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III INTERNATIONAL GREEN BIOTECHNOLOGY CONGRESS

30th September-2nd October

Sarajevo, Bosnia and Herzegovina

BOOK OF ABSTRACTS

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Institute for Genetic Engineering and Biotechnology, University of Sarajevo
Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
www.ingeb.unsa.ba
Phone: +387 33 220-926
Fax: +387 33 442-891
ingeb@ingeb.unsa.ba

Welcoming note,

Dear colleagues,

When University of Sarajevo - Institute for genetic engineering was awarded the honour and privilege to organize the Third International Green Biotechnology Congress, little did we know that the world would experience such a thorough transformation? Once the COVID-19 crisis hit the world, we held our breaths and got ready to wait it out. However, the weeks turned into months and it became obvious that if we were to continue with our plans we needed to adjust.

With conviction that biotechnology would deliver anti-COVID-19 solutions on time, we ventured into organization of the Third International Green Biotechnology Congress as a hybrid event. By the final submission deadline, approximately one third of the participants expressed their desire to visit Sarajevo.

Within the light of global biotechnology rise, the great triumph of science continues, global leaders are striving to more greener governance in order to solve global warming crisis as huge societal challenge ahead. We as scientific community again are expected to deliver new solutions and make a solid stand for future innovations in green biotechnology production.

Finally, 44 abstracts passed review process successfully; 28 of those were by international participants. Substantial portion of the abstracts were submitted by students, which signifies that the time of green biotechnology is yet to come. In addition, we are particularly grateful to our 13 invited speakers, who have taken time to share their views and offer guidelines for the future.

For the Congress planning and realization we have to acknowledge our longstanding supporters and partners, the Federal Ministry of Science and Education, and companies Mikro+Polo d.o.o. Sarajevo and Biosistemi d.o.o. Sarajevo.

This special issue of *Genetic & Applications* is completely dedicated to the contents presented at the Congress. It includes the abstracts of both oral and poster presentations. The select few, innovative papers are published *in extenso* for your perusal.

Dr sc Kasim Bajrović,

Organizing Committee Chairman

Dr sc Lejla Pojskić,

Scientific Committee Chairman

SCIENTIFIC PROGRAMME

Moderator: *Lejla Pojskić*

Thursday, 30th September (CEST 8:30 – 16:30)

08:30 - 09:00	Virtual lobby meeting	
09:00 - 12:00	Online and on-site registrations, technical preparations, uploading presentations, technical check-ups	
12:00 - 12:50	Opening ceremony - INGEB garden cocktail	
12:50 - 13:10	**OPENING REMARKS** **Kasim Bajrović**, Chairman of the Organizing Committee / **Lejla Pojskić**, Chairman of the Scientific Committee **Rifat Škrijelj**, Rector of the University of Sarajevo	
Keynote lectures I		
Chaired by **Ahu Altinkut Uncuoglu** (*Marmara University, Turkey*)		
13:10 - 13:40	**Atanas Atanassov** *(JGC, Sofia, Bulgaria*):	**TOWARDS SUSITAINABLE AGRICULTURE VIA MODERN TECHNOLOGIES AND APPROACHES**
13:40 - 14:10	**Majeti Narasimha Vara Prasad** (*University of Hyderabad, India*):	**INTERNET OF THINGS [IOT] IN ENVIRONMENTAL BIOTECHNOLOGY AND MONITORING**
14:10 - 14:40	**Zahide Neslihan Öztürk Gökçe** (*Niğde Ömer Halisdemir University, Turkey*):	**TWENTY YEARS OF SHAKY RIDE TO ABIOTIC STRESS TOLERANCE**
14:40 - 15:10	**Yelda Özden Çiftçi** (*Gebze Technical University, Kocaeli, Turkey*):	**THE EFFECT OF MICRORNAS ON THE POST-RECOVERY SUCCESS OF *ARABIDOPSIS THALIANA* CRYOPRESERVATION**
15:10 - 15:50	Coffee/Tea break & Poster Viewing	
	Oral presentations - PLANT BIOTECHNOLOGY	
15:50 - 16:10	**Mirna Ćurkovic-Perica** (*University of Zagreb, Croatia*):	**EXTRACELLULAR LACCASE ACTIVITY OF CRYPHONECTRIA HYPOVIRUS 1 – INFECTED CHESTNUT BLIGHT FUNGUS**
16:10 - 16:30	**Nihal Öztolan Erol** (*Sabancı University, Turkey*):	**GENETIC CHARACTERIZATION OF POLYCARPELLARY TRAIT IN A TURKISH ENDANGERED LEGUME SPECIES (*VURALIA TURCICA*)**

Friday, 1st October (CEST 8:30 – 15:30)

08:30 - 09:00	Virtual lobby meeting	
	Keynote lectures II	
	Chaired by **Jelena Samardžić** (*University of Belgrade, Serbia*)	
09:00 - 09:30	**Gavin Sathianathan** (*London, United Kingdom*)	**A REVIEW OF EUROPEAN EFFORTS TO LEGALISE AND INTEGRATE MEDICAL CANNABIS INTO HEALTHCARE PROGRAMS**
09:30 - 10:00	**Fuad Gaši** (*University of Sarajevo, Bosnia and Herzegovina*):	**APPLICATION OF MOLECULAR TECHNIQUES IN SOLVING IMMEDIATE PROBLEMS OF FRUIT PRODUCERS**
	Oral presentations - GENETIC RESOURCES AND BIOTECHNOLOGY	
10:00 - 10:20	**Stuart J Lucas** (*Sabanci University, Turkey*):	**DESIGNING MOLECULAR MARKERS FOR VARIETY MONITORING AND QUALITY CONTROL IN EUROPEAN HAZELNUT**
10:20 - 10:40	**Helena Brekalo** (*University of Mostar, Bosnia and Herzegovina*):	**CHEMICAL COMPOSITION AND SUGAR CONTENT IN FRESH FRUITS OF WILD POPULATIONS *ROSA CANINA* L. IN HERZEGOVINA**
10:40 - 11:00	**Adrijana Filipović** (*University of Mostar, Bosnia and Herzegovina*):	**ALTERNATIVE POSSIBILITIES OF SEED POTATO DORMANCY CONTROL**
11:00 - 11:45	Coffee/Tea break & Poster Viewing	
	Keynote lectures	
	Chaired by **Fuad Gaši** (*University of Sarajevo, Bosnia and Herzegovina*)	
11:45 - 12:15	**Erna Karalija** (*University of Sarajevo, Bosnia and Herzegovina*):	**ENHANCED PLANTS STRESS TOLERANCE BY SEED PRIMING**
12:15 - 12:45	**Jelena Samardžić** (*University of Belgrade, Serbia*):	**SAY SI TO SILICON IN PLANT NUTRIENT STRESS**
	Sponsor's presentation	
12:45 - 13:00	**Igor Pongrac** (*Merck Life Sciences/Research Solutions, Zagreb, Croatia*):	**SUSTAINABLE LAB FOR THE FUTURE WITH MERCK GREEN SOLUTIONS FOR NUCLEIC ACID EXTRACTION AND STERILE FILTRATION**
13:00 - 14:30	Lunch break & Poster viewing	
	Oral presentations - TISSUE CULTURE AND NANOMATERIALS	
14:30 - 14:50	**Tuğba Özgören Can** (*Marmara University, Istanbul, Turkey*)	**BIONANOPARTICLE APPLICATIONS AND THEIR IMPACT ON GROWTH PARAMETERS OF *HELIANTHUS ANNUUS* L.**
14:50 - 15:10	**Ilayda Goktepe** (*Istanbul University, Turkey*)	**SCREENING OF ANTICANCER TAXANES IN TURKISH HAZELNUT SUSPENSION CULTURES**
15:10 - 15:30	**Zeynep Akpak** (*Marmara University, Turkey*)	**CALLUS INDUCTION FROM UNDEVELOPED OVULES OBTAINED THROUGH INTERGENERIC HYBRIDIZATION BETWEEN *PHASEOLUS VULGARIS* AND *VURALIA TURCICA***

Saturday, 2nd October (CEST 8:30 – 15:00)

08:30 - 09:00	Virtual lobby meeting	
	Keynote lectures III	
	Chaired by **Lilijan Sulejmanović** (*London, United Kingdom*)	
09:00 - 09:30	**Zoran Popovski** (*Ss. Cyril and Methodius University, N. Macedonia*)	**GENETIC RESHAPING OF INDUSTRIAL MICROORGANISMS - INEXHAUSTIBLE POTENTIAL FOR NEW PRODUCTS AND CLEANER TECHNOLOGIES**
09:30 - 10:00	**Aydin Yildiz** (*Marmara University, Turkey*):	**ROLE OF DOUBLED HAPLOIDS IN CROP IMPROVEMENT**
10:00 - 10:30	**Mehmet Cengiz Baloglu** (*Kastamonu University, Turkey*):	**CRISPR GENOME EDITING TECHNOLOGY AND APPLICATIONS IN PLANTS: RECENT PROGRESS, CHALLENGES AND PROSPECTS**
10:30 - 11:20	Coffe break & Poster viewing	
	Oral presentations - ENVIRONMENTAL BIOTECHNOLOGY AND MONITORING	
11:20 - 11:40	**Özlem Akkaya** (Gebze Technical University, Turkey):	**RHIZOREMEDIATION OF 2,4-DNT-CONTAMINATED SOILS: A MODEL SYSTEM FOR BENEFICIAL PLANT-BACTERIA INTERACTIONS**
11:40 - 12:00	**Tomica Mišljenović** (*University of Belgrade, Serbia*):	**OPPORTUNITIES FOR NI PHYTOMINING IN SERBIA: EXTRACTION OF NICKEL SALTS FROM THE HYPERACCUMULATING PLANT *ODONTARRHENA MURALIS***
12:00 - 13:30	Lunch break & Poster viewing	
	Keynote lectures	
	Chaired by **Lejla Pojskić** *(University of Sarajevo, Bosnia and Herzegovina)*	
13:30 - 14:00	**Lilijan Sulejmanović** (*London, United Kingdom*):	**BIOTECHNOLOGY INNOVATION STRATEGY - UK EXAMPLE**
14:00 - 14:30	**Ahu Altinkut Uncuoglu** (*Marmara University, Turkey*):	**UNIVERSITY-INDUSTRY COLLABORATIONS AND ENTREPRENEURIAL ACTIVITIES IN PLANT BIOTECHNOLOGY**
	Oral presentations - MICROBIOME POTENTIALS	
14:30 - 14:50	**Omar Zennouhi** (*Moulay Ismail University of Meknes, Morocco*):	**DIVERSITY OF RHIZOBACTERIA ISOLATED FROM ROOT NODULES OF *BITUMINARIA BITUMINOSA* (L) STIRTON, A LEGUMINOUS SHRUB NATIVE OF MEDITERRANEAN AREAS**
14:50 - 15:00	**Congress closing**	
	Rifat Hadžiselimović *(University of Sarajevo, B&H)*	**CLOSING REMARKS**

Poster presentations

I PLANT BIOTECHNOLOGY

Juraj Kleman (Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Nitra, Slovakia): EFFECTS OF STRIGOLACTONES IN RELATION TO PARASITIC PLANT WEEDS

Veronika Mikitová (Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Nitra, Slovakia): ISOLATION AND CHARACTERIZATION OF MYROSINASE GENE FROM THE HOARY CRESS (*CARDARIA DRABA*)

Sinan Meriç (Istanbul Kultur University, Faculty of Science and Letters, Department of Molecular Biology and Genetic, Istanbul, Turkey): INDUCTION OF HAIRY ROOT CULTURES OF Calendula officinalis L. by Agrobacterium rhizogenes MEDIATED GENETIC TRANSFORMATION AND PRODUCTION OF SECONDARY METABOLITES

Belmina Šarić (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): INHIBITORY EFFECTS OF DIFFERENT CONCENTRATIONS OF LAURUS NOBILIS L. ESSENTIAL OIL ON GROWTH OF PATHOGENIC MICROORGANISMS

II TISSUE CULTURE AND NANOMATERIALS

Melike Tepe (Gebze Technical University, Kocaeli, Turkey): PROPAGATION OF HAIRY ROOTS AND CALLI IN SETIS BIOREACTOR

Erva Bacacı (Gebze Technical University, Department of Molecular Biology of Genetics, Kocaeli, Turkey): HAIRY ROOT CULTURE OF VETIVER GRASS

III ENVIRONMENTAL BIOTECHNOLOGY AND MONITORING

Inna I. Horiunova (Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine,, Kyiv, Ukraine): INFLUENCE OF VANADIUM ON MICROTUBULE ORGANIZATION IN *ARABIDOPSIS THALIANA* ROOT CELLS

Svitlana Plokhovska (Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kyiv, Ukraine): NITRIC OXIDE PARTICIPATION IN PLANTS PROTECTIVE EFFECT UNDER HEAT STRESS

Arnela Okić (University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina): GOING GREEN WITH 3D PRINTING: A CASE REPORT OD REDUCING AND REUSING PLASTIC WASTE IN A PLANT PATHOLOGY LABORATORY

Georgieva Dimitrina (Sofia University „St.Kliment Ohridski", Sofia, Bulgaria): SARS-COV-2 VIRUS MONITORING OF WASTEWATER IN BULGARIA

Jasmin Adrović (University of Tuzla, Faculty of Natural Sciences and Mathematics, Tuzla, Bosnia and Herzegovina): CHROMOSOMAL ABERRATIONS AND NUCLEAR ANOMALIES IN ROOT TIP CELLS OF *ALLIUM CEPA* L. CAUSED BY RADON IN WATER

Mujo Hasanović (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): DNA DAMAGE IN PLANTS – SEASONAL VARIATIONS AND LEAF POSITION EFFECTS

Mujo Hasanović (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): PHYSIOLOGICAL ANALYSIS OF *SANGUISORBA MINOR* SCOP. POPULATIONS FROM VARIOUS SOIL TYPES INDICATES EFFECTIVE SURVIVAL MECHANISMS

IV GENETIC RESOURCES AND BIOTECHNOLOGY

Semina Hadžiabulić (Univerzitet „Džemal Bijedić” u Mostaru, Agromediteranski fakultet, Mostar, Bosnia and Herzegovina): EVALUATION OF AUTOCHTHONOUS APPLE VARIETIES (*MALUS DOMESTICA*) IN THE AREA OF TOMISLAVGRAD

Sezgin Mengi (Sabanci University, *Faculty of Engineering and Natural Sciences* İstanbul, Turkey): CHARACTERIZATION OF GENOMICS DATA OF *VURALIA TURCICA* AND UNDERSTANDING ITS POLYCARPELLARY STRUCTURE

Mirzeta Memišević Hodžić (University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina): GENE INTROGRESSION OR GENETIC CONTAMINATION - THE CASE OF BLACK POPLAR (*Populus nigra* L.) IN BOSNIA AND HERZEGOVINA

Selma Vejzagić (University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina): GENETIC CHARACTERIZATION OF THE LAST NATURAL TREES OF THE GENUS *TILIA* IN ČAPLJINA IN THE METAPOPULATION ČAPLJINA-TOMISLAVGRAD IN FRAGMENTED LINDEN HABITATS IN THE CONTEXT OF CLIMATE CHANGE

Madhav K Bhatt (Nirma University, Gujarat, India): PRODUCTION OF BIOSURFACTANT BY FUNGI ISOLATED FROM SOIL

Helena Brekalo (University of Mostar, Faculty of Agriculture and Food Technology, Mostar, Bosnia and Herzegovina: OVERVIEW AND USEFUL VALUE OF WILD PLANTS OF THE CITY OF MOSTAR

Hilal Civelek (Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey): CRISPR/Cas9 MEDIATED SILENCING OF CCDC124 GENE IN *SOLANUM LYCOPERSICUM*

Mariya Borova (Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kyiv, Ukraine): Antibody-conjugated luminescent Ag2S quantum dots for imaging of plant cytoskeleton

Miroslav Klobucnik (Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Nitra, Slovakia): MOLECULAR INSIGHT INTO THE GENETIC STRUCTURE AND THE GENOMIC ARCHITECTURE OF *PINUS SYLVESTRIS* X *MUGO* CONTACT ZONES IN SLOVAKIA

Tamara Ćetković (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): CYTOTOXICITY OF *ARTEMISIA ANNUA* L. COMMERCIAL ETHANOLIC EXTRACT IN NORMAL AND CANCER CELL LINES: CONTRIBUTION TO PHARMACEUTICAL APPLICATION

Nil Türkölmez (Gebze Technical University, Kocaeli, Turkey): MOLECULAR DIFFERENTIATION OF OLIVE CV. KARAMÜRSEL SAMANLI FROM OTHER TURKISH CULTIVARS VIA ISSR MARKERS

V ADVANCED TECHNOLOGIES AND MATERIALS

Ivana Nikolić (University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia): TARGETED MUTAGENESIS OF INTRINSICALLY DISORDERED PROTEIN ATDSS1(V) USING CRISPR-CAS9 APPROACH

Dženan Kovačić (International Burch University, Faculty of Engineering and Natural Sciences, Department of Genetics and Bioengineering, Sarajevo, Bosnia and Herzegovina): INTRODUCTION OF A NOVEL MRNA VACCINE FOR HUMAN TUBERCULOSIS: TARGETING ESAT6 AND THE DOSRST REGULON

Marina Nonić (University of Belgrade, Faculty of Forestry, Belgrade, Serbia): INTRA-POPULATION GENETIC DIVERSITY OF PUBESCENT OAK FROM THE PROTECTED AREA „KOŠUTNJAK FOREST“ ASSESSED BY MOLECULAR MARKERS

Marina Nonić (University of Belgrade, Faculty of Forestry, Belgrade, Serbia): GENETIC DIVERSITY OF EUROPEAN BEECH ASSESSED BY MICROSATELLITE MARKERS

Ali Burak Yıldız (Gebze Technical University, Department of Molecular Biology and Genetics, Gebze, Kocaeli, Turkey): MIR396 KNOCKOUT VIA CRISPR TO ENHANCE EXPRESSION LEVELS OF GROWTH REGULATING FACTORS (GRFS) IN TOMATO

VI MICROBIOME POTENTIALS

Loredana-Elena Mantea (Alexandru Ioan Cuza University of Iasi, Faculty of Biology, Biology Department, Iasi, Romania): THE EFFECT OF PH AND TEMPERATURE ON PHOSPHATE SOLUBILIZATION POTENTIAL AND GROWTH OF A BACTERIAL STRAIN ISOLATED FROM MAIZE RHIZOSPHERE

Anesa Ahatović (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): METAL TOLERANT RHIZOBACTERIA FROM KRIVAJA – KONJUH OPHIOLITE COMPLEX EXHIBIT MULTIPLE PGP TRAITS

VII INNOVATION AND BIOSAFETY POLICIES

Erna Skenderović (Institute for accreditation of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina): THE ROLE OF STANDARDIZATION, ACCREDITATION, CERTIFICATION BODIES AND LABORATORIES IN CONFORMITY ASSESSMENT OF BIO-BASED PRODUCTS

Sarah Hotić (International Burch University, Faculty of Enineering and Natural Sciences, Department of Genetics and Bioengineering): IN SILLICO EVALUATION OF THE APPLICATION OF MUSHROOMS IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

SPONSOR POSTER PRESENTATION

Biosistemi d.o.o Sarajevo (Biosistemi Grupa d.o.o.) PLANT CLONING

KEYNOTE LECTURES

TOWARDS SUSTAINABLE AGRICULTURE VIA MODERN TECHNOLOGIES AND APPROACHES

Atanas Atanassov, Ivelin Panhev

Joint Genomic Center, Sofia, Bulgaria

The impact of the agriculture on the environment, human health, energy crises and climatic changes enforce policy-makers and farmers to rethink the recent model of the agricultural production. The present report is considering the ways to promote a strong ecologisation of the agriculture all over the world. Designing and implementing such agricultural model needs to change deeply the understanding of the modern technologies breakthroughs made recently in the fields of gene editing ,epigenetics , microbiome ets . The management of the farming systems, natural resources, food –chain and scientific approaches in order to meet environmental and societal demands and the link and interrelationship between traditional, organic and new plant breeding technics including GMO and precision farming will be also considered.

Correspondence: atanas_atanassov@jgc-bg.org

INTERNET OF THINGS [IOT] IN ENVIRONMENTAL BIOTECHNOLOGY AND MONITORING

Majeti Narasimha Vara Prasad

University of Hyderabad, Hyderabad School of Life Sciences, Hyderabad, Telangana, India

Developments in information technology, automation, artificial intelligence, remote sensors, and other technologies have played a significant role in Environmental Biotechnology and Monitoring. Several of the emerging contaminants and conventional contaminants such as heavy metals, Nuclear waste and Asbestos etc. require considerable efforts for monitoring and developing treatment technologies. This lecture aims to address global trends in the remediation sector, with an emphasis on the role of environmental biotechnology as a sustainable strategy in different parts of the globe. Global trends in the remediation industry, seeking to highlight the diversity of existing scenarios that often require the application of efficient strategies, which incorporate the concepts of sustainability and resilience. Thus, recent research trends in remediation employing Internet of Things [IoT] with emphasis on pollution/contamination monitoring and control strategies currently applied in the real world are discussed.

Keywords**:** *Air, Remediation industry; Remediation market; Remediation strategies; Soil, Water*

Correspondence: mnvsl@uohyd.ac.in

TWENTY YEARS OF SHAKY RIDE TO ABIOTIC STRESS TOLERANCE

Zahide Neslihan ÖZTÜRK GÖKÇE

Niğde Ömer Halisdemir University, Ayhan Şahenk Faculty of Agricultural Sciences and Technologies, Department of Agricultural Genetic Engineering, Niğde, Turkey

My journey to understand abiotic stress response of plants have started on 2000 while working with Prof. Hans J. Bohnert in University of Arizona, USA. Our work to understand drought and salt stress responses of barley in transcript level with cDNA library construction and sequencing was published on 2002 in Plant Molecular Biology; which, although unbelievable, is still cited by 10 to 15 scientists each year, as being one of the leading studies initiated the search for increasing abiotic stress tolerance through understanding the changes in transcript abundance levels. Through years, the development of new and high-speed technologies increased our knowledge on adaptive changes in plant cellular metabolism upon changes in environmental conditions. Yet, at the same time, the studies indicated that the changes in transcriptome is not representative of how plants adapt and response to abiotic stresses, and new players have been added to the game on post-transcriptome (miRNAs), proteome, metabolome, ionome and lipidome levels. Although over 20,000 studies on abiotic stress response in plants have been published from 2000 and on, our knowledge on understanding the complex structural and metabolic changes in plants, which depends on species, developmental stage, and even, tissue and cell type, is still very limited to a few success stories. Considering global climate change and ever increasing human population, which both decreases water and field resources available for agriculture, finding a key player to increase abiotic stress tolerance of plants is of greater importance. The aim of this talk is to emphasize the complexity of the abiotic stress response and to summarize what have been done, what have been accomplished, what is known and unknown and what other approaches can be used to increase tolerance to changes in environmental conditions, especially drought, salt and heat stresses, which obviously will shape our future.

Correspondence: zn_ozturk@hotmail.com

THE EFFECT OF microRNAs ON THE POST-RECOVERY SUCCESS OF *ARABIDOPSIS THALIANA* CRYOPRESERVATION

Muhammed Hamit Ekinci[1], Doğa Selin Kayıhan[1], Ceyhun Kayıhan[2], Yelda Özden Çiftçi[1,3*]

[1]*Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey*

[2]*Başkent University, Department of Molecular Biology and Genetics, Ankara, Turkey*

[3]*Gebze Technical University, Smart Agriculture Research and Application Center, Kocaeli, Turkey*

Up to now, cryopreservation, which is defined as the long-term ex-situ conservation of plant species at the ultra-low temperatures of liquid nitrogen (LN, -196°C), is known as the best way to preserve plant germplasm as all the metabolic activities of cells cease at such temperatures and no genetic changes occur during storage. Even though more than 200 plant species was successfully cryopreserved by using the most popular cryogenic procedures such as vitrification and encapsulation/dehydration, there are some especially woody plants that poorly recovered after application of such methods. The genetic and molecular bases of post-cryo tolerance are still poorly understood. Moreover, little is known about the roles of the epigenetic processes and microRNAs (miRNAs) involved in the molecular regulation of post-recovery success. Among them, miRNAs are small RNA molecules, approximately 22 nucleotides long, that can not only regulate gene expression in developmental process of plants, but also control transcription in stress conditions by post-transcriptional degradation. Hence,microRNA (miRNA)-based post-transcriptional regulations during different stages of cryopreservation in 48-h and 72-h in vitro germinated *Arabidopsis thaliana* will be discussed deeply in this presentation to shed light on the influence of this small RNAs in post-recovery success after cryopreservation. Besides, we will focus on miR393 since it was up-regulated 15-fold in LN treated 48-h seedlings, while relatively lower amount induction (twofold) was recorded in cryopreserved 72-h seedlings. In conclusion, our results demostrated that the alteration of expression levels of cold- and oxidative stress-induced genes related-miRNAs play a crucial role in development of successful cryopreservation protocols.

Keywords: *microRNAs, cryopreservation, vitrification, Arabidopsis thaliana*

Correspondence: yelda75@yahoo.com

A REVIE OF EUROPEAN EFFORTS TO LEGALISE AND INTEGRATE MEDICAL CANNABIS INTO HEALTHCARE PROGRAMS

Gavin Sathianathan

Alta Flora, London, United Kingdom

Over the past 5 years a number of European governments have taken steps to legalise cannabis, and studies are now being run to build the evidence base for medical cannabis. However, traditional herbal medicine preparations are challenging for Western medicine to integrate. Cannabis can contain up to 500 different components, rather than the single molecules that medicines regulators favour. Furthermore, there is significant variation in response to medical cannabis at the level of the individual patient based on epigenetic and lifestyle factors. These fundamental differences in the MoA of medical cannabis vs. traditional synthetics (e.g. opioids for pain, SSRIs for mental health) mean that a different approach to the Randomised Controlled Trial (RCT) is required to assess the safety and efficacy of these medicines. In this presentation, we will examine the complexity of cannabis medicines and look at novel methods to collect data and build the evidence base for these medicines. We will look at the efforts to assess this data in Denmark, France, Germany and the UK, and outline the means by which genetics data, wearable technology and mobile apps developed by UK-based technology company Alta Flora can be used to optimise a patient's Quality of Life using medical cannabis.

Correspondence: gavin@alta-flora.com

APPLICATION OF MOLECULAR TECHNIQUES IN SOLVING IMMEDIATE PROBLEMS OF FRUIT PRODUCERS

Fuad Gaši

University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina

Various molecular techniques have been widely in use for fruit research purposes for several decades now. However, their application in overcoming different obstacles that commercial fruit production faces, has a much shorter history. In this lecture two current examples of application of molecular techniques in solving immediate problems of fruit producers will be presented, as well as two real life cases. The first examples deals with a very important issue in fruit production, namely genetic identity and purity of fruit seedlings. The success of commercial fruit production relies completely on the planting of fruit cultivars that are suitable for cultivation in certain agro-climatic conditions and give reliable, as well as high yields. Ensuring that the seedling sold under a certain cultivar name possess all the commercial characteristics that genotype is difficult based only on phenotypic observations even in countries with a well-organized seedling certification schemes. The case that will be presented here deals with a plaintiff who bought raspberry seedling from a register fruit nursery and later sued the mentioned nursery for damages caused by sale of falsely labelled seedling.The second example will detail the use of molecular techniques in identifying suitable pollinizers within orchards with unfavourable environmental conditions for pollination. Adequate fertilization represents a prerequisite for seed development, which is in turn essential for growth and development of high-quality fruits. The case that will be presented here entails three contracted research project between Norwegian fruit producing associations and partners from the University of Sarajevo. The mentioned examples and real life cases will portray the current use of molecular techniques in commercial agricultural production.

Keywords: *genetic identity, pollination, contracted research*

Correspondence: fudo01@yahoo.com

ENHANCED PLANTS STRESS TOLERANCE BY SEED PRIMING

Erna Karalija, Arnela Demir, Adisa Parić

University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina

Changes in the environment because of industrialization and urbanization impact negatively plants growth and crops production. During their evolution plants developed a large variety of adaptive strategies and tolerance mechanisms to overcome these stress conditions. With climate change and increase of temperature drought is becoming more and more pressing issue for crop growth. Beside direct effects of temperature increase through drought, secondly climate change induces changes in the soil increasing the rate of heavy metal “leakage” into the ground waters increasing the rate of food chain contamination. Drought and heavy metal-induced stress is the main limiting factor that affects growth and development in plants. Plant and seed priming have become one of the tools to increase plant tolerance against a vast variety of stressors. Laboratory for Plant physiology, Faculty of Science, University of Sarajevo has been investigating seed and plant priming for the past decade and obtained a large number of results. Our investigations of seed priming on seed vigor demonstrated that seed priming induces seed germination synchrony and increases seedling vigor in correlation to different priming agents. What was noted is that the success of seed priming in process of increase of plant tolerance against abiotic stressors is related to priming agent with strong correlation to type of abiotic stress that are plants subsequently exposed. There is no simple solution or magic priming agent, every plant species can react differently making seed and plant priming an exciting area for investigations.

Correspondence: erna.karalija@gmail.com

SAY SI TO SILICON IN PLANT NUTRIENT STRESS

Jelena Samardžić

University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Laboratory for Plant Molecular biology, Belgrade, Serbia

Elemental silicon (Si), after oxygen, is the second most abundant element in the earth's crust, which is mainly composed of silicates. Si is considered non-essential (or quasi-essential) for plant growth and development, however, increasing evidence in the literature shows that this element is beneficial to plants, especially under stress conditions, and in recent publication it is termed the anti-stress element. Nevertheless, the mechanisms underlying the beneficial effects of silicon are still not sufficiently elucidated. Si application in alleviating plant stress caused by the imbalance of micro and macronutrients present in the soil, primarily the toxicity of metals but also the lack of macronutrients such as phosphorus, is of particular importance for the improvement of agricultural production. In our investigations, we focus on the interaction of Si and micro-nutrient deficiency and toxicity, as well as macronutrient deficiencies, with special emphasis on molecular mechanisms. Understanding the molecular processes underlying this cross-talk would be precious for development and wider use of Si-based fertilizers in agriculture.

Correspondence: jelena.samardzic@imgge.bg.ac.rs

GENETIC RESHAPING OF INDUSTRIAL MICROORGANISMS - INEXHAUSTIBLE POTENTIAL FOR NEW PRODUCTS AND CLEANER TECHNOLOGIES

Zoran T. Popovski[1], Milica Svetozarević[2]

[1]Ss. Cyril and Methodius University, Faculty of Agriculture and Food Sciences, Department for Biochemistry and Genetic Engineering, Skopje, North Macedonia

[2]University of Belgrade, Faculty of Technology and Metallurgy, Department of Biochemical Engineering and Biotechnology, Belgrade, Serbia

The utilization of industrial microorganisms spreads over various manufacturing areas for food processing, medical and environmental purposes. Nearly a quarter of food products are processed by industrial microorganisms. Furthermore, synthesis of hormones, immune-modulators, vaccines, other therapeutic agents, enzymes accounting for food industry, and for diagnostic purposes is performed by selected or transformed microorganisms. This indicates that the potential of microbes is unlimited giving opportunity for further development of biotechnology and processes with substantially lower environmental footprint. A permanent imperative in this area is the improvement of industrially important microorganisms through their genetic enrichment which can be achieved through mutations, natural and artificial recombination. Mutations can be natural or induced, where the induced ones can be caused by physical and chemical agents. Natural recombination occurs during the sexual or asexual reproduction of microorganisms depending of their origin and the creation of hybrids. These types of microorganisms are widely used for beer, wine, antibiotic and other bioactive molecules' production. Natural recombination occurs through conjugation, transduction, and transformation. Artificial recombination can be accomplished by protoplast fusion and recombinant DNA technology, or genetic engineering. In this sense, genetic modification is the most common choice in the genetic improvement of viruses, bacteria, yeasts, molds and protozoa. At the molecular level, these interventions come down to the regulation of microbial metabolism through several mechanisms such as: induction, catabolic regulation, and feedback-regulated regulation that can manifest as: retrograde inhibition, retrograde repression, and regulation of branched metabolic pathways. Of course, these mechanisms of genetic control of microbial metabolism must be synchronized with the surrounding factors (temperature, pH, nutrition, etc.). All interventions aim to create safe and genetically stable strains of microbes that will serve to advance production and technological processes in industry. In this manner, alternative, green and cost-effective technologies are developed contributing to biosustainability of manufacturing.

Correspondence: zpopovski@fznh.ukim.edu.mk

ROLE OF DOUBLED HAPLOIDS IN CROP IMPROVEMENT

Aydin Yildiz

Marmara University, Faculty of Science and Arts, Department of Biology, Istanbul, Turkey

Different types of biotic or abiotic stress factors interactions can have a range of effects on plants depending on the nature, severity, and duration of the stresses and may decrease crops yield significantly. The most cost-effective and environmentally friendly approach to reduce yield losses is deployment of effective resistant genes in cultivars. But existing resistant sources may break, over time in plant. Therefore, long-term conventional breeding strategies and the ongoing evolution of pathogen populations in the region would put the success of breeding programs at risk so that there is always a need for speeding up the process of germplasm enhancement through production of doubled-haploid breeding materials. Doubled haploidy is a very efficient tool for the production of completely homozygous lines from heterozygous donor plants in a single step. Obtaining homozygous pure lines using conventional methods takes a long time: 10–12 years for open-pollinated and 6–7 years for self-pollinated plants. This time can be shortened up to 2 years using tissue culture techniques. Development of efficient protocols is the first strategy for the success of the DH production. In vitro and in vivo production of haploid plants followed by doubling of somatic chromosomes is the quickest means to produce pure breeding doubled haploids. The chromosome content of haploid plants can be doubled spontaneously or using colchicine. The spontaneous haploid genome doubling would be possible to directly plant haploid seeds in the field, thus eliminating the need for a greenhouse and the use of dangerous chemicals for chromosome doubling. The objective of this study is to provide an overview of DH production and the barriers in DH production of sunflower from previous studies on different potential methods for haploid induction for sunflower.

Keywords**:** *Haploid, double haploid, in vivo induction, in vitro induction*

Correspondence: ayildiz@marmara.edu.tr

CRISPR GENOME EDITING TECHNOLOGY AND APPLICATIONS IN PLANTS: RECENT PROGRESS, CHALLENGES AND PROSPECTS

Mehmet Cengiz BALOĞLU

Kastamonu University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Kastamonu, Turkey

Recently, the use of genome editing technologies in plants has shown great promise. Novel genome editing technology, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9, allows plants to improve major agronomic properties by modifying any part of their genome. Using sequence-specific nuclease, this new technology triggers a DNA double-strand break (DSB) at specific sequence points within genome. The donor-dependent homology-directed repair (HDR) pathway or the error-prone non-homologous end joining (NHEJ) pathway repairs genetic damage and creates new genetic traits for plants. The HDR technology can be used to replace nucleotides precisely in some plants, whereas its application in recalcitrant plants is limited due to its low editing efficiency. As an alternative and more efficient than HDR in plants, there are new tools available that use deaminase-mediated base editing and reverse transcriptase-mediated prime editing. It is not necessary to form DSBs or to use donor DNA in these new technologies. By employing the cytidine deaminase (cytosine base editor, CBE) and the adenosine deaminase (adenine base editor, ABE), base transitions to C:G>T:A and A:T>G:C can be initiated directly in targeted sites. “Prime Editor or Prime Editing” is yet another extremely promising technology that allows insertions that are up to 44 base pairs and deletions of up to 80 base pairs, along with combinations thereof. Additionally, the system has been improved for plants and can substitute, add, and delete multiple bases simultaneously. The CRISPR/Cas9 technology is becoming an important tool in improving certain traits of crops, such as yield, plant architecture, nutrient composition, disease resistance, and adaptation to different stresses. The knock-down of a negative regulator gene can sometimes make a particular trait stronger. Gene function research in plants and crop genetic improvement have been revolutionized by CRISPR/Cas9 genome editing technology. Clearly, this new technology will have an important impact on plant breeding research.

Keywords***:*** *CRISPR/Cas9, New perspectives in CRISPR/Cas9, Applications in plants, Genetic improvement*

Correspondence: mcbaloglu@gmail.com

BIOTECHNOLOGY INNOVATION STRATEGY - UK EXAMPLE

Lilijan Sulejmanović

British Bosnian and Herzegovinian Chamber of Commerce, London, United Kingdom

Biotechnology offers the promise to help solve a host of the world's greatest challenges, from delivering more sustainable food chains for the 9bn people the Earth will need to support by 2050 to addressing climate change impacts and the growing health and economic threat from pandemics. What are the tools and interventions that can be harnessed to stimulate innovation and, importantly, commercialisation of R&D into solutions capable of mass adoption? How can these innovations be delivered in ways that support the journey towards a net zero carbon economy? This session will look at the example of the United Kingdom whose 2018 National Industrial Biotechnology Strategy to 2030 set out a vision of biotechnology at the heart of national clean growth efforts. The UK's example demonstrates the importance of considering the interplay between government, industry, academia, regulation and finance in creating conditions that are capable of driving innovation and productivity increases in an economically and environmentally sustainable manner. It will highlight the need for a long-term strategy and policy landscape with well-defined roles for all stakeholders – government agencies, industry and educational institutions. The session will also look at examples of successful academic-industrial partnerships in the UK that can be replicated in other countries.

Correspondence: lilijan@osvit.co

UNIVERSITY-INDUSTRY COLLABORATIONS AND ENTREPRENEURIAL ACTIVITIES IN PLANT BIOTECHNOLOGY

Ahu Altinkut Uncuoğlu

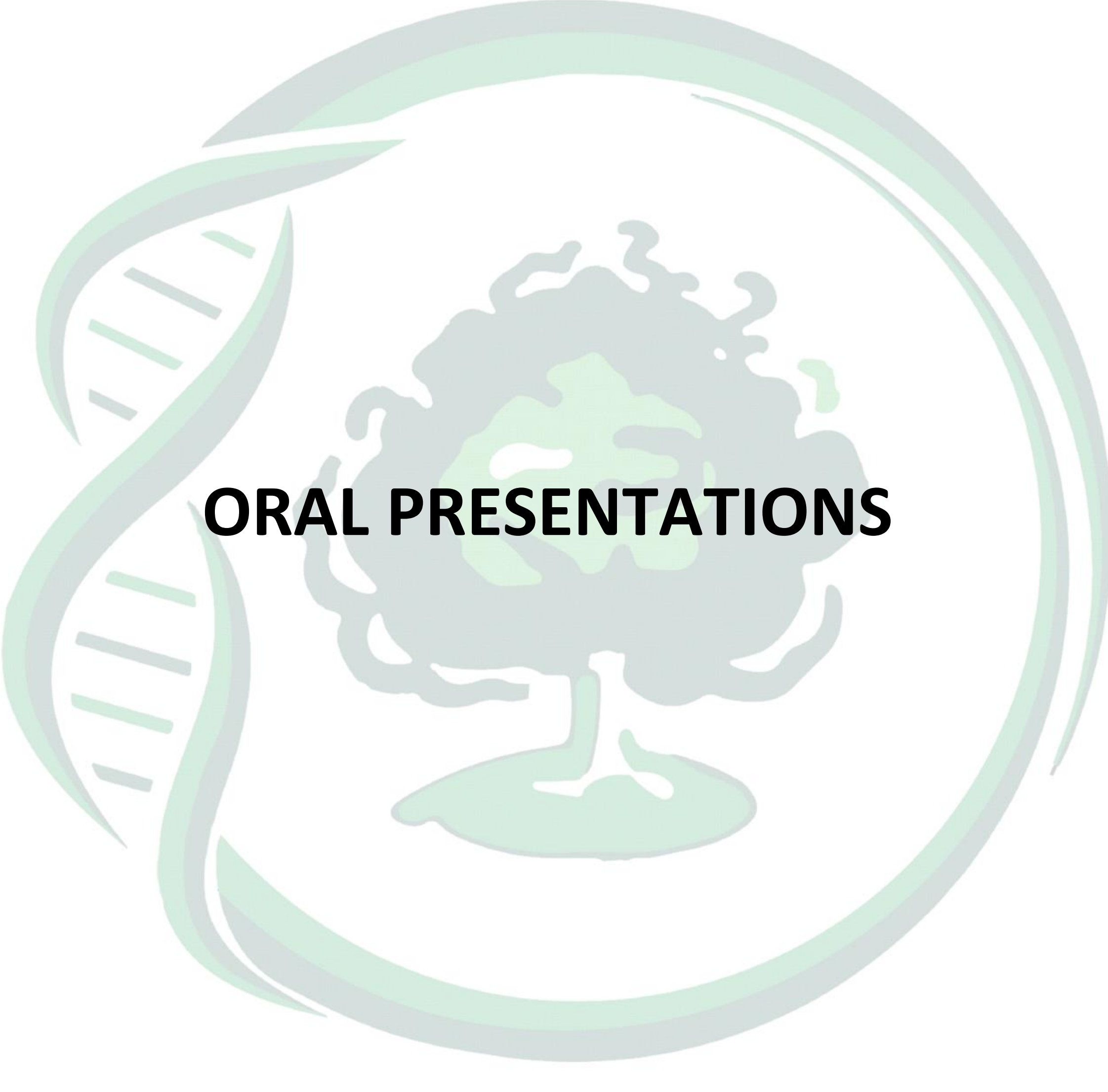
Marmara University, Faculty of Engineering, Department of Bioengineering, Istanbul, Turkey

Multi-disciplinary scientific collaborations in all sub-disciplines of biotechnology are now known to be the vehicle that drives the industry forward. Since sustainable collaborations become crucial for biotechnology research, academic and industrial people act as entrepreneurs by expressing dedication to the potential commercial value of their intellectual capital. There has been a rapid increase in commercial knowledge transfers from universities to practitioners or university–industry technology transfer, through licensing agreements, research joint ventures, and start-ups. In most industrialised countries, the government has played a role in the development of university-industry relationships. Although the mainstream entrepreneurship research activities had neglected the agricultural sector, this situation seems to have changed in the last years with the paradigm changes under pandemic conditions around the World. In plant biotechnology, production of genetically pure lines, engineering for insect and disease resistance, as well as storage protein and other nutritional improvements has been the core topics. Potential benefits from tissue culture, biofertilizers, bio-pesticides, and medicinal plants are also vital. tremendous. Developing countries are already benefiting and should continue to benefit significantly from advances in plant biotechnology. In recent years, the researcher-entrepreneur has become a role model in research institutions and business circles. There is need for would be entrepreneurs to be exposed to the topics most critical to successfully founding, financing and operating a biotechnology company. This talk focuses on understanding the patterns of university-industry collaboration, factors influencing such collaboration and the role of government support in university-industry partnerships in plant biotechnology especially for academic start up formation and creating a commercial channel in the market.

Keywords: *innovation, plant biotechnology, university-industry collaboration, entrepreneurial activities*

Correspondence: ahu.uncuoglu@marmara.edu.tr

ORAL PRESENTATIONS



EXTRACELLULAR LACCASE ACTIVITY OF *CRYPHONECTRIA HYPOVIRUS 1* – INFECTED CHESTNUT BLIGHT FUNGUS

Nuskern L, Tkalec M, Srezović Bijelić B, Ježić M, Ćurković-Perica M

University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

Cryphonectria parasitica is an invasive phytopathogenic fungus, the causal agent of chestnut blight - a devastating disease of chestnut trees in Europe and North America. The disease can be controlled by a mycovirus *Cryphonectria hypovirus 1* (CHV1), which is either present in fungal populations naturally or introduced artificially. CHV1 reduces the virulence and fertility of *C. parasitica*, a phenomenon called hypovirulence which positively affects recovery of the infected trees. Laccase, a polyphenol oxidase, secreted by the chestnut blight fungus *C. parasitica*, is thought to be involved in pathogenesis towards chestnut due to its ability to degrade lignin. Infection with CHV1 has been shown to reduce laccase activity of the infected fungus, but the research has been done only on limited number of fungal isolates and virus strains. Because the role in pathogenesis could have important implications in selection of the best virus strains for human aided biocontrol of chestnut blight, our aim was to determine the effect of CHV1 strains belonging to different virus subtypes on laccase activity in different *C. parasitica* isolates. Four CHV1 strains (belonging to three different subtypes) were transferred to three fungal isolates by hyphal anastomosis. The cultures were subsequently grown on potato dextrose broth. Extracellular laccase activity of virus-free (control) and virus-infected *C. parasitica* isolates was assayed with 2,6-dimethoxyphenol as a substrate. In two *C. parasitica* strains CHV1 infection expectedly reduced extracellular laccase activity, while in one *C. parasitica* strain, regardless of the virus strain utilized, laccase activity was surprisingly elevated. These results indicate that extracellular laccase activity of chestnut blight fungus is affected by factors beyond CHV1 infection, demonstrating that some *C. parasitica* isolates might exhibit unusual and unexpected response to virus infection.

Correspondence: mirna.curkovic-perica@biol.pmf.hr

GENETIC CHARACTERIZATION OF POLYCARPELLARY TRAIT IN A TURKISH ENDANGERED LEGUME SPECIES (*VURALIA TURCICA*)

Oztolan-Erol N[1], Mengi S[1], Lucas SJ[2], Tekdal D[3], Çetiner S[1]

[1]*Sabancı University, Faculty of Engineering and Natural Sciences, Molecular Biology, Genetics and Bioengineering, İstanbul, Turkey*

[2]*Sabancı University, Sabancı University Nanotechnology Research and Application Center, İstanbul, Turkey*

[3]*Mersin University, Faculty of Arts and Science, Biotechnology, Mersin, Turkey*

Vuralia turcica, a member of the Fabaceae family and Papilionoidae sub-family, is a perennial, herbaceous, and endemic species found near Eber and Akşehir lakes in Central Anatolia. It has a polycarpellary gynoecium, a feature that makes *V. turcica* a valuable genetic resource for obtaining multiple carpel trait to increase yield potential in other Fabaceae species, such as faba beans and common beans. To start with, samples from vegetative and reproductive phases of *V. turcica* were collected from Nezahat Gökyiğit Botanic Garden. Transcriptomic analysis was done for all stages using Illumina RNA-seq libraries. Trinity was used for transcriptome assembly, edgeR for differential expression analysis using three developmental stages: vegetative phase, floral meristem differentiation, and reproductive phase to identify candidate genes which were associated with polycarpellary gynoecium. As follow-up analysis, BUSCO showed 96% completeness for transcriptome assembly; Transdecoder identified the candidate coding regions; Trinotate indicated the functional annotation of these coding regions. Differentially expressed genes were analyzed for gene annotation and phylogenetic tree using TRAPID. Results showed that *V. turcica* is genetically close to *Lupinus species*. 1461 out of 1868 differentially expressed genes are found within Fabaceae species-related gene families. We have prioritized differentially expressed transcription factors during the development of the floral meristem. In order to understand the function of these candidate genes, we have gone through *Agrobacterium tumefaciens* mediated CRISPR/Cas9 assays using *V. turcica* and model plants such as *Arabidopsis thaliana* and *Medicago truncatula*. Results will indicate the function of candidate genes, and whether they are associated with polycarpellary trait. This will be very useful for improving yield production of other legume crops and great opportunity to incorporate the natural genetic resources into the agriculture.

Correspondence: nihal.oztolan@sabanciuniv.edu

DESIGNING MOLECULAR MARKERS FOR VARIETY MONITORING AND QUALITY CONTROL IN EUROPEAN HAZELNUT

Oztolan-Erol N[2], Helmstetter A[3], Buggs RJA[34], Lucas S.J.[1]

[1]*Sabanci University, Nanotechnology Research and Application Center (SUNUM), Istanbul, Turkey*

[2]*Sabancı University, Faculty of Engineering and Natural Sciences, Molecular Biology, Genetics and Bioengineering, İstanbul, Turkey*

[3]*Royal Botanic Gardens Kew, Jodrell Laboratory, London, United Kingdom*

[4]*Queen Mary University of London, School of Biological and Chemical Sciences, London, United Kingdom*

Perennial crop species, such as many nut and fruit trees, have usually not been selected and inbred as intensively as most annual crops, resulting in considerable genetic diversity within cultivated varieties. European hazelnut (*Corylus avellana*) is a good model of this as it is wind-pollinated and generally self-incompatible. Turkey is the world-leading producer of hazelnut, which is mostly grown in the Black Sea Region, and maintained largely through clonal propagation. Understanding the genetic variation within and between Turkish hazelnut varieties is essential to assess the resilience of production to future climate change. Samples of commercially important varieties were collected from Akçakoca in the west, and Ordu and Giresun provinces in the east. Every individual was subjected to double digest restriction enzyme-associated DNA sequencing (ddRADseq) and a RADtag library was created. RADtags were aligned to the *C. avellana* var. 'Tombul' reference genome, and Stacks software used to identify polymorphisms. 101 private and six common alleles from nine hazelnut varieties, were identified for diagnosis of these varieties. Phylogenetic analysis and population structure calculations indicated that 'Mincane', 'Sarıfındık', 'Tombul', 'Çakıldak' and 'Palaz' were genetically close to each other; however, individuals within every varietal group were found in different sub-populations. Our findings indicated that years of clonal propagation of some preferred varieties across the Black Sea Region has resulted in admixed sub-populations and great genetic diversity within each variety. In this study, we provide molecular markers suitable for monitoring Turkish hazelnut production and designing breeding programmes to increase the sustainability of hazelnut farming.

Correspondence: slucas@sabanciuniv.edu

CHEMICAL COMPOSITION AND SUGAR CONTENT IN FRESH FRUITS OF WILD POPULATIONS *ROSA CANINA* L. IN HERZEGOVINA

Vasilj V[1], Brekalo H[1], Bakoč M, Petrović D[1], Boras A[2], Batinić K[2], Šic Žlabur J[3]

[1]*University of Mostar, Faculty of Agriculture and Food Technology, Mostar, Bosnia and Herzegovina*

[2]*Agro-Mediterranean Institute Mostar of Federation of Bosna and Herzegovina, Mostar, Bosnia and Herzegovina*

[3]*University of Zagreb, Faculty of Agriculture, Zagreb Croatia*

The rosehip species (*Rosa canina* L.) belongs to the rose family and is widely distributed in Bosnia and Herzegovina. *Rosa canina* L. is relatively insensitive to diseases and pests, thrives on poor soils and is found in Bosnia and Herzegovina mostly as a wild population in various localities characterized by different pedoclimatic conditions. All parts of the rosehip plant, from the root, stem, leaf, flower and fruit can be used for various purposes. The fruit has the greatest popularity for both phytotherapeutic and nutritional purposes. Fresh rose hips have significant nutritional composition, pleasant and refreshing taste, rich in various vitamins, especially C and K, invert sugar, sucrose, organic acids, mineral salts, pectins, tannins and flavonoids. The aim of this study was to determine the differences in chemical composition of fresh fruits of wild rose genotypes collected from different locations: Čitluk, Poljice, Međugorje and Grude in 2020. For this purpose, chemical analyzes were performed to determine the nutritional composition, namely the content of total acids (%), ash (%) and water (%), as well as the content of invert sugar and sucrose (g/100 dry weight). The content of invert sugar and sucrose in a fresh sample of rosehip was determined by liquid chromatography. Significant differences in sucrose content were found in a fresh sample of rose hips with respect to localities. In Medjugorje locality, sucrose content up to 0.30 g/100 g was detected in fresh pomegranate fruits, while no sucrose content was detected in pomegranate fruits at other localities. Higher content of fructose (4.57 g/100 g), glucose (3.26 g/100 g), reducing sugars (7.84 g/100 g) and total acids (45%) was found in fresh rosehip fruits from Poljice locality which values were statistically significantly differend compared to the fresh rosehip samples from other localities.

Correspondence: visnja.vasilj@aptf.sum.ba

ALTERNATIVE POSSIBILITIES OF SEED POTATO DORMANCY CONTROL

Filipović A[1,2], Opančar M[1], Mandić A[1], Crnogorac A[2]

[1]*University of Mostar, Faculty of Agriculture and Food Technology, Mostar, Bosnia and Herzegovina*

[2]*Agro-Mediterranean Institute Mostar of the Federation of Bosnia and Herzegovina, Mostar, Bosnia and Herzegovina*

The aim of this research was to determine the influence of essential oils of fennel, mint and immortelle on dormancy of seed potatoes of three varieties Carerra, Agria and Desiree. The measurement were perform on vegetative parameters (tuber mass, number of germs and germ height). Potatoes tuber seed were weigh, soaked in diluted solutions of essential oil for half an hour, dried and stored in a dark room at a temperature of 18 ° C ± 2 ° C, humidity 50-55% in separate paper bags. The following measurement and germ counting was performed two months after storage. The most significant changes in mass during two months of storage were in the variety Desire while Agria variety had the minor changes in mass. Potatoes soaked in immortelle oil had the largest loss in tuber mass. Tubers mass lose varied in the range of 16.5% to 55%, while the smallest losses were found in the control variant for all three potatoes varieties ranging from 11% to 32.6%. Germ height measurement, at the end of the experiment, showed the longest developed germs in tubers of the Desiree variety in the treatment with immortelle, as well as Carerra and Agria varieties in the control treatment. Both on the appearance of the germ itself, and at germ height, immortelle and fennel oils had the most pronounced inhibitory effect in all three cultivars. The number of germs during the second measurement was highest in the Carerra variety, soaked in essential oil of mint. Based on the results achieved, it is evident that essential oils have influence on the regulation of potato tuber dormancy. Immortelle and fennel essential oil to the greatest extent affected the suppression of tuber germination. The Agria variety had a significant dormancy unlike the other two varieties in the all applied treatments.

Correspondence: adrijana.filipovic@aptf.sum.ba

SUSTAINABLE LAB FOR THE FUTURE WITH MERCK GREEN SOLUTIONS FOR NUCLEIC ACID EXTRACTION AND STERILE FILTRATION

Pongrac I

Merck Life Sciences/Research Solutions, Zagreb, Croatia

Science and technology are at the heart of everything we do at Merck, driving innovations that enable us to contribute to a sustainable future. For us, scientific progress and responsible entrepreneurship go hand in hand, enabling us to create lasting added value for society. GenElute™-E DNA and RNA purification kits employ a negative chromatography method dependent on size exclusion to separate large DNA and RNA nucleic acid molecules from smaller protein, lipid and ionic components in samples. The new approach introduced in these kits allows yielding DNA and RNA preparations with fewer impurities for more robust results. With up to 55% fewer plastic consumables compared to common silica workflows and by eliminating the need for hazardous bind and wash buffers with chaotropic salts and organic solvents, GenElute™-E kits provide an eco-friendly alternative to silica-based purification, resulting in disposal coast savings and reduced environmental impact.Our greener alternative to conventional sterile filtration systems, the Stericup® E filtration units, allow researchers to connect the bottle containing the sample being filtered directly to the filtration unit, thus avoiding the use of a plastic funnel. This product can reduce the amount of plastic used by up to 48% and the amount and size of plastic and corrugated packaging by up to 73%. Each unit is lighter and smaller, which leads to a reduction of CO2 emissions during transport. Taking its entire life cycle into consideration, this can reduce the global warming potential of the product by up to 46%. To conclude, GenElute™-E kits and Stericup® E filtration units are just two examples of what we do at Merck to reduce the environmental and health impacts of our products across their entire life cycle, from their manufacture to use through to disposal. We are striving to bring solutions that help researchers boost the sustainability of their labs for the future.

Keywords: *sustainability, green alternative, eco-friendly, nucleic acid purification, sterile filtration*

Correspondence: igor.pongrac@merckgroup.com

BIONANOPARTICLE APPLICATIONS AND THEIR IMPACT ON GROWTH PARAMETERS OF *HELIANTHUS ANNUUS* L.

Özgören Can T[1], Aydin Y[2], Utkan G[3], Altınkut Uncuoğlu A[1]

[1]*Marmara University, Faculty of Engineering, Department of Bioengineering, Göztepe Campus, 34722, Istanbul, Turkey*

[2]*Marmara University, Faculty of Science and Letters, Department of Biology, Göztepe Campus, 34722, Istanbul, Turkey*

[3]*Marmara University, Faculty of Engineering, Department of Chemical Engineering, Göztepe Campus, 34722, Istanbul, Turkey*

Sunflower is one of the most prominent oil seed production source around the world. Especially in the shadow of global warming and rapid population growth, the demand for its use will increasingly continue due to its tolerance to different environmental conditions, including drought. Although Turkey is one of the leading countries in sunflower cultivation, it still imports a significant amount of sunflower oil to meet the local demand. However, increasing the yield in crop production is possible via environmentally friendly alternatives. In many studies, nanoparticle treatment with appropriate dosages instead of pesticide and chemical fertilizer utilization, have been proved to positively affect the plant growth and crop yield. Furthermore, these nanoparticles can be synthesized via green synthesis by using plant leaves as natural reducing agents. Fe and Zn are essential elements for plants having several roles in vital metabolic activities such as respiration, photosynthesis, chlorophyll and auxin synthesis etc. Although Ti has not an essential role in plants, there are many study indicating that TiO_2 nanoparticles improve seed germination, resistance to biotic stress factors and crop yield in several plants. In the light of all this information, in this study, plant mediated green synthesis of Iron Oxide(Fe_3O_4), Zinc Oxide(ZnO) and Titanium Dioxide(TiO_2) nanoparticles by using *Laurus nobilis* leaves was performed to investigate their effect on local sunflower species. Dynamic light scattering (DLS) analysis and Transmission electron microscopy (TEM) images revealed that nanoparticles with varying size distribution between 5-200 nm were successfully synthesized. After that, the effects of nanoparticles on growth parameters of sunflower plants were examined in hydroponic conditions. Plant groups treated with 1 - 5 ppm Fe_3O_4 nanoparticles gave higher values in terms of average root length, fresh weight, number of leaves and leave surface area compare to others and the control group.

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Keywords: *Helianthus annuus L ., nanoparticle, growth, Iron Oxide, Zinc Oxide, Titanium Dioxide*

Correspondence: tugba.ozgoren@marmara.edu.tr

SCREENING OF ANTICANCER TAXANES IN TURKISH HAZELNUT SUSPENSION CULTURES

Goktepe I, Ari S

Istanbul University, Institute of Graduate Studies in Science, Department of Molecular Biology and Genetics, Istanbul, Turkey

Cancer is one of the most common diseases in the world. Paclitaxel has been successfully used in first-stage chemotherapeutics for many years. The currently used commercial paclitaxel production strategy is the semi-synthesis of taxanes from *Taxus baccata*'s cell cultures. The increasing demand for taxane-derived chemotherapeutics has led researchers to develop alternative production processes. It has been reported that taxanes can be extracted from hazelnut (Bestoso et al., 2006). In this study, we established cell suspension cultures for optimizing the production of 4 different taxanes (paclitaxel, baccatin III, 10-deacetylbaccatin III, cephalomannine) in 4 different hazelnut cultivars (Ham, Sivri Yağlı, Tombul, and Kalınkara hazelnut) according to results of our previous study (Kutluturk, 2019). Cell cultures were treated with elicitors (MeJA and Phe) and they were screened for paclitaxel, baccatin III, cephalomannine, and 10-deacetylbaccatin III by HPLC analysis. Taxane-derived molecules were detected in all elicited cell cultures of hazelnut cultivars studied. The highest amount of taxanes was cephalomannine (331.6 µg/g) in Kalınkara. 10-DAP (6.17 µg/g), baccatin III (3.96 µg/g), and paclitaxel (5.45 µg/g) were also detected in Kalınkara. By elicitor treatments, the amount of 10-DAP and baccatin III, precursors for semi-synthetic paclitaxel production were increased 1.87 and 2.60-fold, respectively in Kalınkara. The cephalomannine increasing ratio was 7.96-fold than the controls in the same conditions. This is the first study in which hazelnut cultivars grown in Turkey were analyzed for in vitro production of taxanes. Our results show that hazelnut cultivars grown in Turkey have the potential to be an alternative for biotechnological production of taxanes.

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Correspondence: ilayda.goktepe@gmail.com

CALLUS INDUCTION FROM UNDEVELOPED OVULES OBTAINED THROUGH INTERGENERIC HYBRIDIZATION BETWEEN *PHASEOLUS VULGARIS* AND *VURALIA TURCICA*

Akça İ[1], Akpak Z[2], Tekdal D[3], Küçükrecep A[1], Çetiner S[4], Hatipoğlu R[5]

[1]*Mersin University, Institute of Science, Department of Biotechnology, Mersin, Turkey*

[2]*Marmara University, Department of Bioengineering, Istanbul, Turkey*

[3]*Mersin University, Faculty of Science and Letters, Department of Biotechnology, Mersin, Turkey*

[4]*Sabanci University, Faculty of Engineering and Natural Sciences, Biological Sciences and Bioengineering Program, Istanbul, Turkey*

[5]*Çukurova University, Faculty of Agriculture, Department of Field Crops, Adana, Turkey*

The study's objective was to investigate whether intergeneric hybridization is possible between *Phaseolus vulgaris* and *Vuralia turcica* and characterize the resulting progeny. Phaseolus vulgaris (2n = 2x = 22) and the distant related Vuralia turcica (2n = 2x = 18) belong to the family of Fabaceae were crossed. In the crossing, *P. vulgaris* was used as a maternal parent, and *V. turcica* was used as a paternal parent. Crossed pistils were collected at different days after pollination and studied under a fluorescence microscope for more information on the interactions of pollen-pistils in these intergeneric crossings. In all crossed samples, no pre-fertilization barrier was found. Pod growth was observed more often in maternal bean parents after pollination, but ovules were not developed. Undeveloped ovules obtained from maternal parent plants were cultured on various culture media, including different concentrations and combinations of 2,4-D and Kinetin to find the best combination of culture media. The callus formation was obtained when the ovules were cultured on the Gamborg's B5 medium, including Kinetin (4 mg L-1) and 2,4-D (1 mg L-1). The results suggest that the medium tested was sufficient for callus induction from intergeneric hybrid ovules. Ploidy levels of callus samples developed from cultured ovule samples were examined at the early stage of their development. Callus was identified as a mixoploid according to flow cytometry analysis. This suggests that spontaneous folds may have occurred. We report the first protocol for callus induction for the ovules obtained from *P. vulgaris x V. turcica hybrids*.

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Correspondence: zeynep8akpak@gmail.com

RHIZOREMEDIATION OF 2,4-DNT-CONTAMINATED SOILS: A MODEL SYSTEM FOR BENEFICIAL PLANT-BACTERIA INTERACTIONS

Akkaya Ö

Gebze Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Kocaeli, Turkey

2,4-dinitrotoluene (2,4-DNT) is a priority environmental xenobiotic pollutant which has toxic, mutagenic, and carcinogenic properties. Thus, its biodegradation by applying recent approaches such as plant-bacteria interactions is crucial. *Pseudomonas putida* (*P. putida*) KT2440 is a reliable host for the biodegradation of many compounds due to its versatile metabolism. In this study, the biotransformation efficiency of 2,4-DNT-contaminated soils on the basis of pot assays using *Nicotiana tabacum* (*N. tabacum*)-*P.putida* interactions was analyzed. Different concentrations of 2,4-dinitrotoluene (2,4-DNT) degrading *P. putida* strain KT.DNT and various growth stages of *N. tabacum* were initially assayed in in vitro tissue culture system and the best conditions for association of plant-rhizobacterium were ascertained to remediation of the soil contaminated with 2,4-DNT. This green remediation technology can be easily used for the effective and low-cost application for sustained cleaning of 2,4-DNT-contaminated soil.

Correspondence: ozlem@gtu.edu.tr

OPPORTUNITIES FOR NI PHYTOMINING IN SERBIA: EXTRACTION OF NICKEL SALTS FROM THE HYPERACCUMULATING PLANT *ODONTARRHENA MURALIS*

Mišljenović T[1], Marković B[2], Ranđelović D[2], Jovanović G[2], Jakovljević K[1], Tomović G[1], Sokić M[2]

[1]*University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden „Jevremovac", Belgrade, Serbia*

[2]*Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia*

Phytomining is a technique that aims to obtain metal(oid)s or their products using hyperaccumulating plant species that grow on substrates rich in heavy metals, but not enough to justify conventional mining. After harvesting, the biomass is combusted and the ash is used as bio-ore for the production of pure metal(oid)s or their salts. Most hyperaccumulating plants accumulate Ni, which occurs naturally in high concentrations in ultramafic soils. A large number of Ni hyperaccumulators are found in the genus Odontarrhena, which is most commonly used in phytomining studies. In order to obtain the largest amounts of Ni and the salts of the higher purity, it is necessary to choose the most suitable ash processing treatment. This study shows results of obtaining Ni salts from the ash of *Odontarrhena muralis* by hydrometallurgical process in the form of ammonium nickel sulfate hexahydrate, Ni(NH4)2(SO4)2x6H2O - (ANSH). *Odontarrhena muralis* from ultramafic sites at West Serbia accumulates Ni at concentrations of 3300 mg kg-1 and is a good candidate for the phytomining process. By biomass combustion, during which biomass weight decreases for 92.7%, ash was obtained as a suitable raw material for Ni extraction. Out of gained mass of crude ash, 11,71% was yielded as ANSH crystals.The obtained purity of ANSH was 73%, which is slightly lower compared to similar studies, but good enough to show the potential for phytomining application. By optimizing the purification process that precedes precipitation of the ANSH crystals, the purity of recovered crystals can be additionally increased, influencing the economic feasibility of the process. Moreover, by selecting appropriate agronomic measures, it is possible to obtain biomass of *O. muralis* with increased concentration of Ni in aboveground parts, which would increase the initial concentration of Ni in bio-ore that is entering the extraction process.

Correspondence: tomica.m@bio.bg.ac.rs

DIVERSITY OF RHIZOBACTERIA ISOLATED FROM ROOT NODULES OF *BITUMINARIA BITUMINOSA* (L) STIRTON, A LEGUMINOUS SHRUB NATIVE OF MEDITERRANEAN AREAS

Zennouhi O

Moulay Ismail University of Meknes, Faculty of Sciences, Meknes, Morocco

In the rhizosphere, there are numerous bacteria which can promote the growth of plants, defend them against harmful agents and can contribute to the improvement of their resistance to abiotic constraints. For this reason, they are called PGPR "Plant Growth Promoting Rhizobacteria». Bacteria belonging to the following genera can be cited as examples Rhizobium, Bradyrhizobium, Azorhizobium Arthrobacter, Azotobacter, Azospirillum, Bacillus, Pseudomonas, Serratia. Therefore, the objective of the present research was the isolation, then the morphological and physiological characterisation of rhizobacteria obtained from the root nodules of the shrubby leguminous *Bituminaria bituminosa* (L.) Stirton commonly named bituminous clover before the use of this species in the rehabilitation of degraded sylvopastoral lands in the mountains of Moroccan Middle Atlas region. Selected on the basis of their performance, these microorganisms can be applied as biofertilisers both during the cultivation of bituminous clover seedlings in the nursery and after their transplantation on the terrains. Gram coloration revealed that these bacteria are predominantly Gram (-) organisms, the majority (80%) of which acidify the medium, can use a wide variety of carbohydrates and are resistant to certain antibiotics, notably Ampicillin and Kanamycin. From a total of 120 isolates, 24 (i.e. 20%) are able to solubilise tricalcium phosphate (maximum solubilisation index: 5.24 and maximum solubilised quantity: 227.27⍰g/ml), synthesize indole acetic acid and siderophors. Also the ecology of the strains indicated that they can grow both within a wide temperature range (4°C to 37°C) pH (4 to 9). Some of them tolerate high levels of NaCl (170 to 855 mM). Such multipurpose and high biodiversity makes these bacteria suitable for use in diverse biotopes where the species *Bituminaria bituminosa* would be established, in view of supporting its growth.

Keywords: *Bituminaria bituminosa, ecology, biodiversity, characterization inoculation isolation, Middle Atlas, Morocco, rehabilitation, Rhizobium, Rhizobacteria, strains, sustainability*

Correspondence: omar.zennouhi@gmail.com

POSTER PRESENTATIONS

EFFECTS OF STRIGOLACTONES IN RELATION TO PARASITIC PLANT WEEDS

Kleman J[1,2], Klubicova K[1], Danchenko M[1], Mikitova V[1,2], Klobucnik M[1,3], Matusova R[1]

[1]*Slovak Academy of Sciences, Plant Science and Biodiversity Center, Institute of Plant Genetics and Biotechnology, Nitra, Slovakia*

[2]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Nitra, Slovakia*

[3]*Comenius University, Faculty of Natural Sciences, Bratislava, Slovakia*

Recent advances in strigolactone related research are continually and systematically expanding our understanding of plants as complex organisms. Progress has been made in understanding their role in the internal plant mechanisms related to their hormonal activity, contributing to modulation of above-ground and below-ground plant architecture, as well as contributing to plant stress response. Serious progress has also been made in regards to strigolactones acting as communication agents in the rhizosphere, facilitating chemical communication of plants and surrounding species of fungi, bacteria, other plants and other organisms. Their importance in establishing mutualistic relationships with arbuscular mycorrhizal fungi or nitrogen-fixing bacteria is slowly being elucidated. However the role they play in the germination process of parasitic weed plant species from the Orobanchaceae family is not fully understood. As it has been known since their discovery that strigolactones act as germination stimulants for these parasites, however their mode of action remains unknown. That is why we approach this process on a proteomic level to better understand how strigolactones affect internal mechanisms of parasitic plant seeds during the crucial stage of their life cycle, which is the host perception and subsequent germination. Since seeds of plants from the Orobanchaceae family tend to only germinate after perceiving signal molecules, like strigolactones, which host plants naturally exude into the rhizosphere. Therefore the changes on proteomic level could help to better understand the internal process that is initiated by interacting with strigolactones.

Correspondence: juraj.kleman@savba.sk

ISOLATION AND CHARACTERIZATION OF MYROSINASE GENE FROM THE HOARY CRESS (*CARDARIA DRABA*)

Mikitová V[1,2], Klobučník M[1,3], Kleman J[1,2], Libantová J[1], Jopčík M[1]

[1]*Slovak Academy of Sciences, Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Nitra, Slovakia*

[2]*Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Nitra, Slovakia*

[3]*Faculty of Natural Sciences, Comenius Universiy, Ilkovičova 6, 842 15, Bratislava 4, Slovakia*

Myrosinases (hydrolytic enzymes, EC 3.2.3.147) and glucosinolates (specific substrates) form a two-component defense system of the Brassicacea family called "mustard oil bombs." When plant tissue is damaged, myrosinases convert glucosinolates into isothiocyanates - an effective defense compound against herbivores and pathogens. In humans, isothiocyanates are known for their anti-cancer, anti-inflammatory, immunomodulatory, antibacterial and cardio-protective properties. Myrosinases in plants are encoded by a multigene family. To date, they have been isolated and characterized from more than 20 Brassica species. In Arabidopsis thaliana, 6 genes for myrosinases have been identified; TGG1 and TGG2 genes with expression in aboveground tissues and TTG4, TGG5 genes with expression in roots. The remaining genes TGG3 and TGG6 were identified as pseudogenes. Here, we focused on the identification of myrosinase genes in the unexplored species *Cardaria draba* (hoary cress), which belongs to the family Brassicaceae. Although this perennial herb is native to South Europe, it is currently distributed throughout Europe where it grows in open areas, fields and meadows. In our strategy, the consensus sequences of myrosinase genes identified in Brassicaceae plant species and available in the NCBI database were used to design myrosinase-specific degenerate primers. These were used in PCR using genomic DNA isolated from *C. draba* as template. The PCR product obtained was verified by sequencing. Then, a complete myrosinase gene was isolated from *C. draba* using the genome walking approach. Next, we focus on isolating other myrosinase genes in *C. draba* and clarifying their expression profiles in individual plant tissues.

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Correspondence: veronika.mikitova@savba.sk

INDUCTION OF HAIRY ROOT CULTURES OF *CALENDULA OFFICINALIS* L. BY *AGROBACTERIUM RHIZOGENES* MEDIATED GENETIC TRANSFORMATION AND PRODUCTION OF SECONDARY METABOLITES

Aydın T, Meriç S, Gümüş T, Ayan A, Atak Ç

Istanbul Kultur University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Istanbul, Turkey

Calendula officinalis known as Marigold, is a flowering plant which has important medical products containing various phyto-chemicals as carotenoids, terpenoids, flavonoids, kinons, kumarins. These phyto-chemicals are widely used for their wound healing, immuno-stimulant, antigenotoxic, anti-inflammatory, anti-bacterial and anti-fungal, antioxidant, antidiabetic, anti-cancerous properties. Hairy root cultures have been preferred for the production of valuable phytochemicals at levels comparable to various plant tissues and undifferentiated cultures. They generally have high genetic stability and growth rates. This pioneer study in which K599 *Agrobacterium rhizogenes* strain was used first time on Marigold plants achieved to induce hairy roots for further phyto-chemical production purposes. In this context, seeds were planted to Murashige & Skoog (MS) medium. In order to initiate hairy root culture, cotyledons and hypocotyls of *Calendula officinalis* were infected with *Agrobacterium rhizogenes* K599 strain by two different methods. In first method, bacterial cultures were grown in liquid yeast mannitol broth medium to OD600 = 1. The cotyledons or hypocotyls from the seedlings were incubated for 30 minutes in a flasks containing 30 mL of bacterial suspension and cultured for 3 days in co-cultivation medium in the dark. In second method, colonies from yeast mannitol agar were used for the infection of plants and inoculated directly into the hypocotyls using a needle. Two weeks later, the developing hairy roots were placed in fresh liquid MS medium supplemented with cefotaxime and timentin antibiotics in flasks for one week. Hairy root lines were maintained in antibiotic-free liquid MS medium at 25 °C on a rotary shaker at 120 rpm and subcultured in the dark every 4 weeks.

Correspondence: s.meric@iku.edu.tr

INHIBITORY EFFECTS OF DIFFERENT CONCENTRATIONS OF LAURUS NOBILIS L. ESSENTIAL OIL ON GROWTH OF PATHOGENIC MICROORGANISMS

Šarić B[1], Jerković-Mujkić A[2]

[1]*University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*

[2]*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*

Essential oils are highly valued natural products that represent a complex of mixtures of volatile and semi-volatile organic components, which originate from a single botanical source that determines the specific aroma or taste and smell of plants. Essential oils contain aromatic substances such as terpenes and non-terpenic molecules. *Laurus nobilis* L. is a plant species which belongs to the Lauraceae family. Laurel essential oil is obtained by distillation of the leaves with water vapor, and the leaves contain 1-3% of the oil. Leaf-derived essential oil has bactericidal, insecticidal and fungicidal properties. The aim of this study was to test the susceptibility of six bacterial and one fungal strain to the laurel essential oil originated from Bosnia and Herzegovina, through the Kirby-Bauer disc diffusion method. In the analysis, the tested essential oil had an inhibitory effect on all tested microorganisms, but with different intensities. Of the total four concentrations of the tested laurel essential oil, the strongest inhibitory effect had undiluted oil on the growth of Gram-positive bacteria. Laurel essential oil has shown the strongest inhibitory effect on *Staphylococcus aureus* (Gram-positive bacteria) and *Salmonella enteritidis* (Gram-negative bacteria). These findings suggest that *Laurus nobilis* L. essential oil could be used as potential antibacterial and antifungal substance in experimental medicine, but further in vitro and in vivo studies are needed to confirm the mechanism of action as well as the potential side effects.

Correspondence: belmina.saric@ingeb.unsa.ba

PROPAGATION OF HAIRY ROOTS AND CALLI IN SETIS BIOREACTOR

Tepe M[1], Süzerer V[2], Gül Şeker M[1,3], Özden Çiftçi Y[1,3]

[1]*Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey*

[2]*Bingol University, Vocational School of Health Services, Department Medical Services & Techniques, Program Medical Laboratory Tech, Bingol, Turkey*

[3]*Gebze Technical University, Smart Agriculture Research and Application, Kocaeli, Turkey*

Echium italicum L. is a medical and aromatic plant that has zygomorphic flowers, basal or lanceolate leaves from Boraginaceae. This plant is common in the world and it grows in more than 30 cities in Turkey. Its leaves and seeds have some oils such as ALA (α-Linolenik Asit Omega-3 (ω-3), SDA (Stearidonik Asit Omega-3) which are used in treatments of diabetes, rheumatoid arthritis, osteoporosis. Moreover, Echium italicum's roots produce some secondary metabolites that have antiviral, anticancer, and wound-healing properties.However, the production of these secondary metabolites are affected by many abiotic and biotic stress factors present in nature. Therefore, usage of biotechnological methods including hairy root cultures and usage of temporary immersion bioreactor systems are becoming alternative to its in vivo production. Hence, callus and hairy roots of Echium italicum were cultured in SETIS bioreactor by using different immersion periods and times while culture in semi-solid medium served as control. At the end culture, hairy roots were propagated as 19 g and 8.7 g in SETIS and semi-agar medium whereas calli were propagated as 4.7 and 5.9, respectively, when cultures were immersed 30 min every 6 h in bioreactor. According to these results, SETIS was found to be more suitable for hairy roots than callus and has potential to be used for secondary metabolites production in plants.

Keywords: *Echium italicum*, hairy roots, SETIS bioreactor

Correspondence: meliketepe@gtu.edu.tr

HAIRY ROOT CULTURE OF VETIVER GRASS

Bacacı E[1],Gül Şeker M[1,2], Özden Çiftçi Y[1,2]

[1]*Gebze Technical University, Department of Molecular Biology of Genetics, Kocaeli, Turkey*

[2]*Gebze Technical University, Smart Agriculture Research and Application, Kocaeli, Turkey*

Medicinal and aromatic plants are used in many fields such as alternative medicine, industry, and cosmetics. Vetiver grass (*Vetiveria zizanioides*) is one of these valuable plants and it can be used in many agricultural and non-agricultural fields. Vetiver oil obtained from the roots of the vetiver has many important effects such as antibacterial, anti-fungal and anti-inflammatory, anti-allergen, anti-itch, insect repellent, skin renewal and hormone regulator as well as its use in perfume essences and cosmetics. Vetiver oil production could be increased by the usage of hairy root formation in which rol genes are transferred to plants by the co-culture of *Agrobacterium rhizogenesis*. Their production can also be further increased in controlled microenvironment conditions by using bioreactor systems. However, there is little research on this subject, more data is needed. Hence, in this study, *Agrobacterium rhizogenes* is inoculated to roots of *Vetiveria zizanioides* to form hairy roots and then those roots were transferred to the temporary immersion bioreactor system (TIS) to increase production and the amount of oil which will be extracted from the roots. This research could be a potential application for mass production of vetiver plant by applying hairy root culture in TIS bioreactor and contribute to the medicinal and aromatic plant sector.

Keywords: *Hairy root culture, Vetiveria zizanioides, Agrobacterium rhizogenes, Temporary immersion system*

Correspondence: erva.bacaci@hotmail.com

INFLUENCE OF VANADIUM ON MICROTUBULE ORGANIZATION IN *ARABIDOPSIS THALIANA* ROOT CELLS

Horiunova I[1], Yaroshenko M[2], Yemets AI[1]

[1]*National Academy of Sciences of Ukraine, Institute of Food Biotechnology and Genomics, Kyiv, Ukraine*

[2]*NSC "Institute of Biology and Medicine", Department of Biotechnology, Kiev, Ukraine*

Plant cytoskeleton orchestrates such fundamental processes in a cell as division, growth and development, cell support, its inner and outer motility, vesicle transport, etc. Vanadium is one of the toxic essential heavy metals and an environmental pollutant. Influence of V as an abiotic stress factor on plant microtubules (MTs) were investigated in this study. 4-day-old seedling primary roots of *A. thaliana* (GFP-MAP4) ecotype Landsberg erecta (Ler) expressing GFP-MAP4 (a green fluorescent protein (GFP)–microtubule-associated protein) was used in this research. The line was grown as describes by us earlier. The seedling primary roots were treated with 1, 5, 10 and 20 µM VCl4 for 1 (to study the effects on the cytoskeleton), 24, 48, 72 h (to study the effect of cadmium on growth and morphology of roots). It was established that a high concentrations of V4+ induce inhibition of growth and changes in the morphology of *A. thaliana* seedlings primary roots after 24,48,72 h of NiSO4 treatment. Root hair development, structure meristematic and epidermal cells of the differentiation zone, root apex and elongation zone, was extremely sensitive to all tested concentrations of V4+. The major reason of these changes is a disruption of the organization of the cytoskeleton, in particular MTs which were visualized in vivo by confocal laser scanning microscope LSM 510 META (Carl Zeiss, Germany). Significant changes during treatment with 1 µM in MTs organization we did not observe. But, vanadium (1-50 µM) provoked a dose-dependent MTs reorganization ranging from randomization to short depolymerization in epidermal as well as cortex cells of all *A. thaliana* primary root zones immediately after the exposure. Similar changes were observed in the meristematic cells, which is one reason for inhibiting the growth and morphology of *A. thaliana* seedlings primary roots. Thus, we have found that MTs is one of the targets for heavy metal action.

Correspondence: inna.horiunova.ukr@gmail.com

NITRIC OXIDE PARTICIPATION IN PLANTS PROTECTIVE EFFECT UNDER HEAT STRESS

Plokhovska S, Pushkarova N, Yemets A, Blume Ya

National Academy of Sciences of Ukraine, Institute of Food Biotechnology and Genomics, Kyiv, Ukraine

It is now well established that nitric oxide (NO) serves as a signaling molecule in plant cells. While, NO is gaining increasing attention from plant science community due to its involvement in plant resistance to various stress, its relation to heat stress tolerance is still unclear. In the present study NO protective effect of against heat-induced stress in *A. thaliana* roots was studied. Endogenous NO formation was detected in the primary roots by 10 µM of the positive fluorescent probe (DAF-FM diacetate) via confocal microscope LSM 510 Meta (excitation 490 nm, mission 515-560 nm). Fluorescence in *A. thaliana* root epidermal cells was observed. It was found that the addition of 100 µm sodium nitroprusside (SNP) increases the level of NO compared to the control. Thus, the fluorescence intensity in *A. thaliana* root cells increased by 26.4% in seedlings exposed to 41°C and after the combined action of SNP and high temperature stress by 69.7% compared to the control. It was found that roots treatment with cPTIO solution (100 µm) decreases fluorescence intensity by 36.7% compared to control, and after the combined action (scavenger and high temperature) by 64.1% compared to seedlings that were only exposed to high temperature (41°C). Therefore, the NO accumulation mechanisms study will allow to alterate its concentration and intracellular localization, allowing plants to show different response to environmental stresses. The obtained results indicate that exogenous NO can effectively increase resistance to high temperature and exhibits plant cells protective effect under stress.

Correspondence: svetaplohovska@gmail.com

GOING GREEN WITH 3D PRINTING: A CASE REPORT OD REDUCING AND REUSING PLASTIC WASTE IN A PLANT PATHOLOGY LABORATORY

Okić A, Konjić A, Grahić J, Mešić A, Mujčinović A, Gaši F

University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina

Research facilities routinely purchase and use vast amounts of disposable plastic consumables. From microtubes, gloves, pipette tips to bottles, racks, and measuring gear - plastic comes in different shapes and sizes in laboratories. Over time, plastic has replaced even more sustainable labware alternatives in life science laboratories. The transition from glass to plastic was made for cost and time effectiveness, but also convenience. Researchers have become reliant on plastic to ensure sterile environments while conducting their scientific experiments. In terms of global plastic pollution, this dependency is quite concerning and raises the question if life science laboratories can curb their plastic consumption? Is there a way for laboratories to be more eco-friendly without compromising research activities? The use of additive manufacturing in the lab, better known as 3D printing, is considered a possible solution. This case report demonstrates how 3D printing can be used in a plant pathology laboratory to curb plastic waste. Base levels of single-use plastics were documented through a 4-week period in the laboratory of plant pathology at the Faculty of Agriculture and Food Sciences University of Sarajevo. After analyzing the different ways of reducing plastic dependency, a two-phase model was implemented in the laboratory and documented. The first phase represents the reduction of plastic consumables through the transition to more sustainable materials, whereas the second phase represents the reuse and recycle approach. The second phase involved setting up a specific recycling framework at the Faculty where plastic is collected and subjected to simple recycling processes of melt extrusion to produce raw material for 3D printing. Afterward, this source material was used for the printing of specifically designed labware and educational material. With this two-phase approach, it was possible to achieve savings in generated plastic waste and financial costs. More importantly, it showcased the importance of small actions in fighting plastic pollution and helped to raise awareness about this ecological problem.

Correspondence: a.okic@ppf.unsa.ba

SARS-COV-2 VIRUS MONITORING OF WASTEWATER IN BULGARIA

Ivanova M, Peykov S, Kitanova M, Georgieva D

Sofia University „St. Kliment Ohridski", Faculty of Biology, Sofia, Bulgaria

Wastewater monitoring for the new SARS-CoV-2 virus is used as a potential tool to support epidemiology. The state is collaborating with laboratories and different wastewater utilities to test the wastewater for virus particles and give health officials early warnings about increases or decreases in COVID-19 cases within a community. The detected RNA fragments of SARS-CoV-2 in wastewater are reported from different independent research groups in nearly all EU Members. Our project aims to consider and optimize the protocols for quantitative analysis of the virus in wastewater. Samples were collected weekly for the period December 2020 – April 2021 from Wastewater Treatment Plant /WWTP/ located in Batanovtsi town and managed by Water Supply and Sewerage Ltd., Pernik, Bulgaria. The subject of WWTP activity is the treatment of domestic, rain, and industrial wastewater coming from a mixed sewerage network of the municipality of Pernik. The samples were analyzed for a set of SARS-CoV-2-specific genes using a real-time quantitative polymerase chain reaction (qRT-PCR). A set of chemical parameters and bioindicators were also analyzed to characterize the quality and composition of wastewater. Our first data suggests wastewater may not be a major route of human transmission, but it is a way of estimating COVID-19 expansion. The results show the total amount of gene equivalents in wastewater correlates with the number of cases of COVID-19 reported in the areas. Wastewater testing can serve as an early warning system and a signal for timely intervention before virus spread in population.

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Correspondence: d.georgieva@biofac.uni-sofia.bg

CHROMOSOMAL ABERRATIONS AND NUCLEAR ANOMALIES IN ROOT TIP CELLS OF *ALLIUM CEPA* L. CAUSED BY RADON IN WATER

Adrović J[1], Eminović I[2], Vidović S[3], Adrović F[1]

[1]*University of Tuzla, Faculty of Natural Sciences and Mathematics, Tuzla, Bosnia and Herzegovina*

[2]*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*

[3]*University of Banja Luka, Faculty of Medicine, Banja Luka, Bosnia and Herzegovina*

In this study, the Allium biotest was used to assess the cytotoxic and genotoxic potential of radon activity concentrations in water, on meristematic cells of *Allium cepa* L. roots. To identify the biological effects of ionizing radiation, which come from the radioactive gas radon, it was necessary to take into account its physico-chemical properties, primarily considering its migratory possibilities. The chromosomes studied were taken from meristematic cells, whose normal cells have 16 (2n = 16) chromosomes, and are suitable lengths for detecting morphological changes. Meristematic cells of *Allium cepa* L. root were treated with a series of 10 different radon activity concentrations in water, ranging from 100.62 to 1,006.25 Bq/L. From a wide range of observed chromosomal aberrations (CA) and nuclear changes, the highest frequency of occurrence were: polyploidy, sticky chromosomes, C-mitosis, bridges, micronuclei, irregular segregation, multipolarity, necrosis, apoptosis, binuclear cells. Trinuclear and tetranuclear cells, as well as stray chromosomes, were observed sporadically. In relation to the negative control, all applied radon activity concentrations in water led to statistically significant genotoxic and cytotoxic effects on meristematic cells of *Allium cepa* L.

Correspondence: biolog@live.com

DNA DAMAGE IN PLANTS – SEASONAL VARIATIONS AND LEAF POSITION EFFECTS

Hasanović M[1], Ćetković T[1], Durmić-Pašić A[1], Čaluk Klačar L[1], Hrelja E[2], Hadžić M[1], Haverić S[1], Haverić A[1]

[1]*University of Sarajevo, Institute for genetic engineering and biotechnology, Sarajevo, Bosnia and Herzegovina*

[2]University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina

As sessile organisms, plants are constantly exposed to numerous environmental factors. Pollutants originating from the atmosphere and soil can induce DNA damage. Therefore, different plants can be utilized in ecotoxicology throughout the monitoring of genetic damage. Recently, as the plant comet assay became more reliable and sensitive, new possibilities for the application of this method in environmental monitoring have emerged. As climate change seems inevitable, it is intriguing to observe how extreme weather and seasonal changes influence plants on a genetic level. Considering the ubiquity of the species and its genome size, as a plant model in this study, we selected *Ligustrum vulgare* L, a semi-evergreen shrub, native to southern parts of Europe, northern Africa and southwestern Asia. The main goal of the study was to investigate whether the seasonal variances and leaves position affect the intensity of genetic damage. Throughout the year, during different seasons, we collected and performed plant comet assay on fully grown leaves positioned in the inner and outer layer of the same shrub. A significant increase in DNA damage was noted in the outer layer of shrub leaves versus inner leaves through all seasons. The results of the ANOVA analysis showed increased DNA damage in the inner leaves from the spring season of 2021, compared to the spring and summer seasons last year during the lockdown period in Sarajevo due to COVID-19. A statistically significant increase in tail intensity was observed in plant leaves collected in winter compared to the summer seasons. Comet assay is a promising method for monitoring DNA damage in plant systems, concerning meteorological data and ecotoxicity, including air pollution biomonitoring.

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Keywords: *plant comet assay, DNA damage, tail intensity, Ligustrum vulgare, seasonal variation*

Correspondence: mujohh@gmail.com

PHYSIOLOGICAL ANALYSIS OF *SANGUISORBA MINOR* SCOP. POPULATIONS FROM VARIOUS SOIL TYPES INDICATES EFFECTIVE SURVIVAL MECHANISMS

Hasanović M[1], Čakar J[1], Ahatović A[1], Murtić S[2], Subašić M[3], Bajrović K[1], Durmić-Pašić A[1]

[1]*University of Sarajevo, Institute for genetic engineering and biotechnology, Sarajevo, Bosnia and Herzegovina*

[2]*University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina*

[3]University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina

Various biotic and abiotic factors, often influence the growth and development of plants throughout different life stages. Harsh environments and sessile life have forced plants to develop a myriad of diverse protective mechanisms, including secondary metabolites, crucial components in plant-environment interactions. Even though *Sanguisorba minor* Scop. (salad burnet) originates from the Mediterranean region, it is also frequently found in different grassland vegetations across Europe. *S. minor* is known as a species with antioxidant, antibacterial, anti-inflammatory and hypoglycemic characteristics. However, data regarding physiological status and heavy-metal uptake potential is scarce. Taking into account previous investigations, the main focus of this study was to assess the physiological response of *S. minor* collected from serpentine and non-serpentine outcrops. Plant and soil samples were collected from four different sites marked as SO1 (Olovo, bedrock), SO2 (serpentine rock outcrop), AS (anthropogenic heavy metal contaminated soil) and NMS (dystric cambisol, non-metalliferous soil). To determine the heavy metal concentration in soil, the Flame atomic absorption spectrometry was performed. Spectrophotometric methods were used to assess the content of chlorophyll *a* and *b*, proline, total phenolics, phenolic acids and flavonoids. Statistical analysis of heavy metals concentrations in soil showed significant differences between serpentine (SO1, SO2) and non-serpentine (AS, NMS) sites. In addition, the concentrations of secondary metabolites, proline and chlorophyll showed statistically significant difference among all investigated sites. The highest concentrations of chlorophyll and total phenolic were detected in plant material from the SO1 site. The concentration of total flavonoids in plant material was highest at the SO2 site, while the plant specimens from NMS had the highest concentration of phenolic acids. Physiological parameters indicate effective adaptation of *S. minor* to various soil types burdened with elevated levels of heavy metals. Considering the obtained results, further studies will include studies concerning plant-microbe interactions, heavy metal uptake and survival mechanisms.

Correspondence: mujohh@gmail.com

EVALUATION OF AUTOCHTHONOUS APPLE VARIETIES (*MALUS DOMESTICA*) IN THE AREA OF TOMISLAVGRAD

Hadžiabulić S[1], Hasanbegović J[1], Aliman J[1], Skender A[2], Hadžić E[1]

[1]*University Dzemal Bijedic of Mostar, Agromediterranean Faculty, Mostar, Bosnia and Herzegovina*

[2]*University of Bihac, Biotechnical Faculty, Bihac, Bosnia and Herzegovina*

The research included seven autochthonous apple varieties in the area of Tomislavgrad, Stipanjići locality, during 2018, which represent only one part of the natural wealth of our country. In seven autochthonous apple varieties: Petrovača bijela, Budimka, Ljutika-Divljaka, Ledarica, Bedrica, Ljepocvjetka-Cvjetača and Jonatanka, phenological characteristics were monitored and analyzed with morphological-pomological properties of fruits. The following phenological characteristics were monitored: flowering phenophases, germination of the first fruits, fruit growth, fruit ripening and full maturity. Five characteristics for the description of apple fruits were determined using the UPOV descriptor for apples (UPOV, 2003) (International Union for the Protection of New Varieties of Plants) - 'Apple'. The results of monitoring the morphological and pomological properties of fruits show that the lowest average fruit weight was in the variety Cvjetača (58.38 g), while the highest average value of fruit weight was recorded in the variety Bedrica (140.59 g). It is evident that the lowest value of fruit width was measured in the variety Bedrica (63.23 mm), and the highest in the variety Jonatanka (70.83 mm). The Cvjetača variety had the lowest average fruit height (47.24 mm), while the Bedrica variety had the highest (59.72 mm). The Budimka variety had the highest average stem length (2.73 cm), while the Divljak variety had the lowest average stem length (1.43 cm). The Jonatanka variety had the largest average cup depression and was (1.28 mm), while the Petrovača bijela variety (0.41 mm) had the lowest average cup depression. Based on the conducted research, the examined autochthonous apple varieties can serve as a valuable genetic material in breeding programs, which can be used in order to create new varieties, as well as for propagation in commercial plantations.

Keywords: *autochthonous, apple, varieties, phenological characteristics, morphological-pomological properties*

Correspondence: semina.hadziabulic@unmo.ba

CHARACTERIZATION OF GENOMICS DATA OF *VURALIA TURCICA* AND UNDERSTANDING ITS POLYCARPELLARY STRUCTURE

Mengi S[1], Oztolan-Erol N[1], Lucas S J[2], Adebali O[1],Tekdal D[3], Cetiner S[1]

[1]*Sabancı University, Faculty of Engineering and Natural Sciences, Molecular Biology, Genetics and Bioengineering, İstanbul, Turkey*

[2]*Sabancı University, Sabancı University Nanotechnology Research and Application Center, İstanbul, Turkey*

[3] *Mersin University, Faculty of Arts and Science, Biotechnology, Mersin, Turkey*

Vuralia turcica is a perennial, herbaceous, and endemic species found near Eber and Akşehir lakes in Central Anatolia. It is a member of the Fabaceae family and Papilionoidae sub-family. *V. turcica* is noted for its polycarpellary gynoecium, a feature that is rarely observed in the Fabaceae family. This feature makes *V. turcica* a valuable genetic resource for obtaining multiple carpel formation to increase yield potential in other Fabaceae species, such as faba beans and common beans, which are very important for their nutritional value. In this study, whole genome sequencing was conducted using 10x Genomics ‘Chromium’ Libraries, sequenced on the Illumina platform, and the assembly was done using ABYSS 2.0 software. After running ABYSS 2.0 with different k-mer values, 100-mer value gave the best N50 (=5592), N80 (=1566), and number of scaffolds more than 500 bp (=231424) compared to other k-mer values. Therefore, the genome size of *V. turcica* was predicted to be about 555 Mbp and this assembled genome of *V. turcica* includes 80% of completed genes according to the BUSCO analysis. After that, the genome size of *V. turcica* will be confirmed by calculating its DNA-C- values based on the absolute quantification of candidate single copy genes (CLAVATA3 and WUSCHEL) of *V. turcica* in a known amount of genomic DNA by utilizing RT-qPCR. Meanwhile, the phylogenetic tree was made using Orthofinder and indicated that, among previously sequenced species, *V. turcica* is genetically close to Lupinus angustifolius. Orthofinder analysis also unraveled the species-specific genes in *V. turcica* that can affect its polycarpellary structure, so the function of these *V. turcica* specific candidate genes will be tested in the future through gene editing methods.

Correspondence: sezginmengi@sabanciuniv.edu

GENE INTROGRESSION OR GENETIC CONTAMINATION - THE CASE OF BLACK POPLAR (*POPULUS NIGRA* L.) IN BOSNIA AND HERZEGOVINA

Memišević Hodžić M, Ballian D

University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina

The survival of the European black poplar, as an indigenous species, has been threatened for two centuries, due to the cultivation of hybrids of Euro-American poplars (*Populus × canadensis*) and East American black poplars (*Populus deltoides*). This study aims to determine the existence of gene introgression on black poplar trees in Bosnia and Herzegovina to protect indigenous black poplars. We analyzed the polymorphism of black poplars in the clone archive in Žepče using ten microsatellite markers. The clone archive was established from a total of 141 trees from 26 localities from six river basins in Bosnia and Herzegovina. Old trees of indigenous black poplars considered not to be contaminated with genes of American black poplars, were used to establish the archives. As a result of microsatellite analysis, a total of 139 different alleles were obtained. Of the 141 trees analyzed, 136 showed autochthonous genetic structure, and 5 trees showed presence of introgressed genes. Three clones were found to show the presence of allochthonous introgressed genes only at the PMGC_14 gene locus. Since the gene locus is typical for PMGC_14 only for *P. deltoides*, we can talk about the hybridization of indigenous poplars with a backcross. The other two clones had more introgressed genes, for the gene locus PMGC_14, WPMS_18, and for PMGC_2163, all with typical alleles for American black poplars. The obtained results confirmed the presence of trees with introgression in populations that will need to be identified and removed from the population. As our sample originated from old poplar trees, the assumption is that in populations of younger plants the situation is even more critical and there are more introgressed individuals. Thus, our populations lose certain adaptive properties, although some spontaneous hybrids can be very promising for poplar plantations.

Correspondence: mirzeta.memisevic.hodzic@gmail.com

GENETIC CHARACTERIZATION OF THE LAST NATURAL TREES OF THE GENUS *TILIA* IN ČAPLJINA IN THE METAPOPULATION ČAPLJINA-TOMISLAVGRAD IN FRAGMENTED LINDEN HABITATS IN THE CONTEXT OF CLIMATE CHANGE

Vejzagić S

University of Sarajevo, Faculty of Forestry, Sarajevo, Bosnia and Herzegovina

The research was conducted in the Čapljina-Tomislavgrad Metapopulation, and shows the structure of linden populations in the territory of Čapljina, Tomislavgrad and their wider surroundings. The focus of the research is the genetic characterization of the last natural linden trees (*Tilia sp.*) in Čapljina. The aims of the study were to identify species, to find potential hybrids, to investigate the Gene pool and to present the meaning and importance of tree-based sampling for DNA analysis of species in their natural fragmented habitats. The research included 62 trees, of which two trees (1.24%) represent the two remaining natural trees of linden in Čapljina (Tanasčići and Struge). A set of 12 nuclear primers (microsatellites) was used in the study: Tc5, Tc31, Tc6, Tc7, Tc4, Tc8, Tc915, Tc963, Tc951, Tc918, Tc920, Tc11. Molecular analysis in the genetic laboratory yielded results that identified the last natural trees in Čapljina as a species of Tilia platyphyllos. By additional "Structure" software analysis, the species *Tilia platyphyllos* was identified, while the remaining trees of the Čapljina-Tomislavgrad Metapopulation were identified as a mixture of trees of the species *Tilia cordata* and *Tilia platyphyllos* (Tomislavgrad and surroundings). Genetic identification of heron trees was performed due to the existence of morphological specifics, which made the leaf an atypical leaf for the species *Tilia platyphyllos*, namely: double leaf tip, uneven serration of leaf edges and dense hairiness (metamorphosed parts of leaves). The results of the research, in accordance with the principles of sustainable management of natural linden ecosystems under the influence of climate change, indicate the need to preserve the Čapljina Gene pool of *Tilia platyphyllos*, due to its high resistance to high temperatures and prolonged drought.

Correspondence: selma.vejzagic@web.de

PRODUCTION OF BIOSURFACTANT BY FUNGI ISOLATED FROM SOIL

Bhatt KM, Pandya BS, Gohel R

Nirma University, Gujarat, India

Biosurfactants are surface active compounds that have sparked interest in recent years because of their environmental advantages over conventional surfactants. The aim of this study is to investigate the production of biosurfactants by fungi (viz. *Trichoderma sp*, *Fusarium sp*, *Aspergilus sp*) isolated from Soil. Biosurfactant production potential of test fungi was examined based on Drop collapse assay, penetration assay, oil spreading assay and emulsification capacity assay. Conventional surfactant (Tween 80) was used as a control.

Correspondence: 20mmb003@nirmauni.ac.in

OVERVIEW AND USEFUL VALUE OF WILD PLANTS OF THE CITY OF MOSTAR

Petrović D[1], Brekalo H[1] Musa A[2], Boban M[1], Medić S[1]

[1]*University of Mostar, Faculty of Agriculture and Food Technology, Mostar, Bosnia and Herzegovina*

[2]*University of Mostar, Faculty of Science and Education, Mostar, Bosnia and Herzegovina*

The plant diversity of urban areas relative to natural ecosystems has been very little researched. Living conditions in urban areas are strongly influenced by man, from direct habitat changes, higher amounts of nitrogen to the impact on other biotic and abiotic factors. Floristic research of urban areas monitors the impact of urbanization on the composition of urban flora, in this case the urban flora of wild plants. During the vegetation season in 2019. and 2020., a floristic survey of wild species was conducted in the area of the city of Mostar (Entity of Federation of Bosnia and Herzegovina, Bosnia and Herzegovina). The research recorded 166 plant species that were classified into 49 families. Taxonomic analysis of wild plant species recorded in the City of Mostar, shows the dominance of species from the family Asteraceae, Fabaceae and Lamiaceae. Life expectancy analysis shows the dominance of herbaceous perennials, and the spectrum of life forms is dominated by hemicryptophytes. According to usable value, the most common are wild plant species used in food, ornamental plant species, and honey plant species. A larger number of plant species has more useful values.

Keywords: *urban flora, medicinal species, honey species, poisonous species, spice species, fodder species*

Correspondence: helena.glamuzina@aptf.sum.ba

CRISPR/Cas9 MEDIATED SILENCING OF CCDC124 GENE IN *SOLANUM LYCOPERSICUM*

Civelek H, Cebrailoğlu N, Tuzlakoğlu Öztürk M, Özden Çiftçi Y, Tazebay UH

Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey

Ccdc124 (Coiled-coil domain-containing 124), a conserved gene in all eukaryotes, is a newly defined centrosome protein. In previous studies, it has been shown that the expression of the Ccdc124 gene occurs in all human tissues. In addition, in the studies conducted in mammalian cell lines, it has been determined that the protein is localized in the centrosome in the interphase stage of the cell cycle. In another study, it was found that multinucleated cells are formed in cytokinesis when the gene is silenced or overexpressed in mammalian cell lines. The main function of the Ccdc124 protein in the cell is still unknown and there are no studies on the Ccdc124 gene in plants. Hence, we aimed to investigate the effects of silencing of the Ccdc124 gene in plants and to approach the cellular events involved in Ccdc124 from a broader and new perspective. In this study, the Ccdc124 gene was silenced in *Solanum lycopersicum* by CRISPR\Cas9 system to study gene function. Initially, guide RNAs (gRNAs) were designed to target the Ccdc124 gene, then cloned into the pYLCRISPR/Cas9P35S-N vector by Golden Gate Cloning. Subsequently, Agrobacterium-mediated stable transformation of the tomato plant was performed. As a result of our study, deletion on the Ccdc124 gene was confirmed by molecular verification. The results showed that SlCCDC124 mutants possessed distinctive morphological differences in comparison with wild-type plants. In the continuation of our study, the effects and basis of these morphological differences will be investigated. Also, different types of mutations will be determined, and their morphological effects will be observed.

Keyword: *Genome editing: Solanum lycopersicum: Ccdc124*

Correspondence: hcivelek@gtu.edu.tr

ANTIBODY-CONJUGATED LUMINESCENT AG2S QUANTUM DOTS FOR IMAGING OF PLANT CYTOSKELETON

Borova M[1], Kapush O[2], Horiunova I[1], Plokhovska S[1], Pushkarova N[1]

[1]Institute of Food Biotechnology and Genomics, NAS of Ukraine, Kyiv, Ukraine

[2]V.E. Lashkaryov Institute of Semiconductor Physics NAS of Ukraine, Kyiv, Ukraine

Ag2S is one of the most important transition metal sulfide semiconductor nanomaterials due to its direct and narrow band gap (1.5 eV) and high chemical stability. It possesses unique semiconducting, optical, and electrical properties and is highly stable. Owing to these features, silver sulfide QDs are broadly used in solar cell batteries, thermoelectric sensors, infrared detectors, and superionic conductors. These QDs can be coated with hydrophilic materials and functionalized with antibodies, peptides, nucleic acids etc. The purpose of the present work is to functionalize Ag2S nanocrystals with monoclonal antibodies against cytoskeletal protein tubulin for microtubule visualization. In our research we modified existing protocol for covalent conjugation of gold nanoparticles with antibodies, which is available at www//nanocomposix.com. The critical reagents for the covalent coupling process are EDC and Sulfo-NHS they increase conjugation efficiency and create a more stable amine-reactive intermediate for binding with antibodies. We have revealed that bioconjugates had a symmetric luminescence spectrum with a maximum at 350 nm. We performed scanning electron microscopy (SEM) of produced Ag2S-antibody bioconjugates and developed a procedure for imaging of microtubules in epidermal root cells of Nicotiana tabacum plants.

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Correspondence: marie0589@gmail.com

MOLECULAR INSIGHT INTO THE GENETIC STRUCTURE AND THE GENOMIC ARCHITECTURE OF *PINUS SYLVESTRIS* X *MUGO* CONTACT ZONES IN SLOVAKIA

Klobucnik M[1,2], Galgoci M[1,3], Kleman J[1.4], Mikitova V[1,4], Kormutak A[1]

[1]*Slovak Academy of Sciences , Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Nitra, Slovakia*

[2]*Comenius University, Faculty of Natural Sciences, Bratislava, Slovakia*

[3]*Constantine the Philosopher University, Faculty of Natural Sciences, Nitra, Slovakia*

[4] *Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Nitra, Slovakia*

The four populations of *Pinus sylvestris × mugo* of uncertain taxonomic status which occur in contact zones in Slovakia, namely in Zuberec, Sucha Hora, Obsivanka and Tisovnica – sometimes referred to as putative hybrid swarms, were studied in order to reveal their genetic structure and genomic patterns of gene flow between hybridizing species. Based on four reference *P. sylvestris* and five reference *P. mugo* populations, our estimates of ML (Maximum Likelihood) hybrid index calculated for 80 iPBS (inter-Primer Binding Site) loci confirmed the presence of intermediate genotypes on the localites. The distribution of individual admixture proportions clearly inclined towards one or the other species, suggesting a rather low frequency of early-generation or F_1 hybrids as compared to the frequency of *P. sylvestris*-like or *P. mugo*-like individuals; the former was dominant in Zuberec, the latter in Sucha Hora, Obsivanka and Tisovnica. Surprisingly, a unimodal and normal distribution of hybrid index, indicative of a hybrid swarm, was only inferred within the Sucha Hora contact zone, where individual trees are morphologically very similar to the 'pure' or typical *P. mugo*. We suppose that the remaining populations are highly introgressed, consisting of different types of introgressants differing in the proportions of genes inherited from the parental gene pools. In respect to Sucha Hora, an inconsistency between morphological observations and the genetic data obtained in our study can be explained by different types and number of genomic regions under consideration. As virtually all molecular (DNA) markers are selectively neutral or nearly neutral, the variation we observed is a reflection of the background genetic variation, rather than polymorphism underlying atypical (hybrid) variants of phenotypic traits that may show strong selection gradient in specific habitats.

Correspondence: miroslav.klobucnik@savba.sk

CYTOTOXICITY OF *ARTEMISIA ANNUA* L. COMMERCIAL ETHANOLIC EXTRACT IN NORMAL AND CANCER CELL LINES: CONTRIBUTION TO PHARMACEUTICAL APPLICATION

Ćetković T, Hadžić M, Čaluk Klačar L, Haverić A, Haverić S

University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina

Artemisia annua L. (Asteraceae), commonly known as “sweet wormwood”, is traditional in Chinese medicine, and it has been used for more than 2000 years for treating a broad range of diseases. Anti-cancer, anti-inflammatory and anti-malarial properties of *A. annua* L. extracts and related compounds, lead to a growing interest for its anti-viral potential and use in treatments of SARS-CoV-2 infections. The aim of this study was to analyse cytotoxic effects of commercial ethanolic extract of *A. annua* L. on human and animal cell lines. The four cell lines including two cancer (5637 human urinary bladder carcinoma and B16-F10 murine skin melanoma) and two normal (HEK 293T human embryonic kidney and MDBK Madin Darby Bovine Kidney), were used for cytotoxicity assessment. Cells were seeded in appropriate growing media and treated with *A. annua* L. extract dilutions of 1:10, 1:100 and 1:1000, 24h upon of cell incubation. The neutral red uptake assay was performed. Results showed significant increase of cytotoxicity ($p<0.001$) in both normal and cancer cell cultures (range from 65% to 24%) treated with the lowest extract dilution of 1:10 compared to the highest dilution of 1:1000. As *A. annua* L. extract dilutions increased, the cytotoxicity in all cell lines decreased. Those preliminary results point to a special carefulness in *A. annua* L. commercial ethanolic extract use, especially when poorly diluted, due to evident cytotoxic potential on normal human and animal cells. Additional assays for determination of harmless doses are certainly suggested for the further evaluation of its anti-cancer potential and pharmaceutical applications, even of commercially available preparations of *A. annua* L.

Correspondence: tamara.cetkovic@ingeb.unsa.ba

MOLECULAR DIFFERENTIATION OF OLIVE CV. KARAMÜRSEL SAMANLI FROM OTHER TURKISH CULTIVARS VIA ISSR MARKERS

Türkölmez N[1], Tanrıkulu MK[2], Mutlu O[2], Çelik O[3], Kaya E[3], Özden Çiftçi Y[1]

[1]*Gebze Technical University, Department of Molecular Biology and Genetics, Gebze, Kocaeli, Turkey*

[2]Karamürsel County Agriculture and Forest Directorate, Karamürsel, Kocaeli, Turkey

[3]Muğla University, Department of Molecular Biology and Genetics, Kötekli, Muğla, Turkey

Olive, which is the evergreen fruit tree species, plays an important role in not only for human health but also the landscape characterization. This autochthonous woody plant of the Mediterranean basin represents the sixth most important edible oil crop worldwide. Although there are a number of molecular studies carried out in olive (RAPD, SSR, ISSR, AFLP etc.) in order to differentiate various olive cultivars previously, there is still only a restricted number of well-known cultivars that have been employed for olive breeding purposes. Therefore, more molecular marker studies should be carried out in Turkish olive cultivars such as cv. Karamürsel Samanlı to differentiate it both from existed cultivars and establish conservation programme. Thus, our study aimed to assess the genetic difference of cv. Karamürsel Samanlı which is only cultivated in Karamürsel province of Turkey among other existing olive cultivars including cv. Gemlik, Ayvalık, Halhali, Memecik by ISSR markers. ISSR primers were selected from our previous olive studies and literature. Our results showed that cv. Karamürsel Samanlı is different from the rest of the studied cultivars and should be considered as a new cultivar. Hence, in vitro propagation and conservation studies are initiated in Gebze Technical University together with Muğla University to conserve its germplasm.

Keywords: *Olive, ISSR, Karamürsel Samanlı cultivar*

Correspondence: nilt82@gmail.com

TARGETED MUTAGENESIS OF INTRINSICALLY DISORDERED PROTEIN ATDSS1(V) USING CRISPR-CAS9 APPROACH

Nikolić I, Samardžić J, Timotijević G

University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia

DSS1 gene encodes small, conserved protein which belongs to an intrinsically disordered protein family. DSS1 protein multifunctionality is mirrored in protein-protein interactions in diverse cellular machinery. For instance, DSS1 interacts with BRCA2 (genome maintenance), 26s proteasome (protein homeostasis), TREX-2 (RNA metabolism) etc. Another DSS1’s role is described as a novel post-translational protein modification in human cells, named DSSylation. This phenomenon is a specific targeting and elimination of oxidative damaged proteins by DSS1. The *Arabidopsis thaliana* genome contains two highly homologous AtDSS1(I) and AtDSS1(V) genes, embedded within chromosomes I and V, respectively. Well-studied function of AtDSS1 in the homologous recombination showed that AtDSS1(I) interacts with AtBRCA(IV) and AtBRCA(V), but AtDSS1(V) only with AtBRCA(IV). Also, direct interactions of AtDSS1 with both TREX-2 complex and 26s proteasome were confirmed and it was suggested that AtDSS1 acts like a linker between the two complexes. Our aim is to examine plant DSS1 involvement in response to oxidative stress and potential functional divergence of AtDSS1 paralogues. Using Agrobacterium-delivered CRISPR-Cas9 targeted mutagenesis with single-guide RNA complementary to the target gene, we obtained a stable line of *Arabidopsis* containing mutations in AtDSS1(V). High resolution melting method followed by Sanger sequencing enabled us to select plants with desired mutations in AtDSS1 gene. The mutant with 18 nucleotides insertion in AtDSS1(V) PAM sequence and altered ORF was chosen for further study. Morphological analysis of Atdss1(V) revealed differences in rosette shape, stem length and stage of silique dehiscence. Atdss1(V) seedlings exposed to H2O2 and UV-C treatment showed increased sensitivity to oxidative stress in comparison to the control plants. In conclusion, the results indicate that AtDSS1(V) might be one of the players in plant cellular maintenance of homeostasis during oxidative stress.

Correspondence: ivana.nikolic@imgge.bg.ac.rs

INTRODUCTION OF A NOVEL MRNA VACCINE FOR HUMAN TUBERCULOSIS: TARGETING ESAT6 AND THE DOSRST REGULON

Kovačić Dž, Jotanović J, Softić A

International Burch University, Faculty of Engineering and Natural Sciences, Department of Genetics and Bioengineering, Sarajevo, Bosnia and Herzegovina

Human tuberculosis (TB), particularly in its respiratory variety, represents an unfortunately placed and persistent thorn in global health; asymptomatically infecting nearly a third of the global population, with roughly 10 million new cases and 1.5 million deaths annually. It continues to hold a place among the top ten causes of death in the world. Such statistics have persisted through the 20th and the entirety of the 21st century, despite the World Health Organization (WHO) placing considerable efforts into distributing anti-TB drugs and the one-hundred-year-old Bacillus Calmette-Guerin (BCG) vaccine across high-burden countries. Though socio-economic and political factors play a considerable role in combating the TB global pandemic and – indeed – countries with a high TB burden are on the lower side of the economic and healthcare ladder, the inefficacy of currently available anti-TB pharmaceutical products, has been, in the very least, substantially discussed in recent years. In spite of the fact that antibiotic treatments for TB pose a complex issue due to the necessity for strict patient adherence for prolonged therapeutic periods in low-income countries, the efficacy of the only approved vaccine for TB (BCG), is modest at best and absent at worst. Evidently, it is quite difficult to design an effective vaccine against TB due to the pathogen's highly efficient and incompletely understood immune evasion techniques. Herein, we report a novel mRNA vaccine design for members of the Mtb complex – a genetically homogenous bacterial group - using currently available immunoinformatic software, and a delivery system based on eukaryotic vaults. We validated the hypothesis that focusing on mycobacterial dormancy-associated proteins belonging to the DosRST regulon, along with the 6 kDa early secretory antigenic target (ESAT6), would allow the host to potentially prime a sufficient immune response to eliminate the pathogen upon infection, thereby preventing both active and asymptomatic TB.

Correspondence: dzenan.kovacic40@gmail.com

INTRA-POPULATION GENETIC DIVERSITY OF PUBESCENT OAK FROM THE PROTECTED AREA „KOŠUTNJAK FOREST“ ASSESSED BY MOLECULAR MARKERS

Nonić M[1], Šijačić-Nikolić M[1], Kerkez Janković I[1], Aleksić J[2]

[1]*University of Belgrade - Faculty of Forestry, Belgrade, Serbia*

[2]*Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade,Vojvode Stepe 444a, Belgrade, Serbia*

By studying the vegetation of the protected area „Košutnjak Forest“ (Belgrade, Serbia), a pubescent oak (*Quercus pubescens* Willd.) population has been recorded. The wide distribution range of this species has been reduced in the past. This research aimed to determine the intra-population genetic variability of pubescent oak in the protected area „Košutnjak Forest“, at the molecular-genetic level. Extraction of total genomic DNA was performed from green leaves, collected from46 genotypes (adult trees), using a commercial peqGOLD Plant DNA Mini Kit (PEQLAB). Multiplication of selected microsatellite markers was performed by polymerase chain reaction using a Multigene Opti Max (Labnet International, Inc.). In total 13 nuclear microsatellites were successfully amplified: PIE239, FIR004, QrZAG90, QrZAG108, MSQ13, QrZAG87, QpZAG104, QrZAG101, MAQ4, PIE242, QrZAG20, QrZAG7, and QpZAG110. Values of genetic diversity parameters were calculated using GenAlEx 6.5 software (Peakall and Smouse 2006). The number of alleles per locus was in the range from 7 (PIE239) to 31 (QrZAG90), while the average number of alleles per locus was 16.538. The number of effective alleles per locus was in the range from 2.919 (QrZAG108) to 23.381 (QrZAG90), with an average of 9.143. A statistically significant deviation of the expected heterozygosity from the observed heterozygosity occurs at 6 loci (PIE239, FIR004, QrZAG108, QpZAG104, MAQ4, and QrZAG101). The average values of the fixation index were positive and statistically significant, which indicated an excess of homozygotes. The obtained data indicate a high level of intra-population genetic variability, which could be a basis for the conservation of the available gene pool of this species. Based on the results, it can be stated that the population is characterized by an increased inbreeding. However, the survival of the Pubescent oak population at a given locality cannot be questioned, but the genetic status can be improved by introducing new genotypes.

Keywords: *Quercus pubescens* Willd., genetic diversity, intra-population variability, microsatellite markers

Correspondence: marina.nonic@sfb.bg.ac.rs

GENETIC DIVERSITY OF EUROPEAN BEECH ASSESSED BY MICROSATELLITE MARKERS

Nonić M[1], Heinze B[2], Šijačić-Nikolić M[1]

[1]University of Belgrade - Faculty of Forestry, Belgrade, Serbia

[2]Federal Research and Training Centre for Forests, Natural Hazards and Landscape Department of Genetics, Unit of Genome Research, Vienna, Austria

Microsatellite markers are very useful in the field of population genetics of beech species, which is a dominant in the growing stock of Serbia. It is characterized by a high variability of numerous traits and genetic studies of beech have been performed in different populations over a wide range of the species distribution. This research aimed to determine the genetic structure of beech (*Fagus sylvatica* L.) population „Crni vrh 2" using microsatellite markers. The population is located in Serbia. Five primer pairs of microsatellite loci (mfc5, sfc_0036, csolfagus_19, csolfagus_31, and DE576_A_0) were used for the analysis of intra-population genetic diversity. The 45 juvenile beech plants were selected from the population and dormant buds were collected from each individual for DNA isolation and PCR amplification. Fragment length sizing and allele determination of the obtained PCR products were performed using an automatic sequencer and data analysis was carried out using the *GenAlEx 6.5 software* (Peakall and Smouse 2006). The number of alleles per locus was in the range from 8 (sfc0036) to 27 (mfc5), with an average of 14. The number of effective alleles per locus was in the range from 5.287 (sfc0036) to 12.736 (mfc5), while the average number was 7.283. Both observed and expected heterozygosity in the studied population were high (the highest Ho = 0.822, at csolfagus19 and He = 0.921, at mfc5). The obtained data indicate a considerable amount of genetic diversity within the population „Crni vrh 2".

Keywords: *genetic structure, Fagus sylvatica L., molecular markers*

Correspondence: marina.nonic@sfb.bg.ac.rs

MIR396 KNOCKOUT VIA CRISPR TO ENHANCE EXPRESSION LEVELS OF GROWTH REGULATING FACTORS (GRFS) IN TOMATO

Yıldız AB[1], Cebrailoğlu N[1], Özden Çiftçi Y[1,2]

[1]*Plant Biotechnology Laboratory, Gebze Technical University, Gebze, Kocaeli, Turkey*

[2]*Gebze Technical University,, Smart Agriculture Research and Application Center, Gebze, Kocaeli, Turkey*

MicroRNAs (miRNAs) are non-coding small RNAs that usually comprised of 21-24 nucleotide length and as a matter of fact they can target specific genes, thus, have a critical role on gene regulation in plants. Among them, MiR396 is one of the conversed miRNAs in plants and has 60 members in various plant species. For now, only 2 members of miR396 have been identified in tomato, miR396a/b and even though they have similar names and similar effects on plants, each of them are expressed on different chromosome and are not complementary to each other. In this thesis, to improve tomato plants through growth regulator factors (GRFs) by the destruction of miR396, it is planned to optimize dual sgRNA CRISPR/Cas9 protocol for tomato. Since in both tomato and Arabidopsis miR396b targets GRFs, this miRNA has direct effect on plant growth and development, regulation of cell proliferation, size of the meristem and leaves, and number of stomata. Thus, CRISPR/Cas9 based gene editing, which is also applicable on tomato and results a permanent and complete destruction of miRNAs, is used to knockout miR396 by targeting both side of MIR gene of miR396b. As the destruction of these miRNA members would lead to physiological improvements in the tomato, the analysis and results will be easily visualized. The development of CRISPR-based protocols for plant breeding is already of great importance in the literature, as CRISPR-based crops are considered GEO (Genetically Edited Organisms) rather than GMOs (Genetically Modified Organisms) in many countries except European Union. This CRISPR-miRNA destruction protocol will not only be used in plant improvement studies in the future, but will also be a useful tool for functional studies on tomato.

Keywords: *CRISPR/Cas9, miRNA, tomato, GEO, plant biotechnology*

Correspondence: abyildiz@gtu.edu.tr

THE EFFECT OF PH AND TEMPERATURE ON PHOSPHATE SOLUBILIZATION POTENTIAL AND GROWTH OF A BACTERIAL STRAIN ISOLATED FROM MAIZE RHIZOSPHERE

Mantea LE, Stefan M

Alexandru Ioan Cuza University of Iasi, Faculty of Biology, Biology Department, Iasi, Romania

Although phosphorus (P) is an essential mineral for growth and development, it is unavailable to plants because is blocked in soil under the form of insoluble phosphates, especially due to the actual climate changes. In normal conditions, rhizospheric phosphate solubilizing bacteria (PSB) are capable to solubilize soil phosphates, increasing in this way P plants uptake. Therefore, PSB which tolerate the abiotic stress could be a reliable solution to the transformation of P into insoluble forms. In this context, the main goal of this study was to identify PSB which can tolerate pH and temperature stress and could be used as biofertilizers. Thus, PSB strains were isolated from the maize rhizosphere and further tested quantitatively in the presence of insoluble tricalcium phosphate (TCP) as the only source of phosphorus. The bacterial strain which solubilized the largest amount of TCP in normal conditions was subjected to abiotic stress caused by variations of pH (4.9, 7.2, 9) and temperature (20°, 28°, 37°). Ten of the isolated PSB strains solubilized P with amounts ranging between 9.82 and 17.06 µg P/ml. Lower P solubilization (9.76 µg/ml) was recorded for P2.1S strain at the initial medium pH of 4.9, compared with pH of 7.2 (17.94 µg/ml). There is no significant difference between the amount of P solubilized at pH 9 (19.38 µg/ml) and pH 7.2. In the case of temperature, the highest amount of phosphorus was recorded after culturing the P2.1S strain at 28°C (19.14 µg/ml). P2.1S strain solubilized TCP regardless of pH and temperature values tested, displaying a biotechnological potential. Further studies are needed to determine the mechanisms of P solubilization before using P2.1S strain as an inoculant for organic agriculture.

Correspondence: mantealoredana9@gmail.com

METAL TOLERANT RHIZOBACTERIA FROM KRIVAJA – KONJUH OPHIOLITE COMPLEX EXHIBIT MULTIPLE PGP TRAITS

Ahatović A, Hasanović M, Durmić-Pašić A

University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina

Rhizobacteria isolated from areas naturally contaminated with heavy metals, such as serpentine outcrops, commonly exhibit metal tolerance as they have adapted to these environments. Some strains of rhizobacteria can enhance plant growth and protect plants from stress through various plant-growth-promoting (PGP) mechanisms. Serpentine rhizobacteria expressing PGP traits have their potential use as biotechnological tools in bioremediation of soils artificially contaminated with heavy metals. The aim of our study was screening for PGP traits in metal tolerant rhizobacteria isolated from three sites in Krivaja – Konjuh ophiolite complex (Bosnia and Herzegovina) that showed extremely high Ni concentrations in soil. Total of 84 isolates were tested for their tolerance of heavy metals (Cu, Ni, Co). Isolates with strongest heavy metal tolerance (33) were initially screened for siderophore production and phosphate solubilization using qualitative methods. While 90.9% of tested isolates showed siderophore production, phosphate solubilization capability was less frequent (21.2%). According to the results of initial screening we selected isolates for downstream analyses. Further screening included quantitative analyses of siderophore and IAA (indole-acetic acid) production and qualitative analysis of ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity. Percentage of siderophore units ranged from 34.2% to even 96.6% with the highest values in two isolates from Bljuva. IAA concentration ranged from 10 to 901.4 µg/ml with the highest concentration in bacterial culture of isolate from Olovo. Where the ability of plants to handle stress is concerned, the most important PGP mechanism is ACC deaminase activity as this enzyme cleaves ACC which is a direct precursor of ethylene. ACC deaminase activity was observed in 22.2% of tested metal tolerant isolates. In addition to metal tolerance, all isolates showed at least two PGP traits. Isolates with expressed PGP traits will be further used for plant inoculation experiments in order to test their potential in promotion of host plant growth as well as plants’ heavy metal tolerance and accumulation.

Keywords: *rhizobacteria, serpentines, PGP, stress, heavy metals*

Correspondence: anesa.ahatovic@ingeb.unsa.ba

THE ROLE OF STANDARDIZATION, ACCREDITATION, CERTIFICATION BODIES AND LABORATORIES IN CONFORMITY ASSESSMENT OF BIO-BASED PRODUCTS

Skenderović E[1], Mujković M[2], Subašić E[2]

[1]*Institute for accreditation of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina*

[2]*Federal Institute of Agriculture Sarajevo, Sarajevo, Bosnia and Herzegovina*

Bio-based economy has recently been developing intensively as an alternative to the use of raw materials derived from oil, aiming to reduce emissions and protect the environment. Bio-based products are produced using raw materials from renewable sources and efforts are being made to ensure that the entire product lifecycle is sustainable. Although bio-based products currently have a small market share, their role is very significant due to the potential to solve environmental problems, e.g. partial replacement of fossil fuels with bio-based alternatives, use of bio-based chemicals, contribution to rural development, reduction of greenhouse gas emissions, etc. Significant amount of biomass in Bosnia and Herzegovina (BiH), that could be used as a feedstock in bio-based production, remain unused. In crop production alone, post-harvest residues in the fields were estimated at close to one million tonnes annually. Production of bio-based products based on biotechnological processes has already been commercialized in some biorefineries in EU (e.g. production of lignocellulosic bioethanol by enzymatic hydrolysis process). Furthermore, energy saving processes were introduced in all stages of production. In order to demonstrate that the process is sustainable and that the products are of bio-based origin, it is expected that there will be a growing interest in third-party confirmation using certification schemes. It is also important to label such products, to ensure their visibility and recognition on the market. To ensure that uniform criteria for conformity assessment of bio-based products is applied, interaction between national standardization body, accreditation body, product certification bodies and testing laboratories is needed. This paper presents the roles and relationships between the main actors in the conformity assessment of bio-based products.

Keywords: *bio-based economy, emissions reduction, standardization, accreditation, conformity assessment*

Correspondence: mirsad.mujkovic@fzzp.gov.ba

IN SILLICO EVALUATION OF THE APPLICATION OF MUSHROOMS IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

Hotić S

International Burch University, Faculty of Enineering and Natural Sciences, Department of Genetics and Bioengineering

The medicinal properties of mushrooms have been studied extensively in recent decades. The effects of their metabolites and other biomolecules have been correlated with a plethora of health benefits, such as anti-carcinogenic, anti-diabetic, anti-fatigue, antihypertensive, antihyperlipidemic, nephroprotective, improvement of cognitive function and mood disorders, and prolonged cell life. These benefits can be attributed to their immunomodulating, antioxidative, and anti-inflammatory effects. Moreover, biochemical activities of these compounds have proven to be beneficial in the prevention and treatments of neurodegenerative diseases. The ethology of neurodegenerative diseases is not completely understood, therefore conventional therapies for the management of NDs are not very effective. Due to this, finding new ways of treatment is paramount. Herein, we've conducted extensive in silico screening of potentially therapeutic mushroom derived biomolecules, and evaluated their biological activities using molecular docking and other methods vis-à-vis the relevant proteins that play a role in neurodegeneration. Interestingly, our results confirm the notion that mushrooms derived biomolecules can potentially play dynamic roles in both preventing and managing neurodegenerating symptoms by effecting the key players in neurodegenerative pathology molecular pathways. Though our initial screening revealed dozens of potential candidates, we opted to include those biomolecules that were implicated in the most well described relevant molecular pathways whose propagation leads to neurodegeneration. Particularly, several compounds could play major roles in beta-amyloid neurotoxicity alongside indirectly preventing neurodegeneration by way of immunomodulation.

Correspondence: sarahbbhotic@gmail.com

PLANT CLONING

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Thermo Fisher Scientific is committed to providing instruments, reagents, and solutions for plant and animal genotyping applications: simple, scalable, and affordable next-generation solutions that will help drive remarkable agricultural innovations. These solutions enable producers to develop healthier, more efficient crops and livestock to help nourish the world's growing population. Cloning plant DNA is a key step for genetic engineering, gene studies, and other applications in plant research, both in discovery and applied settings. Plant biotechnology continues to create new opportunities in agriculture, environmental biology, chemical production, and medicine. The development of genetically modified crops with valued traits like herbicide and pest resistance, drought and salt tolerance, or increased yield requires different combinations of expression elements to achieve optimum effects. Applications like engineering plants for phytoremediation or biofuel research often involve complex design and assembly of DNA elements that require advanced cloning tools. Moreover, if the ultimate goal is expression of foreign genes in plants, there are more extensive technical challenges in plant systems than are encountered with other heterologous expression systems. Our latest technology in Thermo Fisher Scientific for cloning complex DNA elements simultaneously. The construction of T-DNA vectors or vectors for particle bombardment or electroporation in plants cells is now easier with two new cloning and DNA assembly platforms. Make multiple vectors to test the effect of regulatory sequences in your plant of interest, or stack traits on the same expression vector for crop enhancement.

Correspondence: harun.ganic@biosistemigrupa.com

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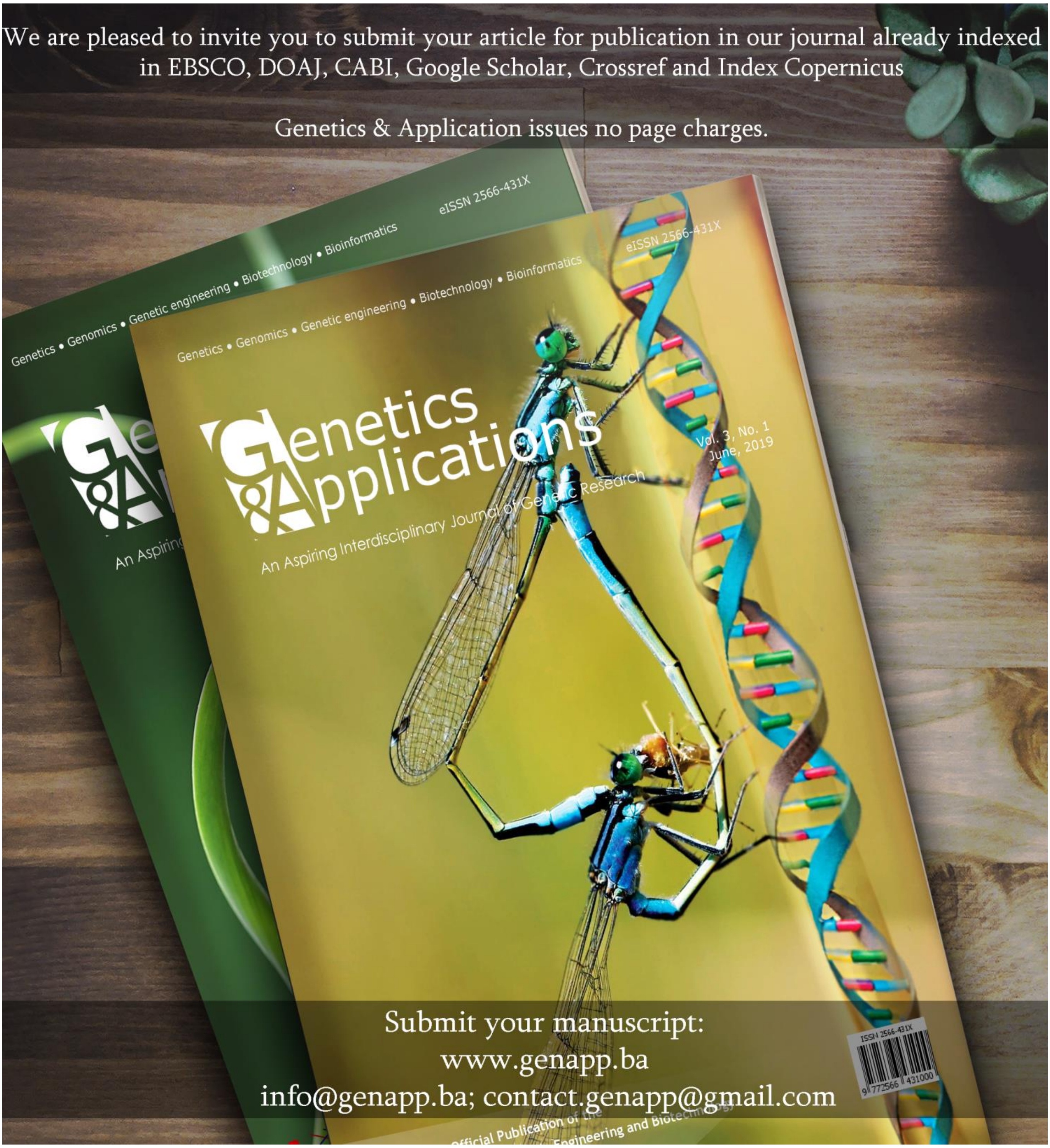
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