and during the propagation phase, tissue browning or necrosis of parts of the proliferating material occurs routinely. Different components including activated charcoal (AC), ascorbic acid, and silver thiosulfate, were added to the proliferation medium to investigate their potential reducing effect on browning. While AC was previously shown to prevent tissue necrosis during somatic embryogenesis in coconut, without interrupting the proliferation process, we demonstrated that the addition of 1g/L AC prevented shoot propagation. Therefore, we opted for components that do not interfere with the plant growth regulators present in the medium, such as ascorbic acid, which prevents oxidation and silver thiosulfate, an ethylene inhibitor. While different concentrations of ascorbic acid were shown not to affect tissue necrosis, silver thiosulfate had a positive trend with increasing concentrations preventing more tissue necrosis. Tissue necrosis mostly started 4-5 weeks after subculture, suggesting that shorter subculture cycles could also help mitigating this problem.

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We also gratefully acknowledge the Directorate-General Development Cooperation and Humanitarian Aid, Belgium (DGD) for the financial support of the project 'Safeguarding vegetatively-propagated crop diversity to nourish people now and in the future'

Keywords: coconut, *Cocos nucifera*, PGR, micropropagation, tissue browning, tissue necrosis, *in vitro* culture

The effect of plant growth regulators on the in vitro regeneration capacity in some horticultural crops and rare endangered plant species

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The screening of PGRs for their influence on in vitro regeneration pathways of some valuable cultivars *Fragaria* × *ananassa*, *Lavandula* × *intermedia*, *Chrysanthemum* × *morifolium*, *Ficus carica*, and rare endangered species, *Aconitum lasiostomum* and *Crithmum maritimum* was carried out. 21 variants of the MS medium were tested. For the chrysanthemum shoot tip development, the MS culture medium with 0.5, 0.75 mg L⁻¹ kinetin, and 0.05 mg L⁻¹ NAA were optimal. The use of 0.73-1.46 mg L⁻¹ metatopoline (mT) promoted better regeneration of explants in chrysanthemum, strawberry, common fig, lavandin, and *A. lasiostomum*. It was found out that using 3.0, 9.0 μ M mT, 6.0, 9.0 μ M TDZ, or 1.5 mg L⁻¹ BAP + 1.5 mg L⁻¹ IBA induced direct regeneration in *A. lasiostomum*. Active development of adventitious buds and root formation on the shoot slices were observed. 0.5, 1.0 mg L-1 BAP + 0.05 mg L⁻¹ NAA, or 1.5 mg L⁻¹ BAP + 1.5 mg L⁻¹ IBA in the culture media stimulated the regeneration up to 4-5 microshoots per explant in lavandin. At 3.0 and 6.0 μ M mT, a high morphogenetic response in *C. maritimum* explants was noted with initiation of development up to 12 buds per explant and spontaneous rhizogenesis. The optimal concentrations of PGRs in the MS culture medium for successful in vitro regeneration of the studied genotypes were determined.

Keywords: cultivar, species, culture medium, explant, morphogenic response

In vitro establishment and multiplication of Vaccinium virgatum Ait. 'Delite'

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Micropropagation in blueberries have been studied, but there are only a few *in vitro* protocols in the group of rabbiteve blueberries (Vaccinium virgatum), which are more adapted to warmer climates. In this study, we evaluated the establishment and the multiplication stages in vitro in 'Delite' rabbiteye blueberry, comparing different sterilization treatments for initial establishment and different orientations of the stem segment in the multiplication culture medium. The surface sterilization was attempted with nodal segments immersed in a varying amount of time in sodium hypochlorite (0.5%). For multiplication, we used five-node stem segments and three treatments (vertical, inclined at 45°, and horizontal orientations). The surface sterilization of nodal segments using 5 min immersion in sodium hypochlorite led to 96.7% of uncontaminated explants, 96.7% of survival rate, and 83.3% of explants with axillary shoot growth. The vertical orientation led to 100% explants with shoot proliferation, 1.8 new shoots per explant, 5.1 cm long with 12.7 leaves per shoot. We concluded that 5 min immersion in sodium hypochlorite was efficient in the establishment and that stem segments in the vertical orientation were more efficient in multiplication. These findings can contribute to the establishment of protocols for in vitro propagation and germplasm conservation of 'Delite' rabbiteye blueberry.

Keywords: blueberry, Ericaceae, initial establishment, in vitro, multiplication, rabbiteye, propagation

A comparative analysis of in vitro responses of anthurium under temporary immersion (RITA®)

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Gene banks employ diversity assessments to characterize the genetic integrity of the collection and design appropriate conservation protocols and efficient maintenance of germplasm. The in vitro behavior of the University of Hawaii anthurium germplasm collection has not been characterized to date, and thus appropriate genotype-specific protocols have yet to be identified. The responses of ten accessions were evaluated under the RITA® temporary immersion system. Two-node cuttings from each accession were placed in RITA® bioreactors with liquid medium containing 0.3 MS salts,0.2 mg L⁻¹ BA, 15% coconut water and 20 g L⁻¹ sucrose. Primary shoots were excised after 45 days to allow axillary buds to develop into secondary shoots. Percentages of explants with shoots, number of primary shoots per RITA[®] vessel, shoot length, and total axillary bud mass volume were noted. Bud masses were placed on a solid medium containing ¹/₂ MS salts with 15% coconut water, 20 g L⁻¹ sucrose and 2 g L⁻¹ gellan gum for further shoot development and growth. The degree of bud formation and number of secondary shoots per RITA® vessel were assessed after another 45 days. Parameters were analyzed using Welch's ANOVA and Two-Step Cluster analysis. Clusters were cross-referenced with pedigree and breeding records. Significant differences in in vitro production of primary and secondary shoots per RITA[®] vessel, degree of basal mass formation, and axillary bud mass volume among the genotypes were observed. Cluster analysis of quantitative and qualitative parameters revealed five clusters, indicating that parentage influenced in vitro shoot production particularly in lines with Anthurium andraeanum, A. amnicola, A. formosum, and A. kamemotoanum in their background. Pedigree and breeding records are valuable resources for predicting response profiles of anthurium in vitro performance. Assessment of proliferative diversity will provide guidelines for future protocol development in the in vitro anthurium germplasm collection.

Keywords: diversity analysis, plant tissue culture, germplasm, bioreactor, micropropagation

Chemotyping and in vitro conservation of strawberry tree (Arbutus unedo L., Ericaceae)

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Arbutus unedo L. (strawberry tree) is an evergreen tree with a circum-Mediterranean distribution, that thrives on dry and rocky marginal lands and is tolerant to low temperatures and drought stress. Its edible red berries are used mainly for the production of a high-value alcoholic distillate. The ability to sprout following fires is crucial to promote the natural recovery of burned areas in Southern Europe forests. Bioactive compounds found on fruits and leaves have been used by the pharmaceutical, cosmetic, and food industries. Due to its great economic and ecological potential, the demanding for high quality strawberry tree plants has increased. Research carried out focused on plant selection based on fruit production but the identification of chemotypes was not yet pursued. Thus, the objectives of this work are: (1) the chemotyping of wild trees and (2) in vitro conservation and multiplication of selected genotypes.

The chemical profile of wild trees was studied by HPLC-PDA and LC-MS/MS. Among the compounds found, hydroquinone and arbutin were quantified and its antifungal effect tested. Both showed antifungal effect and were also effective against *Phytophthora cinnamomi*. Specific chemotypes were identified and those producing high levels of arbutin and hydroquinone were selected. Selected genotypes can be conserved and multiplied in vitro using different micropropagation techniques. Axillary shoot proliferation can be accomplished on solid and liquid medium supplemented with 2 mg L⁻¹ of 6benzylaminopurine (BAP) and a calli with organogenic capacity can be induced on apical leaves with 1 mg L⁻¹ thidiazuron. Rooting and acclimatization rates higher than 90% can be obtained on shoots produced by these methods. Regarding somatic embryogenesis, a one-step induction protocol was established, using a medium containing 2 mg L⁻¹ benzyladenine and 5 mg L⁻¹ of 1-naphthaleneacetic acid. The identification of specific chemotypes accomplished on this work has important repercussions on strawberry tree selection and breeding contributing to strawberry tree valorization. Due to the involvement of some phenolics on plant defense mechanisms, these results can also contribute to improve plant productivity and develop more efficient and environmentally friendly biocontrol strategies for crop production. Funding: Foundation for Science and Technology (Portugal) supported J. Martins PhD fellowship (SFRH/BD/122478/2016). The authors also acknowledge CESAM within PT2020 Partnership Agreement and Compete 2020 (UIDP/50017/2020+UIDB/50017/2020), ReNATURE project (CENTRO-01-0145-FEDER-000007), Foundation for Science and Technology for the project nº 22125 "National Mass Spectrometry Network" (RNEM) of Portugal and Laboratory of Mass Spectrometry (LEM) of UC Node integrated in the RNEM, for MS analyses and project 0377_IBERPHENOL_6_E by Programa de Cooperación Interreg V-A España-Portugal (POCTEP) 2014-2020.

Keywords: Arbutin, HPLC-PDA, hydroquinone, in vitro culture, micropropagation

Prospect and challenges in Rhododendron micropropagation

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Rhododendron L. is a large genus of ornamental shrubs and trees native to Asian and American continents. There are currently 1062 accepted species of Rhododendron; while, an estimated 25% of them are endangered. Due to the importance of micropropagation on conservation and production of Rhododendron, literature on Rhododendron micropropagation was reviewed. 48 research articles on micropropagation of Rhododendron were found, involving 88 different species, subspecies, varieties, and cultivars. The taxa reviewed belongs to Subgenus Hymenanthes Section Pontica (63%) and Pentanthera (2.5%), Subgenus Rhododendron Section Rhododendron (26%) and Schistanthe (3.7%),Subgenus Azaleastrum Section Tsutsusi (2.5%) and Sciadorhodion and Subgenus (1.2%).Choniastrum Section Choniastrum (1.2%). Micropropagation was conducted through in vitro collection of seed and adult parts, shoot induction, callogenesis, root induction, and somatic embryogenesis on tissue culture media, such as Anderson's medium, Woody Plant Medium, Economou and Read medium, Murashige and Skoog Medium, mostly supplemented with sucrose and adjusted to pH 4.5-5.8 before autoclaving. Basal media composition, plant growth regulator and explant choice were most tested and influential factors in Rhododendron micropropagation. Types of cytokinins were significant factor for shoot multiplication, with thidiazuron and isopentenyladenin producing the best results. However, stunted shoot induced on thidiazuron required an extra elongation step. In addition, there were reports of tumorous tissue proliferation from micropropagated plants and certain genotypes. Ex vitro rooting was shown to be a cost-effective alternative to in vitro rooting. Mycorrhizae studies found that inoculation improved growth of rooted microcuttings. Micropropagation should be explored on ornamental and endangered Southeast Asian rhododendrons.

Keywords: Rhododendron, Ericaceae, Vireya, organogenesis, cytokinin, in vitro culture

Biotech 2021

Session V: Physiology and Production (S5)

S5_2

Effects of irrigation levels on growth and chemical constituents in Curcuma alismatifolia

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The irrigation regime affects plant growth and development, as well as phytochemical content. This research was aimed to investigate the effects of irrigation levels on physiological responses and biochemical changes in Curcuma alismatifolia cv. 'Dang Doi Tung'. Plants were grown by using soil, rice husk, and rice husk charcoal (ratio 1:1:1) as the growing media under a plastic greenhouse with averaged 27°C, 80% RH, and 333.20 µmol m^{-2·s⁻¹} light intensity. Plants were prepared by daily watering until shoot sprouting (4 weeks after plating; WAP) then experiment was started. The experimental design was a completely randomised design with four irrigation levels, i.e., 100% of crop evapotranspiration (ETc), 75% ETc, 50% Etc, and 25% ETc. The results showed that at the flowering stage (12 WAP), the plant height, number of leaves per plant, and leaf area of plants irrigated with 100% ETc were higher than other treatments. In addition, the irrigation levels of 100% ETc significantly increased photosynthesis values and chlorophyll contents; while, the low level of irrigation (25% ETc) decreased the chlorophyll content and photosynthetic rate. It was also found that stomatal conductance in the 25% ETc treatment was lower than other treatments. Total phenolic contents were the highest in flowers compared to leaves, rhizomes, and fibrous root parts. Plants irrigated at 100% ETc gave lower total phenolic contents than those at 75%, 50%, and 25% ETc. There were 23 chemical constituents in fresh rhizomes at harvest stage, and the levels of each constituent varied by irrigation treatments.

Keywords: antioxidant activity, chemical constituents, *Curcuma alismatifolia*, irrigation, phenolic, water deficit, water stress

S5_3

Chemical composition analysis of essential oils from black gingers (*Kaempferia parviflora*) by gas chromatography-mass spectrometry (GC-MS)

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Black ginger (Kaempferia parviflora or KP) is one of popular Thai herbs that has valuable benefits for treating a variety of diseases. The essential oils extracted from its rhizome has the pharmacological activities, such as anti-inflammatory, antioxidative, and antimicrobial activities. This study aimed to determine the chemical components of essential oils from ten KP cultivars which were obtained from different production areas located in Phu Tub Berk highland. The essential oils were extracted by steam distillation and their chemical compounds were analyzed by GC-MS. Twenty chemical compounds were identified from the essential oils of KP's rhizomes with 6 major components including germacrene D $(15.4\pm5.5\%)$, α -pinene $(11.2\pm6.4\%)$, camphene $(10.1\pm6.6\%)$, borneol $(9.2\pm2.8\%)$, linalool $(8.0\pm2.5\%)$, and β -pinene $(6.4\pm4.0\%)$. The results showed the variation of the component percentages among ten cultivars. Top three cultivars which have high percentages of those six compounds are Nam Juang-1, Huay Sai, and Khun Nam Khap, respectively. This study showed the chemical compositions of essential oils from ten KP cultivars; however, the quantitative analysis of methoxyflavone in KP's crude extract needs to be further investigated. Both essential oil components and methoxyflavone contents of ten KP cultivars will be beneficial to select the KP cultivars for pharmacological studies and promoting the cultivation in Phu Tub Berk highland communities.

Keywords: Kaempferia parviflora, ten KP cultivars, germacrene D, α -pinene, Tub Berk Model Project

S5_4

Assessment of IAA synthesis by endophytic bacteria in Vanda (Orchidaceae)

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Vanda is an important economic orchid, and some interesting species originated in Thailand. Several factors involved in the growth and development of orchids include nutrition, environmental conditions, plant hormones, and endophytic microorganisms. Endophytic microorganisms influence the germination and adaptation of orchids in various environmental conditions. However, endophytic bacteria in Vanda are rarely reported. Therefore, this research aimed to study isolated endophytic bacteria in Vanda tissue that can produce indole-3-acetic acid (IAA) and affect the growth and development of this plant. An IAA synthetic efficiency assessment of 14 of 208 isolates was done on culturing media with and without L-tryptophan. Without L-tryptophan, isolate 1L1 produced the most IAA (37.75 mg IAA L^{-1} ; whereas, with L-tryptophan, isolate 2R13 produced the most IAA (152.63 g IAA L⁻¹). The endophytic bacteria colonies varied in color (brown, white, pink, or yellow), form (circular or irregular), elevation (flat, raised, convex, or umbonate), surface (smooth or matte), margins (undulate, entire, or filiform), and ooze. In addition, morphological assessment of endophytic bacteria found that nine isolates had a coccus shape, and five isolates had a rod shape. The Gram staining test showed that 13 isolates were Gram-negative, and one isolate, 3R14, was gram-positive. From this research, the results confirmed that there were 14 endophytic bacteria isolates that could synthesize IAA for Vanda.

Keywords: Vandaceous, microorganism, auxin, morphology

Biotech 2021

Session VI: Plant Molecular Research (S6)

S6_1

DNA barcoding and molecular systematics in genus Coelogyne Lindl. (ORCHIDACEAE)

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Subtribe Coelogyninae (Epidendroideae, Orchidaceae) comprises of 16 genera. To study ONE of these genera *Coelogyne* Lindl., reveal sectional relationships and relation with pholidota. DNA bar coding is a technique which helps identification of species even if only small fragment of organism is available at any stage. Present study undertaken to develop DNA barcodes of sub tribe Coelogyninae using *rbcL*. The *rbcL* (Rubisco large sub unit) from chloroplast genome tested for as effective barcode. The inter specific divergence values and species and species discrimination rates were calculated by Kimura 2 parameter (K2P) using MEGA 4.0. The *rbcL* with average interspecific divergence values yielded 72.72% species resolution, thus could be distinguish all the species of *Coelogyne* and *Pholidota*. Invariably the genus *Pholidota* shows close affinity with *Coelogyne*. This supports the inclusion of genus *Pholidota* in the subtribe Coelogyninae of tribe Coelogyneae.

Keywords: DNA barcodes, Coelogyne, Pholidota, rbcl, sectional delineation

S6_2

Plant GARDEN: a portal web site for accessing plant genome, DNA marker, and SNP information

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Various plant species have been sequenced with the advance of NGS technologies. The assembled reference genome sequences are used to analyze polymorphisms and haplotypes within the species by comparisons re-sequenced data of multiple accessions. These data have been contributed plant science and applied industries, such as crop breeding. Plant GARDEN (Genome And Resource Database Entry; https://PlantGARDEN) stores publicly available genome sequences, gene sequences and annotation, marker, QTL, and SNPs. The regular version was opened in July 2020. The data registered in Plant GARDEN in current (December 2020) is 119 assembled genome sequences, 5,890,957 gene sequences, 287,703 DNA markers, 8,217 QTLs, and 3,812 SNP list (vcf files). The aim of Plant GARDEN is providing plant genome information to the fields of plant science, breeding, as well as education. Therefore, we have tried to create simple and user-friendly GUI design. Plant garden target any plant species, which genome sequences are assembled, and continue to increase the number of plant species in the database. We expect Plant GARDEN will assist understanding of genomes and genes diversity by comparing the sequences registered in the DB.

Keywords: plant genome, database

S6_3

Molecular phylogeny and DNA barcode regions efficacy for identification the variety of *Capsicum annuum* L. in Thailand

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Capsicum annuum L. is an important hot chili species for commercial vegetable crop. This species has various varieties that were cultivated in several localities in Thailand. The genetic diversity of C. annuum group in Thailand is poorly known. The aim of this study is to test the efficacy of DNA barcodes for identifying the C. annuum group and to understand the genetic relationships among variety within this species. Seventeen samples were represented from twelve varieties for molecular analysis. All samples were successfully amplified and generated DNA sequences from the internal transcribed spacer (ITS), the large subunit of ribulose bisphosphate carboxylase (rbcL) and Maturase K (matK) regions. Molecular phylogeny was analyzed based on the maximum likelihood (ML) and Bayesian inference (BI) methods with each of single DNA locus. The results showed that two DNA loci (*rbcL* and matK) did not distinguish between species level; while, ITS region showed high genetic diversity within this species. The phylogenetic tree based on ITS region can delimit the C. annuum from other species (C. baccatum, C. chinense, C. frutescens, and C. pubescens). Also, the phylogeny divided C. annuum species into three groups and revealed to this taxa as species complex. The morphological characters did not relate to molecular evidences, which shared between varieties. In addition, C. annuum (Phrik Bang Chang) group demonstrated that more genetic diversity than previous estimate, which separated into four subgroups with strong molecular data supports. This study suggested that DNA barcode via ITS region was suitable for C. annuum identification into the species and variety level. However, the further study needs to investigate on the phylogenetic analysis from other DNA regions and more phenotypic characters, such as chemotaxonomy to clarify the relationship of species complex within C. annuum species.

Keywords: *Capsicum annuum*, DNA barcoding, genetic diversity, molecular phylogeny, Thailand

Biotech 2021

Session VII: Plant Protection (S7)

S7_1

Simple preparation of the dried galangal (*Alpinia galanga*) in ethanol is effective to suppress *Curvularia* sp. which causes grain discoloration of rice (*Oryza sativa*) (var. Suphan Buri 2)

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Galangal (*Alpinia galanga*) is a renowned medicinal plant that has been used for food and medicine. This plant also receives attention to use in agriculture, particularly for controlling plant diseases. The crude extract of galangal, using ethanol as solvent, is effective to suppress plant pathogenic fungi, such as *Curvularia lunata* and *Fusarium sacchari*, the causal agents of grain discoloration (GD) disease of rice (*Oryza sativa*). In a recent study in 2020, the simple preparation of dried galangal, extracted with 60% ethanol, was effective to suppress *Curvularia* sp., the fungus which caused large lesion (LL) symptom of GD. However, the preparation of dried galangal (only at 20% in water) suppressed the same pathogen which caused small spot (SS) symptom in rice (var. Suphan Buri 2) in Phetchaburi, Thailand. The dried galangal, extracted with 60% ethanol, was as effective as the applications of the farmer's concoction which was composed of the commercial chemical fungicide Armure® (mixture of propiconazole and difenoconazole), abamectin, liquid extract of pineapple peel, and the fresh cell suspension of *Bacillus megaterium*.

Keywords: ethanol extract, galangal, grain discoloration of rice, Curvularia lunata

S7_2

In vitro screening of crude extracts from plants against *Fusarium sacchari* and *Curvularia lunata* and testing the efficacy of a selected crude extract of *Alpinia galanga* against grain discoloration and other rice diseases in the fields

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Fifteen plant materials were extracted by 60% ethanol using maceration technique and tested against *Curvularia lunata* and *Fusarium sacchari* using poisoning food technique. The results indicated that dried *Alpinia galanga* rhizome extract at 10% concentration completely inhibited the mycelial growth of *F. sacchari*; while, 20% concentration of *A. galanga* extract highly inhibited the mycelial growth of *C. lunata* (78.3%). Therefore, the most effective one, dried *A. galanga* extract was selected and investigated in the field condition to suppress narrow brown leaf spot disease, neck blast disease, and grain discoloration (GD) disease in two growing seasons. After spraying two times, the results showed that 20% *A. galanga* ethanolic extract significantly reduced the incidence of narrow brown leaf spot disease (0.7) and (1.2) in both seasons and suppressed the severity of neck blast disease (12.5%) in the second season. For GD disease severity, 20% concentration of galangal extract showed the lowest disease severity (3.5) among all treatments. The galangal extract was effective to suppress the other important rice diseases, narrow brown leaf spot, and neck blast diseases under the field condition.

Keywords: Alpinia galanga, Curvularia lunata, ethanolic extracts, Fusarium sacchari, grain discoloration disease

POSTER PRESENTATIONS

Biotech 2021

Session I: Plant Diversity Conservation (S1)

P1

Micromorphology and histochemistry on lip of Orchidantha foetida (Lowiaceae)

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Orchidantha foetida K. Larsen & Jenjittikul (Lowiaceae) is an endemic species distributed in Eastern region of Thailand which possesses an unusual flower with strong stinky odor. Emitted rotten scent, which is released from flowers, plays a critical role in pollinator attraction. Previous studies on pollinator and Orchidantha interaction indicated deceit relationship which plants received benefit for pollination, but pollinator did not. However, study on floral characters and putative compounds of Orchidantha have not been investigated yet. Here we show the micromorphology and histochemistry of O. foetida which may be involved in pollinator attraction. Lip transverse section presented papillae on adaxial epidermis with large intercellular space at subadjacent layer. Idioblasts with raphide crystal was commonly distributed in palisade cell layer. For SEM investigation, conical papillae and bullose epidermis with rugose cuticular striation were found on adaxial lip surface. Several minute droplets of putative secreted remnants were observed on papillose and bullose epidermal cells. Histochemical results revealed location of scent-containing structure on adaxial epidermal cells which presented mucilage, phenolic compounds, and terpenes inside cells. These characters indicated that adaxial epidermal cells on lip may act as osmophores which was a scent releasing function. These results revealed the floral characters of O. foetida which may be related and help in pollinator attraction. Further studies would emphasize the importance of habitat protection and conservation of unique deceit relationship between animals and this plant.

Keywords: anatomy, coprocantharophily, labellum, osmophores, papillae, SEM

The morphological characteristic of *Amaranthus* spp. for conservation in DOA genebank of Thailand

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The objective of this study was to characterize morphological characteristics of 125 accessions of *Amaranthus* spp. collected from southern and eastern regions of Thailand. Characterization of plant height (cm), number of leaves per plant at flowering stage, and stem and leaf colour of a plant were done at Biotechnology Research and Development Office, Department of Agriculture from February 2020 to June 2020. It was found that there were statistically significant differences in all characters among accessions. N31 (green stem and leaf) has the highest stem height (162 cm) and its average number of leaves per plant was 40. The highest number of leaf per plant was 46 which was found in N116 (green stem and leaf) and its average stem height was 98 cm. In addition, it was revealed that there were green and reddish-green colours found in collected samples. As a result, further studies on plant improvement should be done in the future.

Keywords: Amaranthus spp., conservation, morphology, characterization

Seed storage of Ipomoea alba L. in DOA genebank Thailand

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Study on influence of seed moisture and storage temperature for seed germination and seed storage period of *Ipomoea alba*. This study was separated into four experiments based on the condition of storage temperature as follows; room temperature, 5 °C, -10 °C and freezing in liquid nitrogen (-196 °C). These experiments were designed in Split Plot Design with 4 replicates, including 2 factors as main plot with 4 levels of percent moisture in seed at 6, 8, 10, and 15.4 (start seed humidity) and sub plot with 7 levels of period for seed storage at 0, 3, 6, 9, 12, 15, and 18 months. The results showed that seed moisture and period of seed storage affected the seed germination percentage of *I. alba* in all storage temperatures. The room temperature with 6, 8, and 10 percent of seed moisture (storage for 18 months) showed the seed germination at 77, 82, and 60 %, respectively; while, non-reduced seed moisture could be stored about 9 months. The seed moisture did not affect seed germination of *I. alba*, which was kept at low temperature for 18 months. In addition, seed moisture of 6, 8, 10, and 15.4 % at 5 °C, -10 °C, and -196 °C showed the seed germination at (85, 83, 85, and 49 %), (94, 83, 89, and 78 %), and (83, 75, 75, and 67 %), respectively.

Keywords: Ipomoea alba, seed moisture, seed storage temperature, germination

Biotechnological methods of reproduction and preservation of species and cultivars of the genus *Syringa* L

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Biotechnological methods, in particular the creation of in vitro gene pools, are among the most effective ones for plant biodiversity conservation. One of the most representative in Russia in vitro collection fund of the genus Syringa L. was formed in Tsitsin Main Botanical Garden. Nowadays, it consists of 257 taxa (19 species and subspecies and 238 cultivars and selective forms). The collection of lilac species includes samples obtained from different native locations and represents most series of the genus. The collection of cultivars was formed for preservation and dissemination of retro-cultivars, unique cultivars with unusual morphological traits and the most significant achievements of national and foreign lilac selection. Consequently, the fund represents cultivars from all color groups with both simple and double florets. The purpose of the study was to improve the methodology of in vitro reproduction and preservation of the genus Syringa representatives. The generally accepted and developed in the Laboratory of Plant Biotechnology of MBG biotechnological methods were used in the work. As the result of the research, the plant morphogenesis under the impact of the nutrient medium components was studied, as a consequence, the optimal conditions at most stages of clonal micropropagation for the effective reproduction and conservation of valuable lilac genotypes were established. The efficiency of using the modified Quorin-Lepoivre medium with combined addition of 6-Benzylaminopurine and Indole-3-acetic acid as the plant growth regulators for obtaining high reproduction coefficient was proved for the studied genotypes. Nowadays, the molecular-genetic researches most on the genus Syringa representatives are conducted.

Keywords: Syringa, in vitro collection, clonal micropropagation, morphogenesis

Session II: Cryopreservation (S2)

P6

Cryopreservation of Sesame indicum L. seeds

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The study on cryopreservation of *Sesame indicum* L. seeds was carried out at Biotechnology Research and Development Office from October 2019 to September 2020. This research was conducted to study seed moisture content (%) which is the main factor affecting seed storage by comparison with ambient conditions for a period of 1 month. Split split plot design was laid out with 4 replicates of each condition. The main plot consisted of 6 certified sesame varieties of Department of Agriculture: 1) White-seeded cultivar "Roi et 1", 2) White-seeded cultivar "Mahasarakhram 60", 3) White-seeded cultivar "Ubonratchathani 2", 4) Black-seeded cultivar "Ubonratchathani 3", 5) Red-seeded cultivar "Ubonratchathani 1", and 6) Red-seeded cultivar "Ubonratchathani 2". The sub plot consisted of 3 storage periods: 0 day, 7 days, and 1 month. Seed viability by monitoring changes in percent of seed germination and oil content were recorded. The results showed that all varieties of sesame could be kept in cryopreservation but the seed moisture content should be reduced to 6 percent or lower to maintain seed viability and oil content of sesame seeds.

Keywords: cryopreservation, sesame seeds, seed viability, oil content

Biotech 2021

Session III: Breeding (S3)

P7

Intergeneric hybridization of Seidenfadenia mitrata (Rchb.f.) Garay with Ascocentrum spp.

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Intergeneric hybrids of *Seidenfadenia mitrata* (Rchb.f.) with Ascocentrum spp. were developed using *Seidenfadenia mitrata* (Rchb.f.) as a female parent and *Ascocentrum* spp. including Ascocentrum curvifolium (L.) Schltr. and Ascocentrum miniatum (L.) Schltr. as a male parent. Pollinia of Ascocentrum spp. were collected from the flowers after 2 days of blooming and then were pollinated with the Seidenfadenia mitrata (Rchb.f.) flowers by handpollination. The results showed that the percentage of successful fruit formation was the crosses between Seidenfadenia mitrata (Rchb.f.) x Ascocentrum curvifolium (L.) Schltr. and Seidenfadenia mitrata (Rchb.f.) x Ascocentrum miniatum (L.) Schltr. about 80 and 20%, respectively. Sizes of Seidenfadenia mitrata (Rchb.f.) x Ascocentrum curvifolium (L.) and Seidenfadenia mitrata (Rchb.f.) x Ascocentrum miniatum (L.) Schltr. mature fruits were about 3.75 cm in length x 0.50 cm in width and 2.69 cm in length x 0.48 cm in width after 168 and 180 d of pollination, respectively. Fruits of two hybrids produced seeds. Seeds of Seidenfadenia mitrata (Rchb.f.) x Ascocentrum curvifolium (L.) and Seidenfadenia mitrata (Rchb.f.) x Ascocentrum miniatum (L.) Schltr. germinated into protocorms after sowing 60 and 40 d on MS agar medium (1962), respectively. Protocorms developed into shoots and roots after tranfered to modified VW agar medium (1949) supplemented with 150 ml L⁻¹ coconut water, 10 g L⁻¹ sucrose, 2 g L⁻¹ activated charcoal, 7 g L⁻¹ agar, and pH at 5.2. Plantlets of two hybrids were successfully acclimatized and grew in greenhouse conditions.

Keywords: orchid, pollinia, pollination, fruit, intergeneric hybridization

Apricot cultivars of the Chinese breeding

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The research involved eight apricot cultivars of the Chinese breeding from the collection of the Nikita Botanical Gardens (NBG). All genotypes were studied for a number of economically valuable traits. The study was conducted in 2015–2020. The control variant was one of the best cultivars by economically valuable characters - Krymsky Amur - zoned in the south of Russia. The site is located in the Southern Coast of the Crimea at 200 m above sea level. The aim of the work was to conduct a comprehensive assessment of the Chinesebred apricot cultivars in the conditions of the Southern Coast of the Crimea and establish the prospects for their use as sources of valuable traits. According to the results of the studies carried out, it was found that most genotypes have large fruits, obtain increased drought resistance, low susceptibility to clasterosporium disease and medium one to moniliosis, early flowering, and fruit ripening. To create cultivars resistant to spring frosts, it is advisable to involve in breeding sources of late plant development, especially those distinguished by late flowering. With late flowering, cultivar Da-Huang-Hou was selected. Plants bloom 10 days later than the control cultivar. In order to extend the period of consumption of fresh fruits, cultivars with early and late fruiting periods are of interest as sources for breeding. Lanyhou Jim Mama, Yin-Bei-Xin, and Mai-He-Sin are distinguished by early ripening of fruits. Their fruits ripen at the end of June. With late fruiting, the Da-Huang-Hou cultivars is of interest $(22.07 \pm 6 \text{ days})$. Most of the Chinese cultivars are characterized by large fruit size, light skin color with a bright blush, and a fusion-fibrous pulp. Genotypes with a non-separable kernel and bitter seed are common. Among the studied genotypes, Yuan-Xin (100.8 \pm 16 g) and Lanyhou Jim Mama (109.1 \pm 21.9 g) cultivars have the largest fruits. On the basis of a pomological description of taste (tasting score 4.2 points), three cultivars are distinguished; there are five large-fruited cultivars (59.6-109.1 g) and six cultivars with a bright integumentary color, occupying 25-75% of the fruit surface. The cultivars are selected for use in further breeding as sources of various valuable traits.

Keywords: apricot, Chinese cultivars, flowering time, ripening period, frost resistance, drought, breeding

Session IV: Micropropagation (S4)

P9

Effect of auxins and cytokinin on micropropagation of *Impatiens sirindhorniae* Triboun & Suksathan *in vitro*

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Impatiens sirindhorniae Triboun & Suksathan (Balsaminaceae) is an endemic species to Krabi and Suratthani provinces. This study is the first report on *in vitro* micropropagation of *I. sirindhorniae* from the reservoir of Ratchaprapa Dam, Khao Sok National Park. The objective of this study was to investigate effect of explants and plant growth regulator treatments on shoot induction. *I. sirindhorniae* collected from the reservoir of Ratchaprapa Dam as were used as explants. The explants were sterilized and cultured on MS agar media supplemented with 0, 0.5 or 1.0 mg L⁻¹. BA in combination with 0 or 1.0 mg L⁻¹ IBA or NAA for 4 weeks at 25 ± 2 °C, under 16 h photoperiod with a light intensity of 60 µmol m⁻²s⁻. The results showed that all the treatments survived 100%. The highest number of multiple shoots was obtained from the shoot explants cultured on MS agar mediau supplemented with 0.5 mg L⁻¹ IBA (2.22 shoots per explant). However, the longest shoot was obtained form the shoot explants cultured on MS medium without plant growth regulator (1.48 cm).

Keywords: Impatiens sirindhorniae, BA, NAA, shoots, nodes

A reliable protocol for micropropagation of an ornamental aquatic plant *Staurogyne repens*

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Staurogyne repens is a commercially important ornamental aquatic plant species with traditional uses. Due to the low growth of plantlets, propagation by conventional means has been met with many difficulties. In this study, a reliable protocol for micropropagation of an ornamental aquatic plant Staurogyne repens was established. The explants were surface sterilized using 15% Clorox® (5.25% sodium hypochlorite, NaOCl) for 5 minutes followed by rinsing 3 times with autoclaved double distilled water. The surface sterilization of explants was rendered by 10% Clorox® for 10 minutes and 5% Clorox® for 15 minutes. The aseptic nodal segments (1.5 cm in length) were transferred to MS medium supplemented with varying level of 6-benzylaminopurine (BAP; 0, 1.0, 3.0, 5.0 or 7.0 mg L⁻¹) alone or in combination with 1-naphthalene acetic acid (NAA; 0.15 mg L⁻¹). A maximum mean number of shoots per explant (48.75 ± 0.27) and mean shoot length (21.42 ± 0.70 mm) were induced from about 1 aseptic nodal segment within 45 days in the presence of 0.15 mg L⁻¹ NAA and 1.0 mg L⁻¹ BAP. In vitro regenerated shoots were rooted on full strength MS medium conjunct with 3-Indolebutyric acid (IBA) singly at different concentrations. The best rooting response was observed on full strength MS medium containing IBA at 3.0 mg L⁻¹. This medium yielded maximum number of roots (26.20±0.32 roots/shoot) with 9.53±0.44 mm average length. In vitro grown plantlets were successfully acclimatized in pots containing a rockwool under greenhouse conditions at 100% survival and grew vigorously without any morphological abnormalities during acclimatization in the greenhouse. The current protocol was the first report on successful establishment of *in vitro* clonal propagation of *Staurogyne repens*.

Keywords: aquatic plant, 6-benzyl-aminopurine, micropropagation, naphthaleneacetic acid, rockwool

Organogenesis and efficient in vitro plantlet regeneration from nodal segments of an ornamental aquatic plant *Lobelia cardinalis* L. using BAP

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Aquatic plant is a high diversity of plant species in the world. Aquatic plants are grown in aquaria for their beauty and to maintain the quality of water. High demand for aquatic plants mainly from the developed countries has created an aquatic plant industry in both developed and developing countries. The effect of 6-benzylamino-purine (BAP) has been investigated in shoot multiplication for a simple, efficient, rapid, and commercially applicable regeneration protocol of an important ornamental aquatic plant, Lobelia cardinalis L. Multiple shoots were induced in nodal segments obtained from young plantlets on Murashige and Skoog (MS) medium containing cytokinins in various concentrations (BAP; 0.5, 1.0, 2.0, or 3.0 mg L^{-1} and Kinetin; 0.5, 1.0, 2.0, or 3.0 mg L^{-1}). The highest shoot proliferation (100%) and maximum number (7.20±0.12) of shoots per explant with shoot length of (8.96±0.10 mm) and maximum number (56.40±0.97) of leaves per explant was recorded on MS medium supplemented with 1.0 mg L⁻¹ BAP after 45 days of culture. The highest rooting in the micro shoots (3 cm) was achieved on full strength MS medium supplemented with 0.5 mg L⁻¹ α -naphthaleneacetic acid (NAA) which produced 28.00±0.46 mean roots/shoot with 18.69±0.10 mm mean root length. Regenerated plantlets were subsequently hardened, acclimatized, and successfully established in aquariums after 30 days of transfer to the greenhouse with 100% survival rate. The present in vitro propagation protocol would facilitate an alternative method for rapid and large-scale production of this important ornamental aquatic plant.

Keywords: aquatic plant, micropropagation, naphthaleneacetic acid, shoot multiplication

Large scale *in vitro* micropropagation of an *ornamental* plant, *Oxalis triangularis* A.st.-Hil for commercial application

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Oxalis triangularis A.st.-Hil is an attractive ornamental plant propagated by bulbs and has no natural viable seeds. *In vitro* micropropagation and acclimatization for the ornamental *Oxalis triangularis* A.st.- Hil, are reported. Plantlet regeneration ability on MS medium supplemented with 6-benzylaminopurine (BAP) at different concentrations (0, 1.0, 3.0, and 5.0 mg L⁻¹) either singly or in combination with α-naphthalene acetic acid (NAA) (0.5 mg L⁻¹) or 2,4-dichlorophenoxy acetic acid (2,4-D) (0.5 mg L⁻¹) was evaluated using petiole explants. Prolific shoot multiplication (28.40±0.12 shoots per explant), mean shoot length (28.94±0.14 mm), maximum number of roots (16.00±0.12 roots/shoot), and average root length (26.45±0.16 mm) was achieved on MS medium supplemented with 3.0 mg L⁻¹ BAP, and 0.5 mg L⁻¹ 2,4-D. Micropropagated plantlets were successfully transferred in sterilized mixture of soil : vermiculite (3:1) with 100% survival rate under field conditions. Regenerated plantlets were morphologically identical with mother plants. This system can be used for rapid mass clonal propagation of *Oxalis triangularis* A.st.-Hil, for commercial production, conservation strategies, and producing phytomedicines.

Keywords: 6-benzyl-aminopurine, dichlorophenoxy acetic acid, *in vitro* propagation, multiple shoots, plant growth regulator
Effect of N⁶-benzyladenine on in vitro shoot multiplication of *Iris collettii* Hook.f. and *I. domestica* (L.) Goldblatt & Mabb.

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Iris collettii Hook.f. and I. domestica (L.) Goldblatt & Mabb. are perennial plants that have a high potential for employing as new economic ornamental plants due to their beautiful flowers. However, these plants are typically propagated through seeds or bulbs which cannot achieve the large volumes of plant to the floriculture industry. Thus, to prevent the problem of smuggling these plants from their natural habitat, in vitro shoot multiplication method for both Iris species was developed. In vitro plantlets at the height of 5-6 cm were used as starting materials. Leaf bases at 1.5 cm in length were excised from starting materials and used as explants. At the eighth weeks of culture on Murashige and Skoog (MS) agar medium augmented with 0 (control), 1, 2, and 4 mg/L N⁶-benzyladenine (BA), leaf bases of I. collettii that cultured on MS medium supplemented with 1 mg/L BA showed the greatest shoot formation (3.13 shoots/explant and 2.31 cm). Whereas, I. domestica provided maximum new shoot numbers per explant (5.60) from leaf bases culturing on MS medium augmented with 2 mg/L BA. This study also found that the media containing BA resulted in shorter regenerated shoots of I. domestica when compared to the control. Root formation was not found in any treatments except I. domestica that cultured on BA-free medium. These results could be used as basic information for optimizing complete in vitro propagation protocol and germplasm conservation method of both Iris species in further study.

Keywords: Iridaceae, ornamental plant, N⁶-benzyladenine, in vitro propagation

Terpenoid Screening and *In vitro* Tissue Culture of the medicinal orchid, *Dendrobium fimbriatum* Hook

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Dendrobium fimbriatum Hook., one of the native orchids to Thailand, has shown to possess highly various important secondary metabolites which have been used in the traditional medicine. Interestingly, this orchid species having the terpenoid group, especially sesquiterpenoids, exhibits the anti-viral activities against SARS-Co V-2 (COVID-19). Preliminary studies of terpenoid screening and surface disinfection of the apical bud explants were carried out. Plant growth regulators (PGRs) affecting multiple shoot induction of D. fimbriatum was also conducted and described next. The apical buds were cultured on modified Vacin and Went (MVW) medium containing thidiazuron (TDZ) (0, 0.1, and 0.2 mgL^{-1}) in combination with 2,4-dichlorophenoxeacetic acid (2,4-D) (0 and 0.5 mgL^{-1}) for 8 weeks. The CRD experiment was designed with 6 replications. It was found that the presence of terpenoid could be noticed. A successful protocol for surface sterilization was recorded as follows: apical bud was dipped in 70% ethyl alcohol for 1 minute. After that, the pretreated bud was then surface-sterilized with 15% Clorox, 10% Clorox, and 0.1% mercuric chloride for 15, 10, and 5 minutes, respectively. This effective sterilization also provided 95% sterilized-, 70% survived-, and 25% brown explants after culture on MVW medium without PGR for 4 weeks. The Influence of PGRs on shoot induction was shown to be significantly different among the treatments. The maximum average shoot number (3.04±0.53 shoots/explant), the highest shoot (6.30±0.81cm/shoot), and the maximum average leaf number (8.04±0.65 leaves /shoot) were obtained from MVW medium supplemented with TDZ 0.1 mgL^{-1} .

Keywords: clonal propagation, COVID-19, medicinal plant, multiple shoots, sesquiterpenoids

Preservation of sweet potato (Ipomoea batatas) using slow growth techniques for genebank

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Sweet potato [*Ipomoea batatas* (L.) Lam] is an important food crop in Thailand and germplasm conservation is mostly in the field. The preservation of plant genetic resources by slow growth technique is a useful method for *in vitro* conservation of sweet potato genotypes that should be developed to save the important accessions of sweet potato germplasm for breeding and genebank. Four genotypes were used (PJ265-1, PJ0106-6, PJ65-3, and PJ284-17). They represent in the different pulp color with 3 experiments are induced by the concentrations of MS salts (1/2MS, 1/4 MS) and sucrose (30, 60, and 90 g/L), using plant growth regulator (ABA 0, 2, 4, 6, 8, and 10 mg/L) and using plant growth retardants (ancymidol 0, 5, 10, 15, and 20 μ). The survival (%) was evaluated every three months. The four genotypes of sweet potato were obtained over nine months by using 1/2MS medium plus 30 mg/L of sucrose, MS medium plus ABA 2-6 mg/L, and MS medium plus ancymidol 10 μ . *In vitro* plantlets should be sub-cultured after nine months.

Keywords: Ipomoea batatas, sweet potato, slow growth technique, preservation

Thidiazuron induces high-frequency plant regeneration from shoot tip explants of an ornamental aquatic plant, *Anubias barteri* var. *nana*

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Anubias barteri var. nana is a commercially important ornamental aquatic plant that has low multiplication rate and in vitro regeneration difficulties. Anubias barteri var. nana is widely used for making ornamental fishes healthier and decorating aquariums in more natural surroundings in aquaria. In the present study, a reproducible and highly efficient protocol for obtaining shoot organogenesis from shoot tip explants of an ornamental aquatic plant, Anubias barteri var. nana has been developed. Shoot tip explants were excised from 90-dayold plantlets and cultured on MS (Murashige and Skoog, 1962) medium supplemented with various concentrations of growth regulators namely 6-benzylaminopurine (BAP; 5.0, 7.0, and 9.0 mg L⁻¹), 6-furfurylaminopurine (Kinetin; 5.0, 7.0, and 9.0 mg L⁻¹), and thidiazuron (TDZ; 5.0, 7.0, and 9.0 mg L^{-1}). The highest frequency (100%) of multiple shoot formation with maximum number of shoots (4.50 shoots/explant), shoot length (9.50 mm), number of leaves (16.75 leaves/explant), leaf length (13.22 mm), leaf width (6.58 mm), root induction efficiency (100%), and number of roots (25.25 roots/explant) was achieved on MS medium supplemented with 7.0 mg L^{-1} TDZ. The regenerated complete plantlets were successfully acclimatized into small clay pots containing a rockwool under greenhouse conditions at 100% survival and grew vigorously without any morphological abnormalities during acclimatization in the greenhouse. This phytohormones and shoot tip explants based micropropagation can open up the route for in vitro clonal multiplication of this commercially important Anubias species.

Keywords: aquatic plant, Araceae, regeneration, thidiazuron, tissue culture

Micropropagation of an endangered medicinal Plant, ant-plant; *Myrmecodia tuberosa* Jack. for Conservation in Thailand

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Tubers of Myrmecodia tuberosa Jack. have been used in Thai traditional herbal medicine. Myrmecodia tuberosa Jack. (Rubiaceae) is an endemic epiphytic species which was found only in Southern part of Thailand. This plant has a mutualism relationship with ants so it is commonly called ant-plant. Ant-plant was traditionally used by local people as a medicine for several diseases for a century. Ant-plant contains important compounds such as glycosides, vitamins, minerals, flavonoids, tocopherols, polyphenols and tannins, which is very useful as an antioxidant and anti-cancer. However, Myrmecodia tuberosa Jack. in natural source have been decreased due to deforestation and forest smuggling for using as ornamental plant. It is rare in Thailand; therefore, availability of raw material is limited and an effective method of producing in vitro-derived plants for pharmaceutical reasons would be desirable. The stem segments (1.0 cm) were excised from 45-day-old aseptically seedlings of Myrmecodia tuberosa Jack. and cultured on MS (Murashige and Skoog, 1962) medium augmented with 6-benzylaminopurine (BAP) at different concentrations (0.0, 1.0, 3.0, and 5.0 mg L⁻¹) either singly or in combination with α -naphthalene acetic acid (NAA) (0.5 mg L⁻¹) for 90 days. The results revealed that the stem segments which were cultured on MS medium supplemented with 3.0 mg L⁻¹ BAP, in combination with 0.5 mg L⁻¹ NAA gave the highest frequency (100%) of multiple shoot formation with maximum number of shoots (86.25 shoots/explant), shoot length (8.50 mm), root induction efficiency (100%), number of roots (16.25 roots/explant), and root length (21.54 mm). The regenerated complete plantlets of Myrmecodia tuberosa Jack. were also successfully acclimatized under greenhouse conditions at 95% survival and grew normally within 45 days.

Keywords: ant-plant, anti-cancer, medicinal plant, plant growth regulators, plant tissue culture

In vitro shoot organogenesis in *Anthurium andraeanum* cv. HC 028-a high valued a cutflower and potted ornamental plants

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A high-frequency clonal propagation protocol was developed for *Anthurium andraeanum* cv. HC 028, a high valued a cut-flower and potted ornamental plants. Shoot tip explants of *Anthurium andraeanum* cv. HC 028 were cultured on MS medium fortified with different concentrations of BAP (1.0-5.0 mg L⁻¹) and Kinetin (1.0- 5.0 mg L⁻¹) individually for shoot induction. The highest frequency (100%) of multiple shoot regeneration with maximum number of shoots (12.90 shoots per explant) was noticed on MS medium supplemented with 4.0 mg L⁻¹ BAP after being culture for 60 days. Elongated shoots dissected out from the *in vitro* proliferated shoot clumps were cultured on MS medium fortified with various concentrations (1.0-3.0 mg L⁻¹) of auxins (NAA and IBA) for root induction. Highest frequency of rooting (100%) was noticed on MS medium augmented with 2.0 mg L⁻¹ IBA. The plantlets were successfully acclimatized in the greenhouse with more than 95% survival rate. Regenerated plantlets were morphologically identical with mother plants. The present in vitro propagation protocol would facilitate an alternative method for rapid and large-scale production of *Anthurium andraeanum* cv. HC 028 a cut-flower and potted ornamental plants.

Keywords: auxin, cytokinin, micropropagation, plant growth regulators, regeneration

In vitro propagation of the genus Actinidia Lindl. representatives

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The genus Actinidia Lindl. includes about 40 species. The in vitro Actinidia collection of Tsitsin Main Botanical Garden is represented by 42 cultivars of 3 species (A. kolomikta (Rupr. Et Maxim) Maxim., A. arguta Planch., and A. polygama (Sieb. Et Zucc.) Maxim.), most of them were bred by E.I. Kolbasina. The purpose of the study was to identify the features of regeneration of Actinidia species and their forms at the propagation stage. The methodology of the research was based on the generally accepted methods of plant tissue and organ culture. The nutrient medium, Quorin-Lepoivre (QL) was used with the addition of 0.0...1.0 mg·L⁻¹ 6-Benzylaminopurine as plant growth regulator. The determining value of characteristics of species and their forms (male, female, and bisexual) in the realization of morphogenetic potential was established. It was revealed that A. kolomikta was characterized by lower multiplication coefficient (5.0) compared to A. arguta (8.0) and A. polygama (7.7). This correlates with the growth features of these species in field collections. The female forms of A. kolomikta (multiplication coefficient 5.2) and A. arguta (7.0...9.0) had higher morphogenetic potential than the male and bisexual forms (4.3 and 6.3, respectively). This was not the same for A. polygama: its female form had lower multiplication coefficient than other forms. The efficiency of using QL medium with $0.5...1.0 \text{ mg} \cdot \text{L}^{-1}$ 6-Benzylaminopurine at the propagation stage for the studied species and forms was revealed.

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Keywords: Actinidia, in vitro collection, clonal micropropagation, morphogenetic potential

Response of Phalaenopsis species to different types and concentrations of cytokinin in vitro

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Phalaenopsis is one of the favorite genera in Orchidaceae family. In vitro culture is a very useful method for orchid propagation and conservation. The study evaluates the response of three species of Phalaenopsis to different types and concentrations of cytokinin. The plant materials used were in vitro shoot originated from seed protocorm germination. The experiment used a randomized complete block design with two factors. The first factor was the species of Phalaenopsis (P. cornu-cervi, P. amboinensis, and P. tetraspis). The second factor was the types of cytokinin (control without cytokinin, benzyl aminopurine 22.2 µM, kinetin 11.1 µM, and thidiazuron 11.1 µM). Murashige and Skoog (MS) medium supplemented with 15% coconut water were used as a basal medium. The results showed that the highest percentage of explant forming shoot on P. amboinensis (66.7%) and P. cornucervi (55.6%) were at kinetin 11.1 µM; while, P. tetraspis (66.7%) was in control (without cytokinin). The interaction between Phalaenopsis species and cytokinins did not significantly affect the number of shoots, leaves, and roots. However, in general, the 11.1 µM kinetin was the best treatment to produce the highest number of shoots and leaves in *P. cornu-cervi* and *P.* amboinensis, and roots in all of Phalaenopsis species. The 11.1 µM TDZ treatment gave the highest number of shoots and leaves on P. tetraspis. The kinetin 11.1 µM and TDZ 11.1 µM resulted in a better visual performance of shoot and leaf color than BAP treatments. From this we conclude that the genetic background experiment. is the main factor (Phalaenopsis species) to determine the explant response in vitro.

Keywords: shoot induction, multiplication, BAP, kinetin, thidiazuron

Session V: Physiology and Production (S5)

P21

The effect of dehydration on the functional state of the leaf apparatus in some apricot (*Prunus armeniaca* L.) cultivars

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The article presents the results of evaluating the activity of the photosynthetic apparatus in the leaf blades of some apricot (Prunus armeniaca L.) cultivars by the method of chlorophyll a fluorescence induction kinetics change. Two cultivars of Russian breeding (Yaltinets and Naslazhdenie) and one interspecific hybrid of apricot and cherry plum - Briol 38 were compared. A cultivar of foreign breeding - Vardaguin Vagdaas was used as a control cultivar. The photosynthetic activity of the leaves was studied under native conditions, as well as after artificial dehydration. The studies of chlorophyll fluorescence were carried out on a MINI-PAM II portable pulsed fluorimeter (Heinz Walz, Germany, 2017). The parameters of photosynthetic activity in the leaves were measured at time intervals of 4, 8, 12, and 24 hours. Water content, water holding capacity, water deficiency of leaves and turgor recovery rate were also considered. The loss of water in leaves per day was 22.2% for the cultivar Yaltinets, 23.9% for the cultivar Vardaguin Vagdaas, 29.4% for the cultivar Naslazhdenie and 34.3% for the hybrid Briol 38. The cultivar Yaltinets is superior to all studied cultivars in the ability of the photosynthetic apparatus to withstand dehydration and maintain high photosynthesis productivity. After 24 hours of leaves dehydration in Yaltinets cultivar, the index (Fm-Ft)/Fm decreased by 8.3%, Rfd by 31.2%, Fv and Fv/Fm remained unchanged, and Y(II) increased by 6.3 % The cultivar Vardaguin Vagdaas also demonstrated high resistance of PS II to arid conditions. An interspecific hybrid of apricot and cherry plum Briol 38 is tolerant to shortterm dehydration of leaf tissues, but longer dehydration leads to significant malfunctions and damages in PS II.

Keywords: drought tolerance, fluorescence, chlorophyll, turgor, water-holding ability

Assessment of drought tolerance in some peach cultivars by chlorophyll fluorescence induction method

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Water deficit stress has a significant effect on the growth and yield of peach plants. The aim of the study: to assess the effect of leaf tissue dehydration on the changes in chlorophyll fluorescence parameters and to identify cultivars highly tolerant to drought. The photosynthetic activity of the leaves was studied under native conditions, as well as after artificial dehydration. The studies of chlorophyll fluorescence were carried out on a MINI-PAM II portable pulsed fluorimeter (Heinz Walz, Germany, 2017). The investigations were made on leaves of four peach cultivars: 'Asmik' (Armenia), 'Zempush' (Azerbaijan), 'Naryadny Nikitsky' (Russia), and 'San Lorenzo' (Italy). The weight method was used to determine water content, water holding capacity and fluorescence indexes were calculated. The loss of water by the leaves per day was 34.5% in the 'San Lorenzo' cultivar, 35.4% in the 'Zempush' cultivar, 35.8% in the 'Asmik' cultivar and 40.5% in the 'Naryadny Nikitsky' cultivar from the initial weight of the samples. Despite the greatest water loss, 'Naryadny Nikitsky' cultivar demonstrated better activity of the photosynthetic apparatus under the stress, compared to the other studied cultivars, and maintained high production processes. After 24 hours of 'Naryadny Nikitsky' leaf dehydration, index Fv decreased by 23%, Y(II) by 31%, and Y(NPQ) increased by 23%. Indexes PA, F_v/F_m, Rfd and Y(NO) did not change. According to the results of the experiments, the cultivar 'Naryadny Nikitsky' was characterized by high adaptability of the photosynthetic apparatus to water stress. In addition, the cultivar 'Naryadny Nikitsky' was characterized by the highest levels of chlorophyll a + b (5.06 mg / g dry matter).

Keywords: Photosystem, water stress, Prunus persica.

Ex vitro acclimatization of Lavandula angustifolia Mill. plants

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The success of large scale micropropagation depends on the ability of the in vitro regenerated plants to acclimatize in the greenhouse and in the open field. The objective of this study was to determine the complex of structural and functional changes in regenerated plants of valuable Lavandula angustifolia cultivars under their acclimatization ex vitro. Rhizogenesis of lavender cultivars, 'Belyanka', 'Record', 'Prima', and 'Sineva' was induced on MS medium with 0.5-1.0 mg/L IBA or NAA. By histological analysis, it was found that the root regeneration started with the formation of a tiny morphogenic zone by division of cambium cells in the shoot. For plantlet adaptation, three types of relative air humidity regulation, such as culture vessel without an insulator, with a perforated insulator, and with an entire insulator were used. After 15 days, the insulators were removed in the second and the third types. Changes in morphological, anatomical, and physiological parameters of the vegetative organs after 7, 15, 30, 60, and 90 days of ex vitro culture were noted. In the leaf structure, an enhancement of mesophyll densening, leaf thickness increase, epidermis and cuticle thickness increase, and the formation of a number of non-glandular trichomes were noted. Significant increase in the water-holding capacity of the leaf blades subjected to almost equal water content of in vitro and ex vitro plants was shown. The maximum photochemical quantum yield and photosynthetic activity in the acclimatized plants after 30 days were the same as in the open field plants. Maximum intensity of transpiration was under in vitro conditions and it had negative correlation with the water-holding capacity of leaf tissues. The effective quantum yield was increased. After 90 days of plant growing in the greenhouse, their morphology and organ anatomy corresponded to those of the mother plants. The most favorable method for the control of the relative air humidity at the initial stages of the adaptation was the usage of perforated insulators for the first 15 days of ex vitro acclimatization.

Keywords: lavander, cultivar, in vitro micropropagation, ex situ adaptation, gas exchange, photosynthesis

Extending the vase life of cut chrysanthemum flowers with herbal extracts

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The chemical holding solution is widely used in extending the vase life of cut flowers. Some chemicals are unsafe and not ecologically friendly. This research was aimed to investigate the effect of water extracts of herbs on extending the vase life of cut chrysanthemum (Dendranthema grandiflora, yellow standard type) flowers. There were six treatments with and without 5% sucrose were applied to holding solution, including reverse osmosis (RO) water (control), 200 mg l⁻¹ 8-hydroxyquinoline sulfate (8-HOS), 5% sodium carbonate (SC), 200 mg l⁻¹ crude extract of *Mentha cordifolia* leaves, 200 mg l⁻¹ crude extract of *Eryngium foetidum* leaves, and 200 mg l⁻¹ crude extract of *Zingiber officinale* rhizomes. Cut chrysanthemum flowers were performed at 25±1°C and the changes of flower quality were determined every day for 20 days. The results showed that the longest vase life of flowers was 18.2 days with 8-HQS. The vase life of flowers holding in solution of E. foetidum, Z. officinale, SC, RO water, and M. cordifolia was 10.8, 9.2, 9.8, 6.2, and 5.4 days, respectively. The holding solution with sucrose did not significantly increase the vase life. The long vase life of cut flowers related to the high rate of solution uptake and relative fresh weight. These results indicated that the holding solutions with crude extract of E. foetidum and Z. officinale can be used to prolong the vase life of cut chrysanthemum flowers.

Keywords: water extract, sodium carbonate, 8-hydroxyquinoline sulfate, holding solution, Chrysanthemum flowers

Photosynthetic activity of olive leaves before and after treatment with an experimental mixture of pesticides

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The article presents the results of evaluation of chlorophyll fluorescence of leaf blades for cultivars of olive (Olea europaea L.) before and after pesticide treatment. Five cultivars were compared, namely 'Bidza', 'Kaliniot', 'Kokermadh I Berat', 'Nisiot', and 'Pulyazekin'. The photosynthetic activity of the leaves was studied under native conditions (Control), as well as after 24 (Variant 1) and 72 (Variant 2) hours exposure to an experimental mixture of chemicals consisting of the "Fufanon" insecto-acaricide (manufacturer and registrant - "Cheminova A/S" Denmark), a fungicide with an additional bactericidal action of "Fitolavin" (manufacturer and registrant - "Pharmbiomedservice", Russia.), and the "Falcon" fungicide (manufacturer and registrant - "Bayer" Germany). Under cultivation in open ground (ex situ) in the collection plantings of the Nikita Botanical Gardens, most active production processes were found in the 'Kaliniot' and 'Kokermadh I Berat' cultivars, as evidenced by the high coefficients of the maximum (0.80 and 0.74 relative units) and effective (0.47 and 0.41) photochemical quantum yield of PSII, photosynthetic activity (0.65 and 0.73), and fluorescence decay coefficient (1.86 and 2.55). After exposure to an experimental mixture of chemicals, no significant changes in the maximum and effective photochemical quantum yield of PSII, photosynthetic activity, and fluorescence decay coefficient were observed. This indicates the absence of a negative effect of chemicals on the activity of photosynthetic apparatus of the leaf in the olive cultivars used in the study.

Keywords: olives, cultivars, photosynthetic activity, fungicide, Olea europaea L.

Evaluation of cannabinoid content in different varieties of Cannabis indica L.

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Our study on planting ten varieties of *Cannabis indica* L. seeds (KKU-01, KKU-02, KKU-03, KKU-04, KKU-05, KKU-06, KKU-07, KKU-08, KKU-09, and KKU-10) obtained from Khon Kaen Provincial Health Office gave in various amounts of canabinoids content. The seeds of 8 males and 16 females were planting in a close system (the container cabin) by controlling soil material, light, water, and temperature. The cannabis plant flowers were collected after planting for 90 days to yield dry weight of plant 45 - 88 grams. The samples were subjected to determine for two major cannabinoids, Δ 9-tetrahydrocannabinol (THC) and cannabidiol (CBD) by high performance liquid chromatography (HPLC). The KKU-01, KKU-09, and KKU-10 yielded only THC at 7.55, 18.40, and 12.6 % from dried weight, respectively. While KKU-03 and KKU-08 produced mixtures of THC and CBD at 5.28 and 4.55 % from dried weight, respectively. In addition, KKU-05 and KKU-07 yielded only CBD at 8.33 and 6.70 % from dried weight, respectively.

Keywords: container farm, tetrahydrocannabinol, cannabidiol

Phytochemical and biological activities of extracts from *Impatiens sirindhorniae* Triboun & Suksathan

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Impatiens sirindhorniae Triboun & Suksathan is a succulent plant with pink-purple flowers in the family Balsaminaceae. It is a new endemic species to limestone mountains in Krabi and Suratthani provinces. It has many bioactive compounds. The purpose of this study was to investigate phytochemical compositions and biological activities in leaves of natural I. sirindhorniae, tissue cultured I. sirindhorniae, and I. balsamina L. extracted by ethanol and water. The results showed that the extracts of natural *I. sirindhorniae* presented higher numbers of phytoconstituents than tissue cultured *I. sirindhorniae* and *I. balsamina* L. Ethanol extracts of natural habitat I. sirindhorniae have the highest amount of total phenolic contents (752.94±9.56 mg GAE/ g extract) and total flavonoid contents (1468.84±50.58 mg Qu/ g extract). Furthermore, the ethanolic extracts of natural I. sirindhorniae showed the strongest antioxidant activity with IC₅₀ value 6.49 μ g/ml.; tyrosinase inhibitory activity with IC₅₀ value 12.01 μ g/ml and xanthine oxidase inhibitory activity with IC₅₀ value 24.16 μ g/ml. The natural I. sirindhorniae and tissue cultured I. sirindhorniae extracts presented higher biological activities than I. balsamina L. This study revealed that I. sirindhorniae may be a source of natural antioxidant, anti-tyrosinase, and anti-xanthine oxidase compounds which can be used in pharmaceutical and cosmetic formulations following further investigation.

Keywords: Impatiens sirindhorniae, phytochemical, antioxidant activity, enzyme inhibition

The influence of canopy on the amount of light under the canopy shade

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Thailand is in a hot climate. As a result, Thailand has high temperatures and strong sunlight all year round which tends to have more heat in Thailand every year. The strong sunlight can burn the body's skin, so most people avoid direct exposure to sunlight. Therefore, most people opt for air conditioning to help alleviate hot weather. For landscape architecture, it is found that using shade of trees to reduce the intensity of sunlight and the heat from the sun is effective. Therefore, planting trees for shade is essential to reduce the amount of sunlight and direct heat from the sun. It will also reduce the heat accumulation of the concrete walls of the building, which makes the building cool all day and will save on electricity bills. Planting trees for shade is a great way to protect them from the heat of the sun.

This research project is interested in studying the influence of tree canopy on the amount of light under the canopy by selecting 60 kinds of ornamental trees planted in the park for two groups: perennial and shrub. Light intensity measurements under a canopy are performed using the LI-250A light meter. The experiment conducted a completely randomized design (CRD) with three replications.

The results of the experiment showed that the intensity of the light under the canopy of the tree group compared to the light intensity outside the canopy. It was found that the perennial group had the highest light intensity under the canopy at 48.64%, namely *Tabebuia argentea* Britton and the lowest light intensity under the canopy at 1.04%, namely *Melodorum fruticosum* Lour. The group of shrubs had the highest light intensity under the canopy at 9.14%, namely the *Tecoma stans* (L.) Kunth, and the lowest light intensity under the canopy at 3.32%, namely the *Dracaena cochinchinensis* (Lour.) S.C. Chen. This information will make it easier for interested people who plant shade trees to have information in deciding on tree types for shade.

Keywords: canopy, ornamental plants, canopy shade, light intensity

Session VI: Plant Molecular Research (S6)

P30

Development of transient expression system for gene functional analysis in Setaria viridis

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An efficient transient expression system to enable functional gene analysis was developed in *Setaria viridis*, a new C4 model plant. A promoter deletion analysis of C4 phosphoenolpyruvate carboxylase (PEPC) was used to demonstrate the protoplast transient expression system mediated by polyethylene glycol (PEG). Protoplasts were isolated from young leaves of *S. viridis*. A series of promoter deletion fragments ranging in size from 243 bp to 859 bp were fused to firefly luciferase and co-transfected with Renilla luciferase. Luciferase levels were used to determine promoter activities based on the ratio of firefly/Renilla to normal cell number and transfection efficiency. Luciferase activities of the longer PEPC promoter constructs exhibited higher promoter activities. Results revealed potential uses of the protoplast transient expression system for promoter analysis in *S. viridis*.

Keywords: green foxtail, protoplast, promoter analysis

Molecular genetic diversity of *Lavandula* × *intermedia* Emeric. ex Loisel. in the Nikita Botanical Garden's collection detected by microsatellite markers

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The prerequisites for the successful conservation and use of various species and cultivars of agricultural crops is the identification and control of their genetic variability, for the studies of which various methods are used, including methods of molecular genetic analysis. Currently, a highly productive hybrid Lavandula angustifolia Mill. and L. latifolia Medic. — lavandin (L. × intermedia Emeric. Ex Loisel.) is actively used in the production of lavendin essential oil. In the Nikita Botanical Gardens, breeding works have been carried out for this crop and 10 cultivars have been originated. In this regard, the aim of the presented work was to search for marker systems for the determination of intervarietal polymorphism of lavandin using microsatellite DNA loci. DNAs were isolated from young leaves of three lavandin cultivars 'Rabat', 'Temp', and 'Snezhnyi Bars'. Amplification was made with primers LAF, LAL, LINT, and LAB. A total of 486 alleles (270 alleles of the LAF series, 91 - LINT, 83 - LAB, and 42 - LAL) were identified in the studied group of lavandin genotypes according to 45 SSR markers, from 30 to 2000 bp. The markers LAB 009, LAB 029, LAB 030, LAB 039, LAB 042, and LAB 051 did not form an amplification product. The number of alleles per locus for the rest of the markers varied from 1 (LAB 014, LAB 050) to 11 (LAF 14). The maximum degree of polymorphism was observed for the loci LAF 5, LAF 10, LAF 14, LAF 16, LAF 19, LAL 2, LAB 10, and LAB 62.

Keywords: lavandin, cultivar, DNA, SSR, diversity

Development of microsatellite markers for Trema micrantha, species of tropical America

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The reduction of forest areas poses a risk of biodiversity loss for the planet, affecting the genetic diversity for tree species. Microsatellite markers have proved to be excellent tools for to investigate genetic diversity and population structure, and to provide support for natural population management and conservation genetics. The purpose of this study was to develop the first set of microsatellite markers for Trema micranta (L.) Blume, a species of tropical America. Sixteen microsatellite loci were isolated from a microsatellite-enriched genomic library and used to characterize 30 individuals of T. micrantha collected in a natural population in the state of Amazonas. Genetic diversity was determined by the number of alleles per locus, observed and expected heterozygosity and the test for Hardy-Weinberg Equilibrium. The allele number of the polymorphic loci ranges from 2 to 6 with an average of 3.13 per locus. The observed and expected heterozygosity ranged from 0.11 to 1.000 (mean 0.31) and 0.10 to 0.71 (mean 0.33), respectively. The investigated loci displayed high polymorphism for the T. micranta population. The information derived from the microsatellite markers provides support to the implementation of several conservation strategies for this species with the goal of preserving their genetic diversity and evolutionary process for the future.

Keywords: Cannabaceae, molecular markers, pioneer tree, medicinal tree, conservation

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Session VII: Plant Protection (S7)

P34

Paenibacillus polymyxa reduces leaf spot disease caused by *Cersocpora* sp. and has potential to promote growth in green oak lettuce (*Lactuca sativa* var. *crispa*) in the commercial hydroponic farm

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Cercospora sp. caused leaf spot disease in green oak lettuce (*Lactuca sativa* var. *crispa*) grown hydroponically in Phetchaburi, Prachuap Khiri Khan and Rayong, Thailand. *Paenibacillus polymyxa* (isolate 4), which was also reported to inhibit the mycelial growth of *Pythium helicoides*, was effective in inhibiting the mycelial growth of *Cercospora* sp. on potato dextrose agar (PDA). The simple culture of *Paenibacillus polymyxa* (isolate 4) was prepared and tested with green oak lettuce grown in the nutrient film technique (NFT) in the commercial farm. The bacterial culture not only reduced the severity of leaf spot disease caused by *Cercospora* sp., but also statistically increased dry weight of root when the bacterium was applied at 1:100 proportion (v/v, bacterial culture/nutrient solution). The application of this bacterium also potentially impacts positively the fresh weight of shoot and root, as well as the dry weight of shoot.

Keywords: simple culture, *Paenibacillus polymyxa*, *Cercospora* sp., antagonism, growth promotion

Bacillus megaterium promotes both growth of seedlings in the laboratory and nodulation of the yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis*) in the pot under outdoor condition

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The culture of *Bacillus megaterium* was prepared using simple culture technique in the laboratory. The aerated culture of this bacterium using Knorr® as the medium yielded the bacterium at 1.3×10^5 CFU/mL; while, the bacterial culture using shaken method produced 1.2×10^2 CFU/mL after four days of cultivation. This simple preparation of the culture was used to soak the seeds of the yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis*). In the sand tray test in the laboratory, *B. megaterium* had potential to promote growth of the yardlong bean when the dried biomass of the seedlings was assessed. Seeds of the yardlong bean were soaked with the bacterial culture before they were sown to the growing medium in the pots. The bacterial culture was applied to the seedlings of the yardlong bean by drenching the growing medium under outdoor condition. Based on observation, the bacterium induced early flowering and increased yield of the yardlong bean. The yardlong beans which received the bacterial culture through drenching had number of nodules higher than other treatments with statistically significant difference. Diluted aerated-culture of *B. megaterium* has potential for improving growth and yield of yardlong bean in the farm.

Keywords: Bacillus megaterium, nodulation, Vigna unguiculata subsp. sesquipedalis

Plant host selection for spore production of Glomas mosseae

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Five species including *Ipomoea aquatica*, *Triticum aestivum*, *Oryza sativa*, *Zea mays* and *Vigna radiata* were used as plant hosts for spore production of *Glomas mosseae*. Seeds of all plant species were surface sterilized and grown in sterilized growing medium with a ratio of 1-part soil to 8-part sand. Each pot of each plant species was inoculated with 30 spores of *G. mosseae* and compared with the control without inoculum. Each treatment consisted of five replicates containing four pots. Half-strength Hoagland solution was applied once a week and plant growth parameters including diameter, height, and number of leaves were recorded every week for 2 months. Spores of *G. mosseae* in the rhizosphere, root colonization and plant dry mass were measured after 2 months of inoculation. Results showed that *G. mosseae* improved plant growth of all plant species, with maximum increase in plant diameter found in *Z. mays*, while maximum effects on plant height and root dry mass were observed in *V. radiata*. Results of spore formation suggested *Z. mays* as the most suitable host for mass spore production with highest spore numbers of 97.4 per 100 g soil; however, no significant differences in root colonization were shown among the five plant species.

Keywords: mycorrhizal fungi, host preference, mass spore production, pot culture

Management based on POLC framework to promote the adoption and use of beneficial bacterium *Bacillus megaterium* for growers in Thailand

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Empowering the groups of people and organizations involves (1) planning (2) organizing (3) leading, and (4) controlling. These four steps process is called POLC framework which will be applied to manage the group of growers in Thailand. There are four main stakeholders, such as target growers, local agricultural staffs, Sub-district headmen, and researchers. In this study, there are nine target growers, three local agricultural staffs, two Sub-district headmen, and four researchers who have participated in the project to promote the adoption and use of beneficial bacterium Bacillus megaterium in horticultural crops, such as vegetables and fruits, based on POLC management framework in two provinces, Uttaradit and Phetchaburi. A purposive sampling technique was employed to select these nine growers. The interview was conducted using in-depth interview and focus group from January to December 2019. The data from the in-depth interview and the focus group was used to score the relative role of each stakeholder in the project and describe the key activities in which each stakeholder must carry out under POLC framework accordingly. Based on the score of a relative role of each stakeholder, the target growers played the most active leading role. The score also corresponded with the number of the key activities. The local agricultural officers are the "change agent", whilst the researchers are the catalyst assisting the "change agent". The sub-district headmen are the facilitator of the project. For a project to succeed, there must be a common understanding of the plan, stakeholder roles, and approach to management of the process.

Keywords: Management, POLC framework, *Bacillus megaterium*, fungal diseases in vegetable crops

Evaluation of processing tomato cultivars for resistance against tomato yellow leaf curl Thailand virus and high yield production

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Tomato Yellow Leaf Curl Thailand Virus (TYLCTHV), which is caused by whiteflytransmitted begomoviruses, is very destructive and causes heavy losses in tomato yields, especially in sub-tropical and tropical regions including Thailand. This research aimed at evaluating the yields of 28 tomato varieties and their resistance to TYLCV. The varieties used were collected from the Plant Breeding Center for Sustainable Agriculture at the Faculty of Agriculture of Khon Kaen University in Thailand. The study was conducted under drip irrigation at the Vegetable Research Station of the Department of Horticulture, Faculty of Agriculture at Khon Kaen University during the period of October 2019 to February 2020. Screening against TYLCTHV was carried out under both induced natural disease conditions and whitefly mediated inoculation, with symptoms that ranged from symptomless to very severe infections. The study indicated that four cultivars had shown the highest yields per plant, and these consisted of KKU-T278, KKU-T193, KKU-T233, and KKU-T204 (3,265.7, 3,135.1, 2,926.3, and 2,898.2 grams, respectively). Under the natural disease conditions, five cultivars appeared symptomless until the fruit maturation stage. The results for resistance were classified into 2 groups based on their responses to TYLC-THV. The first group (i.e., KKU-T224, KKU-T249, and KKU-T276) were identified as being 'Resistant' (R); while, the second group (i.e., KKU-T245 and KKU-T233) were identified as having a 'High resistance' (HR).

Keywords: whitefly, high resistance, drip irrigation

Morpho-biological features of oriental persimmon pollen

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It is known that a large number of persimmon cultivars are characterized by parthenocarp; however, the palatability of the fruit improves, and the yield increases significantly with cross-pollination. The main aim of this research is to study the viability of pollen of polygamous persimmon genotypes and to determine the ratio of normally developed and giant pollen grains in the analyzed samples. The studying results of the structural features for the pollen of five persimmon cultivars of the Nikita Botanical Gardens selection (Yuzhnaya Krasavitsa, Yaltinskaya, Mechta, Prelestnaya, Zolotistaya) growing in the gene collection, which is located in the soil and climatic conditions of the Southern coast of the Crimea, made it possible to reveal a significant difference between the degree of germination of pollen grains. On an artificial environment containing 20% aqueous solution of sucrose, the maximum level of this indicator was recorded for Yuzhnaya Krasavitsa cultivar - 33.7%, and the minimum - 21.5% for Zolotistaya cultivar. A similar tendency is observed in the process of germination of grains and the formation of pollen tubes on a medium with 15% sucrose solution: cultivar Yuzhnaya Krasavitsa - 22.4% (maximum), cultivar Zolotistaya minimum - 21.5%. Depending on the genotype, the diameter of pollen grains varies from 29.73 µm (Yuzhnaya Krasavitsa) to 68.13 µm (Yaltinskaya). In the pollen samples of four genotypes, giant grains were found: Yalta - 8.0%, Mechta - 7.9%, Prelestnaya - 9.8%, Zolotistaya - 15.4%. On the basis of the obtained results, diagrams were constructed that reflect the specifics of the distribution of pollen grains depending on their diameter. The use of the analyzed persimmon genotypes as pollinators is not effective due to the low degree of viability of the pollen that they form. The presence of unreduced pollen (giant grains) in the cultivars Yaltinskaya, Mechta, Prelestnaya and Zolotistaya allows them to be used in the breeding process to obtain new polyploidy seedless hybrids.

Keywords: persimmon, pollen, vitality, cultivar

Influence of edaphic conditions on the productivity of eastern Persimmon

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The dependence degree of the eastern persimmon productivity on the edaphic conditions of the southern coast of the Crimea has been studied. Experimental areas were laid on the territory of the Nikita Botanical Gardens and Koreiz. The study of the architectonics of the root system of persimmon plants performed by the "cut" method according to V.A. Kolesnikov, showed that the roots have mastered almost the entire soil profile to the depth of the dense rock. At the same time, up to 80% of the root cuts, both conducting and absorbing, were localized in the meter layer of the soil profile. The analysis of the research results showed that in the experimental areas where normally developed persimmon trees grow, which characterized with high fruit yields, the degree of density of the fine earth ranged from 1.21 to 1.67 g/cm³, and under a group of oppressed trees - from 1.35 to 1.70 g/cm³. An increase in the density of fine soil and a decrease in soil porosity leads to oppression of trees and a decrease in the productivity of persimmon plantations. It was revealed that the reserves of fine earth in a meter layer of soil in the studied areas under normally developed persimmon trees averaged 15 thousand t/ha, humus reserves - 279 t/ha; under trees in a depressed state - 6 thousand t/ha and 178 t/ha, respectively. The granulometric composition of the fine-loamy part of the analyzed soils ranged from heavy loamy to light-loamy with a predominance of silty fractions (up to 31.7%). The reaction of the aqueous soil suspension is alkaline, pH is in the range of 7.80-8.50. The average CaCO3 content in a meter layer of soil in areas with normally developed trees is low - from 0.3 to 5.0%, under oppressed plants - the soil is highly carbonate (up to 55%). The soils in all experimental plots, regardless of the state of the persimmon trees, are low-humus, the humus content is not more than 3.3%. The study of the reaction of persimmon plants to the properties of brown planted soils on the southern coast of Crimea made it possible to identify the main factors influencing the productivity of plantations: heavy granulometric composition, high density of fine earth, low humus reserves and high carbonate content.

Keywords: persimmon plants, edaphic conditions, root system, productivity