Institute of Cell Biology and Genetic Engineering
NAS of Ukraine
Ukrainian Radiobiological Society

10th International Meeting on Recent Advances in Plant Biotechnology

25-26 June, 2024
Kyiv, Ukraine

Dear Colleagues:

We are pleased to invite you to take part in the 10th International Meeting on Recent Advances in Plant Biotechnology (RAPB 2024) **dedicated to the 75th anniversary** of the prominent scientist and the founder of Institute Cell Biology and Genetic Engineering (Kyiv, Ukraine) as well as Icon Genetics GmbH, Nomad Bioscience GmbH and Nambawan GmbH (Halle, Germany) **Professor, Dr. of Science Yuri Gleba**.

RAPB 2024 will be held on **25-26 June 2024**, in **Kyiv, Ukraine**. This conference continues the tradition of the Meetings which have been already organized by scientists of the Institute of Cell Biology and Genetic Engineering NAS of Ukraine since 1986. Despite russia's brutal aggression against Ukraine, we continue our work for the development of plant biotechnology in our country. So we hope RAPB 2024 will become an event which will bring together scientists who are working in the fields of **plant genetic engineering** and **plant biotechnology**.

Welcome to the RAPB 2024!

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Organizing committee is whole-heartedly grateful:

- for the all Speakers and Participants as only our joint efforts made this Meeting possible, important and interesting one

- for Ukrainian Scientific Institute of Plant Breeding (VNIS) for their substantial support in any possible way

- for Kyiv Palace of Children and Youth for providing the opportunity to broadcast the Meeting while Kyiv was suffering from long-term power outages

- for the Armed Forces of Ukraine as we owe them every hour of this Meeting and every second of our lives

Tuesday, June 25, 2024 (offline and online)

Plenary Session.
Moderator - Mykola Kuchuk

09:00–09:20 — **Mykola Kuchuk,** Welcome speech
09:20–10:20 — **Yuri Gleba,** 50 Years of Plant Biotechnology
10:20–11:00 — **Julian Ma,** The development of a next generation of recombinant antibodies using plant biotechnologies

11:00 – 11:15 — Coffee break

Session MF. Molecular Farming
Moderator - Mykola Borysyuk

11:15–11:40 — **Anatoli Giritch**, NOMAD Bioscience: Plant-Made Recombinant Proteins for Medical, Food and Technical Applications
11:40–12:05 — **René Schlesier**, Plant-produced lectins for prevention and therapy of respiratory diseases
12:05–12:30 —**Franziska Jarczowski**, Made in … plants: Reinventing the Wheel or Developing New Pharmaceutical Products
12:30–12:55 — **Frank Thieme**, Icon Genetics: A Pioneer in Plant-Based Biotechnology
12:55–13:15 — **Olga Ovcharenko**, Biologically-active recombinant human interferon in "long-shelf life" tomatoes
13:15 –13:35 — **Natalia Shcherbak** , Antibacterial activity of recombinant colicin M in edible transgenic plants
13:35–13:55 — **Valeria Tonova**, Plant-produced SARS CoV-2 Nucleocapsid and chimeric RBP as diagnostic reagent candidates
13:55–14:15 — **Vaiva Kazanavičiūtė**, Plant-expressed antimicrobial against Cutibacterium acnes

14:15 – 14:40 — Coffee break

Session GE. Genetic Engineering
Moderator - Victor Klimyuk

14:45–15:15 — **Yaroslav Blume**, With the permission of microtubules: paradigma of lucky 13 protofilaments for cell biology
15:15–15:45 — **Mykola Borysyuk**, Modulation of leaf surface features for improved wheat drought tolerance
15:45–16:15 — **Volodymyr Sidorov**, In vitro floral culture for transformation/editing

Wednesday, June 26, 2024 (on-line)

Session GE. Genetic Engineering (and a little bit MF)
Moderator – Kateryna Lystvan

09:20–09:50 **Bogdan Morgun**, Genetic improvement of cereals to ensure foor secutiry
09:50–10:25 — **Olena Kischenko**, Transient expression of antigens from koi herpesvirus in duckweed
10:25–10:50 — **Volodymyr Radchuk**, Understanding mechanisms of grain filling for yield improvement in cereals
10:50–11:15 — **Stefano Torti**, Transient reprogramming of crop plants for agronomic performance.
11:15–11:40 — **Stanislav Isaenkov**, Exploration Genetic Potential of Halophytic Barley Relatives as a Promising Source of Salinity Tolerance for Modern Crops

11:40 – 11:55 — Coffee break

Session E. Epigenetics
Moderator - Stanislav Isaenkov

11:55–12:35 — **Etienne Bucher**, Crop genome and epigenome dynamics in the context of climate change
12:35–12:55 — **Alexandra Kravets**, Epigenetics reveals better genomes
12:55–13:15 — **Iryna Zhuk**, Transgenerational effect of oxalic acid and sodium nitroprusside as elicitors in Triticum aestivum
13:15–13:35 — **Sergii Litvinov**, Ionizing radiation affects aggregated proteins and amyloidogenesis in *Pisum sativum* l.

Session SM. Secondary Metabolism
Moderator - Stanislav Isaenkov

13:35–14:05 —**Adam Matkowski**, Recent advances in polyphenols biotechnology

14:05 – 14:20 — Coffee break

14:20 –14:50 — **Sylvestre Marillonnet**, Tools and strategies for engineering of anthocyanin and betalain biosynthetic pathways in plants
14:50–15:20 — **Sylwia Zielinska**, Recent advances in Papaveraceae biotechnology
15:20–15:40 — **Anton Stepanenko**, Resolving the status of the duckweed genus *Lemna*, section Alatae, based on sequence and karyotype variability
15:40–15:55 — **Carmen Laezza**, Elicited callus cultures from apple peel: a unique reservoir of antioxidants

Sessions E + GE (continued)

15:55–16:25 — **Igor Kovalchuk**, Epigenetic regulation of transgenerational response to stress
16:25–16:55 – **Andrii Bilichak**, Implementation of the CRISPR/Cas9-mediated gene editing in spring wheat (*Triticum aestivum* L.) – challenges and opportunities
16:55–17:25 - **George Rudenko**, Development and Manufacturing of Medicines with Synthetic Biology

50 YEARS OF PLANT BIOTECHNOLOGY

By Yuri GLEBA

The presentation is a personal, biased, fragmentated and short (one year per minute) account of my colleagues' and my presence in plant biotechnology over periods of my work at the Institute of *Cell Biology & Genetic Engineering,* Ukraine, *Icon Genetics,* USA, Germany, *Nomad Bioscience,* Germany, Lithuania, *Nambawan Biotech,* Germany, Spain and *Ethnos Kalos,* Germany (1974 -2024). Colleagues' names are not mentioned to preserve equidistance and semblance of fairness to hundreds of people I've had an honour and joy to work with. It's about ideas, science, but mostly about search for technologies and products that people need (or want). It is pre-'genome editing' so should be boring to younger colleagues, but do remember: 'if you don't go to other people's funerals, thy won't come to yours' (graffito).

THE DEVELOPMENT OF A NEXT GENERATION OF RECOMBINANT ANTIBODIES USING PLANT BIOTECHNOLOGIES

Julian K-C. Ma

Institute for Infection and Immunity, St. George's, Univ. of London, UK

The first products of molecular pharming (the manufacture of recombinant pharmaceutical biologics using plant biotechnology) are now on the market and many others are in the pipeline. In Europe, regulatory approval of a manufacturing process for monoclonal antibodies in tobacco plants in 2015 dispelled concerns around quality control of plant-derived biologics. In 2022 a plant-made SARS-CoV-2 vaccine completed clinical trials and was authorised for use. Regulatory hurdles are not the major barrier that many originally feared and whole plant manufacturing systems have been shown to match conventional cell culture based production for quality and reproducibility.

The focus now for Molecular Pharming is to identify the products for which plant manufacturing offers significant advantage over other manufacturing platforms. This may be speed, cost and scalability, or it could be pharmaceutical molecules that are unattainable by other systems. This talk will explore how recent developments in molecular pharming can provide solutions, particularly for complex recombinant antibodies that are not possible to produce by other manufacturing platforms. Recent developments in the expression of secretory IgA antibodies for the control of infection at mucosal surfaces, could introduce a new class of protein biologic to impact on global health.

NOMAD BIOSCIENCE: PLANT-MADE RECOMBINANT PROTEINS FOR MEDICAL, FOOD AND TECHNICAL APPLICATIONS

Anatoli Giritch and Yuri Gleba

NOMAD Bioscience GmbH, Halle (Saale), Germany

NOMAD Bioscience is a plant biotechnology company founded in 2008 in Halle/Saale, Germany as a split-off of ICON Genetics GmbH. The company has its fully owned subsidiary UAB Nomads in Vilnius, Lithuania.

NOMAD is active in the field of Plant Molecular Farming (PMF) - production of recombinant proteins in plants. The company utilizes several platforms based on transient, inducible and constitutive expression in leaves and seeds of green plants.

Nomad explored plant-based expression systems for production of various recombinant proteins including antimicrobials, antivirals, sweet-tasting proteins, technical enzymes, etc. as well as for transient delivery of agronomic traits.

Currently, NOMAD focuses on development of several products for different markets including 1) antimicrobial proteins (bacteriocins) for food safety and medicinal use, 2) antiviral proteins for medicine and 3) sweet-tasting proteins (thaumatins and brazzeins) for food industry. A number of corresponding transgenic hosts were created.

NOMAD's recombinant bacteriocins to control *Escherichia coli* and *Salmonella* during food processing were designated as GRAS (Generally Recognized as Safe) products by US Food and Drug Administration (FDA). Similarly, NOMAD's recombinant sweet-tasting protein thaumatin obtained GRAS status from US FDA and FEMA (Flavor and Extract Manufacturers Association of the United States) to be used as a sweetener and a taste modifier. GRAS regulatory approval paves the way towards industrial production and commercialization of these products.

Nomad has created transgenic tobacco host and developed upstream and downstream processes for seed-based production of thaumatin II. For the past three years, Nomad has been conducting ongoing industrial greenhouse and open field trials on growing transgenic tobacco expressing recombinant thaumatin in seeds. For industrial production and commercialization of sweet-tasting proteins, the split-off company NAMBAWAN Biotech (Halle, Germany) with its fully owned subsidiary NAMBAWAN Spain (Badajoz, Spain) were created.

PLANT-PRODUCED LECTINS FOR PREVENTION AND THERAPY OF RESPIRATORY DISEASES

René Schlesier, Stefano Torti, Anka Thümmler, Anett Stephan, Anatoli Giritch, Yuri Gleba

NOMAD Bioscience GmbH, Halle (Saale), Germany

Lectins are naturally occurring sugar binding proteins, discovered in many organisms (e.g., animals, plants, algae). Some of them are being extracted from edible plants or algae. Based on their ability to bind glycosylated molecules or surfaces with glycans, lectins have several potentially useful therapeutic and preventive properties including antiviral, antibacterial, insecticidal activities; there are also known to exert antitumoral and mitogenic effects. It was shown that some lectins have antiviral activity, at as low as nanomolar concentrations, against coronaviruses, influenza viruses, Ebola virus, and HIV even. Using TMV and novel PVX vector constructs, we were able to transiently express several algal and plant lectins in both *Nicotiana benthamiana* and crop plants. Two plant-produced algae lectins were extracted, purified in high amounts and examined in more detail for their antiviral activity. Using preclinical analyzes including analytical characterization, *in vitro* and cell-based evaluation of antiviral efficacy, *in vitro* toxicity studies in cell culture, and *in vivo* efficacy studies in animal model we could show that both lectins interact strongly to the spike proteins of several respiratory viruses and thus reduce their infectious potential. Moreover, the stability of these lectins in physiological solutions and their mode of action allow an easy and fast application by spraying or inhalation without medical care. We can show that our transient transformation procedure offers the possibility to produce high amounts of lectins in a fast, flexible, and cost-effective way allowing a fast and uncomplicated usage of antiviral lectins as preventive or therapeutic agents if there is a suspicion of a possible infection or an infection that has already occurred.

MADE IN … PLANTS: REINVENTING THE WHEEL OR DEVELOPING NEW PHARMACEUTICAL PRODUCTS

Franziska Jarczowski

Icon Genetics GmbH, Halle (Saale), Germany

Plants have been the source of medicines since ancient times. They are specialists in organic biosynthesis of extremely complex cyclic organic compounds with pharmaceutical activity. In recent decades, however, other classes of molecules, such as proteins and RNA, have been used with great success in medical treatment. For some time now, plants were used as production hosts for recombinant proteins. With some detours, plant biomass can be introduced into standardized biotechnological platform processes for production under current Good Manufacturing Practice (GMP).

Icon Genetics' Head of Manufacturing responsible for GMP-compliant process development and production at one of the world's leading manufacturers for plant-made recombinant proteins will give her personal perspective on the key questions: Where are we today? What are the challenges? Where have we discovered that plant expression can be used to create meaningful products that are truly game-changers?

ICON GENETICS: A PIONEER IN PLANT-BASED BIOTECHNOLOGY

Frank Thieme, Victor Klimyuk

Icon Genetics GmbH, Halle (Saale), Germany

Icon Genetics is a fully integrated clinical stage biotech company that specializes in the production of recombinant proteins in green plants. The company has a dominant patent portfolio for plant-based expression and its application. In its 25-year history, Icon Genetics has achieved several key milestones, including the development of the magnICON® plant-based transient expression technology, the development and clinical testing of personalized cancer treatments, and the development and clinical testing of a norovirus vaccine. The presentation provides an overview of the company's history, capabilities, and achievements, as well as its research on virus-like particles, the development of a bivalent vaccine candidate and the results of the first-in-human phase I study in more detail.

PLANT-EXPRESSED ANTIMICROBIAL AGAINST *CUTIBACTERIUM ACNES*

Vaiva Kazanavičiūtė, Audrius Misiūnas, Aušra Ražanskienė

UAB Nomads, Vilnius, Lithuania

Cutibacterium acnes is a gram-positive bacterium associated with skin acne. Traditional acne treatments often involve antibiotics, which can lead to resistance. This study explores the potential of a plant-expressed antimicrobial protein, ALT, as an alternative treatment for acne.

ALT protein, containing peptidoglycan binding and glycosyl hydrolase family 25 (GH25 BacA-like) domains, was expressed in plants and purified. The antibacterial activity of ALT was evaluated against eleven different strains of *C. acnes*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined, and a semi-quantitative spot on lawn activity assay was conducted. Protein stability was assessed at various temperatures (4°C to 40°C) over extended periods.

The MIC of ALT against *C. acnes* ranged from 0,24 ng/ml to 2 ng/ml, and the MBC ranged from 0,24 ng/ml to 8 ng/ml. The spot on lawn assay demonstrated antibacterial activity at concentrations between 0,4 µg/ml and 1,5 µg/ml. Stability studies showed that ALT retained antibacterial activity after incubation at 4°C and 22°C for 13 months, and at 37°C and 40°C for 3 months. Noteworthy, antibacterial activity of protein was observed at concentration of 6,2 µg/ml after incubation at 37°C for three months and at concentration of 12,5 µg/ml after incubation at 40°C for three months. Importantly, ALT was not active against *Staphylococcus epidermidis*, *Staphylococcus aureus*, or *Cutibacterium granulosum* bacteria.

ALT is a stable, plant-expressed protein with specific antibacterial activity against *C. acnes*. This protein has the potential to be developed as an alternative to traditional antibiotics for the treatment of acne, addressing the need for novel treatments in the face of increasing antibiotic resistance.

TRANSIENT EXPRESSION OF ANTIGENS FROM KOI HERPESVIRUS IN DUCKWEED

Olena Kishchenko[1,2], Anton Peterson[1,2], Anatoli Giritch[3], Ingo Schubert[2] and Manuela Nagel[2]

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[3] NOMAD Bioscience GmbH, Halle / Saale, Germany

Vaccination is an important integral part of health management, also concerning intensive production systems such as aquaculture. Oral delivery of vaccines is recognized currently as potentially a better approach for mass vaccination of fish than the commonly used injection and emersion, which are time-consuming, labor-intensive and highly stressful for fish (Mičúchová et al., 2022). Plant-made vaccines provide natural bio-encapsulation of expressed recombinant proteins that are analogs of pathogen antigens, and, therefore, are of high gastrointestinal stability if delivered orally, and can be easily stored and transported without requiring cooling. Lemnaceae, commonly called duckweed, are efficient host organisms for transient expression of recombinant proteins under fully contained, controlled, and aseptic conditions that can be easily adapted to fulfil the requirements of bio-safety, environmental protection and current Good Manufacturing Practice (Peterson et al., 2021). As platform for oral vaccines, duckweeds have some advantages over majority of other plants used for plant-based recombinant protein production: 1) easy propagation and high growth rate; 2) a high protein content and high nutritional value; 3) natural feed component for herbivorous fish species including carps (common carp, grass carp) and tilapia; 4) duckweed biomass contains compounds, such as the pectic polysaccharide lemnan, which appeared to be useful as a natural adjuvant for oral immunization, as reported for mice.

Here, we report *Agrobacterium*-mediated transient expression of recombinant proteins - analogs of antigens from koi herpesvirus KHV. KHV causes highly contagious koi herpesvirus disease that leads to mass mortality in carp aquaculture worldwide. The envelope proteins ORF25, ORF81, ORF131, ORF136 and ORF149, and the capsid proteins ORF72 and ORF92 of KHV were chosen for expression in duckweed because of their potential immunogenic activity. Coding sequences of these antigens were codon optimized, fused with 6His-tag and cloned into based on potato virus X deconstructed vector backbone for transient expression (provided by the Nomad Bioscience GmbH). Two expression vectors harboring coding sequences for fused *ORFs ORF136::ORF72* and *ORF25::ORF81* were also assembled. Transient expression of the recombinant antigens in duckweed *Landoltia punctata* 7260 was analyzed by SDS-PAGE and Western Blot using anty-6His-tag monoclonal antibodies and polyclonal antibodies specific to cloned KHV antigens (provided by Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health).

Western Blot analysis confirmed accumulation of serologically recognizable aggregates and products of partial degradation of the target recombinant antigens in duckweed biomass, except ORF92. After transient expression of fused ORF136::ORF72, both recombinant products were detected by SDS-PAGE analysis (≈40 kDa corresponding to ORF72, and ≈17kDa corresponding to ORF136) as specific major bands, indicating high level accumulation of non-degraded recombinant proteins.

This work is dedicated by the authors to Prof. Yuri Gleba at the occasion of the 75th anniversary of his birthday

References

Mičúchová, A., Piačková, V., Frébort, I., & Korytář, T. (2022). Molecular farming: Expanding the field of edible vaccines for sustainable fish aquaculture. *Reviews in Aquaculture*, *14*(4), 1978–2001. doi:10.1111/raq.12683.

Peterson, A., Kishchenko, O., Zhou, Y., Vasylenko, M., Giritch, A., Sun, J., et al. (2021). Robust Agrobacterium-Mediated Transient Expression in Two Duckweed Species (Lemnaceae) Directed by Non-replicating, Replicating, and Cell-to-Cell Spreading Vectors. *Front. Bioeng. Biotechnol.* 9, 761073. doi:10.3389/fbioe.2021.761073.

DEVELOPMENT AND MANUFACTURING OF MEDICINES WITH SYNTHETIC BIOLOGY

George Rudenko

Purissima (United States)

The development of technologies for a sustainable, secure, low-cost, and scalable supply of established and emerging plant-based medicines, as well as novel chemical scaffolds for new therapeutics, has a major impact on drug development, drug discovery, and overall human health and wellness. With over 15,000 medicinal plants threatened by extinction due to global warming and intensive agricultural practices, access to natural therapeutic producers is becoming a significant hurdle for the pharmaceutical industry. As a viable solution, Purissima has developed a biosynthetic platform utilizing microalgae—single-celled, plant-like organisms—as a chassis for the discovery and manufacturing of diverse, complex chemical scaffolds. Microalgae are ideal for supporting plant gene-based biosynthesis and are well-suited for the efficient large-scale production of plant-derived chemical scaffolds using industrial fermentation.The initial focus was on the production of meroterpenoids, a diverse group of prenylated plant lipids that include phytocannabinoids and related compounds. These compounds possess pain-relieving, antiviral, antibacterial, and anticancer properties, and could find applications in the treatment of a broad range of neurological and metabolic diseases. Overall, this new technology enables year-round, market-responsive, and consistent production of valuable active pharmaceutical ingredients (APIs). It promises to broadly transform the approaches to the provision, discovery, and development of essential medicines and future drug candidates.

ANTIBACTERIAL ACTIVITY OF THE RECOMBINANT COLICIN M IN EDIBLE TRANSGENIC PLANTS

Nataliia Shcherbak[1], Heike Prochaska[2], Kateryna Lystvan[1], Olga Ovcharenko[1], Yelizaveta Prokhorova[1], Anatoli Giritch[2] and Mykola Kuchuk[1]

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Bacteriocins comprise a large family of antimicrobial proteins produced by bacteria that exhibit bactericidal activity against closely related bacterial strains. Bacteriocins are notable for their specificity which makes them a potential alternative to traditional antibiotics. Colicin M (ColM) is a type of bacteriocin produced by *Escherichia coli* - a unique member of colicins family, characterized by its ability to disrupt the integrity of bacterial cell walls. This property makes it more effective in controlling clinically prominent pathogenic strains than other colicins. Recombinant ColM is particularly of interest in the food industry for their potential to control foodborne pathogens. In plants ColM was first produced in *Nicotiana* species using transient gene expression and genetic transformation methods. Antibacterial activity of purified plant-produced ColM was confirmed against a variety of pathogenic strains, including clinical isolates of *E. coli* and *Klebsiella pneumonia*. However, the stable expression of antimicrobial proteins in transgenic edible plants allows the use of such vegetables as a ready-to-use antibacterial food or feed additive without prior purification and preparation of a protein product.

The goal of our work was to create edible transgenic plants producing recombinant ColM and thus obtain the plants with antimicrobial quality. Transgenic plants of several edible species were developed using *Agrobacterium*-mediated transformation with a vector containing the ColM-coding gene. Extracts of transgenic lettuce, mizuna, kale and carrot expressing ColM showed essential activity against two strains of enterohaemorrhagic *E. coli* (O157:H7 and O104:H4) as well as three multidrug-resistant strains of *E. coli* that produce beta-lactamases and carbapenemases. Results of our study showed that the antibacterial activity persists across generations of transgenic edible plants carrying the ColM gene. Western blot analysis was performed in transgenic plants and their descendants to confirm transgene expression and quantify ColM accumulation. Our results also show that the antibacterial activity of dried (up to 40°C) biomass of transgenic plants remained stable without a decrease for at least three months.

Our work demonstrated the potential of edible plants expressing ColM to be used as food or feed additives effective against pathogenic and multidrug-resistant *E. coli* strains.

PRODUCTION OF BIOLOGICALLY ACTIVE HUMAN INTERFERON A-2B IN TRANSGENIC "LONG-SHELF LIFE" TOMATO AND ITS INFLUENCE ON IMMUNOLOGICAL CHARACTERISTICS IN MICE

Olga Ovcharenko[1], Volodymyr Rudas[1], Nadiya Zholobak[2], Mykhaylo Guzyk[3], Olha Yaroshko[1], Elyzaveta Zozulya[4], Vladyslav Moseiko[4], Natalya Shcherbak[1], Bogdan Morgun[1], Mykola Kuchuk[1]

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Human interferon α-2b (HuIFNα-2b) belongs to type I of interferons that enhance the immune response, inhibit viral replication and cell growth, and therefore have many clinical applications. Plants of *Solanum lycopersicum* L. cv. Shedevr 1 with "long shelf life" fruits were used for the transformation with *HuIFNα-2b* gene. Among other host plants, used for biopharming of this interferon, tomatoes used in our research have a number of advantages. High level biological activity of the protein of interest in tomato tissues, its long term stability in stored fruits as well as transmission of transgenic traits through generations were confirmed. Beneficial antiviral influences of orally administered plant-based HuIFN α-2b from fresh tomato fruits on survival and immunological characteristics of VSV-infected balb/c mice were demonstrated.

BIOLOGICAL ACTIVITY OF COLICIN M-CONTAINED PLANT EXTRACTS OF TRANSGENIC LETTUCE (*LACTUCA SATIVA* L.) T_1 GENERATION

Valeriia Halkina, Nataliia Shcherbak, Mykola Kuchuk

Institute of Cell Biology and Genetic Engineering of the NAS of Ukraine, Kyiv, Ukraine

Food products contaminated with enterohemorrhagic *Escherichia col*i are one of the main causes of bacterial intestinal infections worldwide. Currently, except for thermal inactivation, there are no effective methods of combating pathogenic bacteria in food products. Since green leafy vegetables are the frequent source of pathogenic *E. coli* infections, we obtained transgenic lettuce (*Lactuca sativa L.*) plants producing recombinant colicin M - an antibacterial protein that specifically destroys disease-causing bacterial strains.

In this work, we studied the offspring of the obtained transgenic lettuce plants, namely, the preservation of antibacterial activity in subsequent generations of transgenic lettuce. In addition, to strain *E. coli* XL-1 blue used in previous experiments, we also tested three more *E. coli* strains: Neb turbo, BL 1 and DB 3.1. It was demonstrated that strain DB 3.1 as well as XL-1 blue was sensitive to colicin M-containing transgenic plant extracts while Neb turbo and BL were resistant to colicin M. Antibacterial activity of extracts of the T_1 generation plants from 8 transgenic lettuce lines against *E. coli* strains DB 3.1 and XL-1 blue were analyzed by a spot-on-lawn soft-agar overlay assay. Our results show that two lettuce transgenic line retained significant antibacterial activity in all plants of the T_1 generation. In six transgenic lettuce lines, a split ratio of approximately 3:1 was observed.

We also conducted a study to examine the ability of the colicin M-containing transgenic plant extracts to inhibit the growth of suspension culture of sensitive bacterial strains. An overnight bacterial culture of *E. coli* strains XL-1 blue and DB 3.1 diluted to OD=0.01 was used for analysis. Plant extracts were added to the bacterial suspension in ratios of 1:5, 1:10, 1:50 and 1:100 (extract:suspension). We carried out measurements of the optical density of bacterial suspensions every 40 minutes and made 5 consecutive measurements. The colicin M-containing transgenic plant extracts showed suppression of bacterial growth compared to the control.

In summary, we demonstrate sensitivity to plant-produced colicin M of laboratory *E. coli* strain DB 3.1. We also confirmed the inheritance of the heterologous colicin M gene and the antibacterial activity of extracts from the generation of transgenic T_1 lettuce plants both on agar medium and in suspension culture.

COLICIN PRODUCTION IN *NICOTIANA BENTHAMIANA* PLANTS IS DETERMINED BY THE EFFICIENCY OF GENETIC EXPRESSION CASSETTES USED FOR TRANSIENT GENE EXPRESSION

Yana Sindarovska, Diana Kulybko, and Mykola Kuchuk.

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Plant cells can be a good system for obtaining of valuable recombinant proteins as they are low-cost eukaryotic systems free from human pathogens and bacterial toxins, and they can produce physiologically active complex proteins. One of the ways to produce recombinant proteins in plants is *Agrobacterium*-mediated transient gene expression (TGE) which allows to increase the levels of target proteins. In this study we tested efficiency of two genetic expression cassettes carrying the *cma* gene coding colicin M protein (bacteriocin) in *Nicotiana benthamiana* plants through TGE technology. In a simple vector system (35S-Col), the target gene expression was controlled by viral 35S CaMV promoter and bacterial nopaline synthase terminator. While in an advanced vector system (PVX-Col), the target gene expression was controlled by multiple genome elements of potato virus X. The expression vectors were incorporated in *A. tumefaciens* cells and delivered into *N. benthamiana* plant cells by agroinfiltration. Colicin activity was measured by the inhibition of bacterial cell growth of *Escherichia coli* strain XL1Blue: by measuring of an inhibition zone diameter on agar nutrient media (for the leaf cuts and plant protein extracts), and by measuring the OD600 of liquid bacterial suspension grown in presence of protein extracts. We confirmed that both genetic constructs were active in plant cells and produced physiologically active recombinant colicin. We determined that the colicin levels were slightly varied depending on the used constructs. Approximate estimation of recombinant colicin level showed that about 1.2-2.5% of total soluble protein (TSP) (100–200 μg/g of fresh weight (FW) can be obtained with 35S-Col system, and about 1.9-3.8% of TSP (150–300 μg/g FW) can be obtained with PVX-Col system. We made several dilutions of the protein extracts and found that extracts diluted 200-fold still inhibited the *E. coli* growth. All dilutions of the initial extracts showed that the PVX-Col system had stronger (approximately 12-18%) inhibition of bacterial growth than the 35S-Col system. 100-fold and 200-fold diluted extracts (prepared from leaves infected with 35S-Col and PVX-Col expression cassettes, respectively) reduced bacterial growth in liquid culture by approximately 70%, while control protein extracts from uninfected leaves didn't inhibit bacterial growth. We estimate that purified plant-made colicin is a more active biomolecule than kanamycin (an antibiotic) because it inhibits the growth of *E. coli* with the same efficiency at the concentrations that are 100-200 times lower. We also observed that storage of protein extracts at +4^{o}C for two months reduced drastically colicin activity. In conclusions we elucidated that the levels of recombinant colicin production in plants through TGE depend on the expression cassette used; we confirmed that plant-made colicin inhibits growth of *E. coli* in liquid and agar nutrient media and it is a more active biomolecule than kanamycin; we also concluded that colicin M is unstable protein and must be storage under special conditions.

NICOTIANA BENTHAMIANA ROOT CULTURES: A PLATFORM FOR RECOMBINANT PROTEIN PRODUCTION AND FUNDAMENTAL RESEARCH

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Nicotiana benthamiana is a model plant species commonly used for recombinant protein production through transient gene expression (TGE) technology, especially due to its high sensitivity to a wide range of plant viruses. Recently, we established *in vitro* culture of *N. benthamiana* plants that produces recombinant GFP through TGE, and high GFP levels were detected both in plant leaves, and roots [1]. While the production of recombinant proteins in stably transformed hairy root systems was elucidated in many publications, the production of recombinant proteins in root systems through TGE is poorly studied. The aim of the study was to establish untransformed root cultures and hairy root cultures from *N. benthamiana* plants and assess them as systems for recombinant protein production through TGE. To obtain untransformed root cultures producing GFP through TGE, we detached roots of plants growing *in vitro* and producing GFP in their roots due to the systemic distribution of PVX-based expression vector carrying the *gfp* gene. To obtain hairy root cultures we transformed leaves of plants growing *in vitro* and producing GFP through TGE with wild type soil bacteria *Rhizobium rhizogenes* strain A4. GFP production in hairy root culture appeared due to systemic distribution of PVX-based expression vector carrying the *gfp* gene. Then roots were transferred on nutrient media for growth and further experiments. As a result of the work, we obtained untransformed root cultures and hairy root cultures of *N. benthamiana* producing recombinant GFP through TGE. We noticed that untransformed root cultures can grow on MS or ½ MS nutrient media without adding phytohormones. Adding phytohormones (BAP & NAA) promoted formation of brown calli on the roots and rapid decrease of GFP production. Cultivation of untransformed root cultures on ½ MS liquid nutrient medium with continuous shaking promoted fast root growth and rapid decrease of GFP expression as well. In contrast, untransformed roots cultivated on MS agar nutrient medium without phytohormones showed a slow rate of root growth, but GFP expression was continuous and PVX-based vector distribution followed the growth of the root apical meristem. Moreover, under light conditions green (chlorophyll-containing) compact calli expressing high levels of GFP were formed on the untransformed roots. The untransformed roots maintained on MS agar nutrient medium under light conditions were white with green areas. We also obtained hairy roots expressing GFP through TGE from the leaves of *N. benthamiana* plants producing GFP. During the study, we observed that gene silencing occurred in the cultures, and GFP production ceased in old roots, while it was still visible in young roots. Our results showed that root systems must be improved to prevent gene silencing and stabilize uniform expression of recombinant proteins in all cells. On the other hand, these root systems can be used for fundamental research, for example in virology.

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OBTAINING OF TRANSGENIC TOBACCO PLANTS WITH SALMOCIN GENE UNDER THE CONTROL OF THE ETHANOL-INDUCIBLE *ALC* GENE-EXPRESSION SYSTEM

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Plant biotechnology has taken various directions, including obtaining edible vaccines, pharmacological proteins, crop improvement, etc. However, strong constitutive promoters using due to control gene expression do not always provide the required amount of the target protein. Therefore, the gene-of-interest designs may often include tissue-specific or inducible promoters. It makes it possible to regulate the time of gene expression and enrich targeted protein accumulation, also avoiding potential cytotoxicity and gene silencing. For example, the ethanol-inducible gene regulation system is both flexible and accessible and could be used as one of the approaches [1].

Bacterial resistance to antibiotics has been considered a global public health problem. The constant increase in the number of multi-resistant bacteria has become a challenge both in public health and in veterinary medicine or agriculture [2]. Therefore, it is extremely important to identify and produce recombinant proteins with antimicrobial properties, such as bacteriocins, in plants. Recently, several specific bacteriocins derived from *Salmonella enterica* — the salmocins — have been discovered. Also, it was shown that plant-driven salmocines have a broad spectrum of activity against different strains of *E. coli* and the main pathogenic serotypes of *Salmonella enterica* ssp. *enterica* [3].

The aim of the work was to obtain transgenic tobacco plants with salmocin gene under the control of the ethanol-inducible *alc* gene regulation system. The vector kindly provided by Nomad Bioscience GmbH also contained viral polymerase sequences (TMV-based binary vector) allowing the accumulation of large amounts of recombinant protein after ethanol induction. Transgenic plants were obtained by tobacco leaf discs transforming using *Agrobacterium tumefaciens* GV3101 strain. The PCR results confirmed the presence of the salmocin gene in the tobacco plants regenerated on the selection medium. After treating of transgenic tobacco plants with ethanol vapor, the antibacterial activity of the extracts against *E. coli* strains XL1-blue and BL21 was observed. These results allow us to confirm the ethanol-dependent expression of the salmocin gene under the control of *alc* promoter. However, further research is necessary to investigate the possibility of using *Salmonella`s* bacteriocin-expressing transgenic plants to fight these pathogenic bacteria.

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IN VITRO FLORAL CULTURE FOR TRANSFORMATION/EDITING

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New transformation system based on *Agrobacterium*-mediated transformation of *in vitro* floral culture was developed and demonstrated for quinoa and *Arabidopsis*. For a first-time successful transformation was confirmed for quinoa which is one of the most important ancient grain crops. On cytokinin-containing medium inflorescences, after chopping quickly produced meristematic cultures which mainly consist of flower buds, flowers on different stage of development (for quinoa), and real inflorescences (for *Arabidopsis*). Floral culture is meristematic culture that can be used for transformation and plant production without the need for callus generation or plant regeneration. Transformation of floral cultures, like transformation of apical meristems requires stringent and extended selection to prevent the production of chimeric shoots. We demonstrated the production of non-chimeric and stably transformed quinoa and *Arabidopsis*. For *Arabidopsis* developed method can be a good alternative for floral dip transformation. It is efficient, eco-friendly (no contamination of environment with *Agrobacterium*) and greenhouse independent (all work from seeds to T1 seeds can be done in vitro). Our results suggest that floral culture transformation has a great potential as a transformation system for other species, particularly for other *in vitro* recalcitrant species.

WITH THE PERMISSION OF MICROTUBULES: PARADIGMA OF LUCKY 13 PROTOFILAMENTS FOR CELL BIOLOGY

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Recently, the 125th anniversary of the discovery of double fertilization in plants (1898) by Kyiv University professor Serhiy Navashin (1857-1930) was celebrated. Against this background, the fact that, describing double fertilization, S. Navashin paid considerable attention to the study of mitotic division in the generative cells of the pollen tube of the lily (Lilium martagon L.) remained inconspicuous. Although these studies, coinciding in time with the works of the Polish-German scientist Eduard Strasburger (1844-1912), who introduced the terms "prophase", "metaphase", "anaphase" and "meiosis" into scientific circulation (1884), and other researchers, demonstrated the same role of mitosis in the division of the generative nucleus as in the division of meristematic cells, researchers were still unable to visualize and identify microtubules as the main structural element of the mitotic spindle for a long time.

Much later, in the second half of the 20th century, during a new wave of instrumental and methodical development of cytology, which, in fact, gave birth to a new biological discipline called "cell biology", it became clear that microtubules are an indispensable component of the cytoskeleton of any eukaryotic cell and form not only the mitotic spindle, but also the interphase network of microtubules. Having basic structural conservancy (due to the formation of polymer filaments of the heterodimeric protein tubulin) with simultaneous dynamic plasticity (due to associated proteins and fine signaling regulation), microtubules not only enable mitotic division, but also support cell shape, intracellular transport and motility of flagella and cilia, participate in the positioning of organelles, etc. The basis of any cytoplasmic microtubule is 13 protofilaments formed from heterodimers of tubulin. There are, however, deviations from this magic number (and such exceptions as specialized microtubules of flagella and cilia), but the rule of the "lucky" number 13 is quite strict, especially in plants.

The structural and functional plasticity and dynamism of microtubules is ensured by: the expression of different tubulin genes at different stages of tissue development and in different types of cells; the course of post-translational modifications of tubulin; the presence of structural and motor proteins associated with microtubules. The combination of modern research methods, which are the basis of cell biology, genomics, bioinformatics and structural biology, makes it possible to make a new qualitative leap in the understanding of the fine mechanisms of microtubule functioning. In particular, this presentation will consider our own results on obtaining plant mutants with altered tubulin and their somatic hybrids, conducted out us under the initiative support of Yuri Gleba. The issues of obtaining transgenic lines of plants with mutant tubulin and the use of such mutant tubulin genes as selective markered genes in biotechnology will also be covered.

The question of our discovery of such post-translational modifications of plant tubulin, such as phosphorylation on tyrosine residues and nitrotyrosylation, and the study of their functional significance will be separately covered. The possibilities of involving the analysis of big data for the structural-biological analysis of the molecular structure of plant tubulin in comparison with tubulins of other origins and their functional significance will be analyzed. Issues of modeling the structure of intact microtubules will be raised to clarify their dynamic properties and the specificity of interaction with antimicrotubule drugs and cellular structures, in particular, those involved in the development of autophagy

TRANSIENT REPROGRAMMING OF CROP PLANTS FOR AGRONOMIC PERFORMANCE

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Modern biotechnology holds many promises for the future, not only in the medical field but also in plant breeding and the food industry. Genome editing is a popular example of those advancements. However, such technology still must go through the generation of transgenic organisms, which is extremely time consuming, and it still suffers from limitations such as possible off-target issues. At NOMAD, we took an approach based on transient expression using viral vectors (VVs). VVs are modified viruses which we engineer to encode and express specific plant proteins. We hypothesized that such tools could make it possible to manipulate plant phenotypes without the need to modify the plant genome. Traditional VVs created by us and several other research groups in the past, were able to infect mainly *Nicotiana benthamiana* and a few other plant species. To be effective on important crops, we created several novel VVs. Some of them will be presented here. Analyzing gene networks and regulatory pathways which influence plant performance, we selected several groups of gene candidates to be tested for modification of agronomic traits. Those genes were inserted into the viral vectors and tested on plants upon infection. This resulted in a large screen evaluating various genes, VVs, traits and plant species. As an outcome, we obtained effects on several agronomic traits: time of flowering, plant architecture, drought tolerance, and others. We also conducted some pilot studies in open fields, to test our technology outside of laboratories and greenhouses. Those experiments demonstrated the feasibility of such transient reprogramming of plants in a real field scenario. The last aspect opens the possibility of a revolutionary approach for future agriculture.

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IMPLEMENTATION OF THE CRISPR/CAS9-MEDIATED GENE EDITING IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) – CHALLENGES AND OPPORTUNITIES

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Bread wheat (*Triticum aestivum* L.) is a staple crop, providing more than 20% of calories to the population worldwide. Canada and Ukraine are among the world's top ten wheat grain-producing countries (~1 billion tonnes combined for both countries in 2023, world-grain.com). At the same time, due to climate change and limited genetic diversity in the elite breeding lines, wheat faces plateauing yields and outbreaks from rapidly evolving pathogens. These factors compromise its ability to meet the growing human demand. Wheat improvement programs utilize genetic diversity from available germplasms and wild relatives (from *Triticum* and *Aegilops* species) to confer beneficial traits into elite wheat cultivars. Unfortunately, the process is complex and lengthy, the genetic mechanisms of the desired traits are not yet fully understood, and the introgression of beneficial genes from wild relatives frequently leads to linkage drag. Additionally, wheat has a large genome (16 gigabase (Gb)) and is allohexaploid ($2n = 6x = 42$; AABBDD genome) with high sequence similarity among subgenomes and an abundance of repetitive elements (about 85% of the genome). Gene editing (GE) through CRISPR/Cas9 can introduce mutations into a targeted part of the genome for gene functional discovery, genetic diversity expansion, and crop improvement. GE requires the knowledge of the target gene sequence and tissue culture, which, until recently, only worked well with a few genotypes. We will present our ongoing studies to increase wheat genetic transformation and GE efficiency for functional genomics studies and improve desired traits in elite bread wheat lines.

EXPLORATION OF HALOPHYTIC BARLEY RELATIVES' GENETIC POTENTIAL AS PROMISING SOURCE OF SALINITY TOLERANCE FOR MODERN CROPS

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Salinity and drought are significant threats to global agriculture, adversely affecting crop productivity. Over 1125 million hectares of arable land worldwide (nearly 20% of irrigated land) are salt-affected [1]. Among wild crop relatives, the Triticeae tribe, which exhibits a halophytic phenotype, stands out due to its anatomical, physiological, and genomic similarities to major cereal crops [2]. *H. marinum*, commonly found in coastal areas and salt marshes, can grow and reproduce at salinity levels exceeding 1.8%. This species is considered the primary source of salinity tolerance for wheat and other cereals. Our recent study shows elevated expression levels of genes encoding anion channels and transporters (SLAH and NRT1/PTR), the potassium channel SKOR, the borate transporter BOR4, and the cation exchanger CHX [3]. The BOR4 and CHX-like16 genes have been successfully cloned for further functional analysis. Transformation of yeast strains deficient in K^+ uptake with CHX-like16 demonstrated partial restoration of wild-type phenotypes in the mutant lines. Additionally, ion content analysis of transformed yeast revealed higher potassium accumulation within 4 hours of incubation in K^+-deficient media, suggesting that the CHX-like16 transporter is involved in K^+ transport. Transient expression of BOR4 and CHX-like16 mCherry fusions in barley protoplasts revealed a Golgi-specific pattern for both transport proteins. The creation of CHX-like16 and BOR4-expressing plants, as well as CRISPR-Cas9 modified plants (barley and Arabidopsis), is in progress. Furthermore, we have selected another wild barley species, *H. intercedens*, for further comparative analysis of adaptation strategies between the model barley halophyte *H. marinum* and new halophytic species. *H. intercedens*, an increasingly rare annual species found in Californian saline beds and alkaline soils, will be studied for its physiology, ionome, and gene expression profiles. Our primary focus on transcript profiling of salt/sodicity-treated halophytic barley species aims to facilitate the discovery of novel stress tolerance genetic traits as well as the identification and isolation of stress-specific ion transporters.

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INTEGRATION OF NON-INVASIVE MAGNETIC RESONANCE IMAGING AND GENETIC APPROACHES TO UNDERSTAND GRAIN FILLING IN CEREALS

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Cereals are grown because of their grains which represent the basic of human nutrition. The cereal grains accumulate large amounts of starch, proteins and lipids in their endosperm, the main storage organ in a grain. Because developing endosperm is covered by maternal tissues, sophisticated mechanisms exist to transfer assimilates and nutrients from the mother plant towards endosperm. Using ^{13}C-labelled sucrose, we monitored assimilate allocation in the developing barley (*Hordeum vulgare*) grains *in vivo* by Magnetic Resonance Imaging (MRI) and discovered that nucellar projection (NP) and endosperm transfer cells (ETC) are the primary tissues responsible for assimilate transfer within cereal grain[1]. Continuous cell turnover and programmed cell death (PCD) occur on NP margins[2]. Three much similar genes encoding Vacuolar Processing Enzyme (VPE), the protease responsible for PCD execution in plants, specifically expressed in NP with similar transcriptional profile. The simultaneous repression of these genes by RNA-interference resulted in a disturbance to the wild-type progression of PCD, thereby compromising grain filling and realizing in lower-weighted grains due to decelerated accumulation of starch, proteins and lipids[2]. MRI analysis revealed delayed sucrose transport into the developing endosperm and increased sucrose accumulation in maternal parts of a grain. This investigation has shown that PCD at maternal–filial borders of the barley grains serves to widen the rather narrow post-vascular assimilate route, thereby accelerating assimilate allocation[2].

Sugars Will Eventually be Exported Transporters (SWEETs) have been considered to facilitate the transport of sugars but may be also involved in the movement of phytohormones. Of 23 SWEET genes in barley, the *SWEET11b* transcription is restricted to the NP while the sucrose transporter 1 (*SUT1*) mRNA largely accumulates in ETC. A radiotracer assay revealed that expressing barley *SWEET11b* in *Xenopus* oocytes facilitated the bidirectional transfer of not only just sucrose and glucose, but also cytokinin[3]. Barley plants harbouring a loss-of-function mutation of *SWEET11b* could not set viable grains, while the distribution of sucrose and cytokinin was altered in developing grains of plants in which the gene was knocked down. These data indicate that SWEET11b mediates the movement of both sucrose and cytokinin across the maternal–filial boundary. Decreasing *SWEET11b* expression in developing grains reduced grain size, sink strength and the contents of starch and protein. The control exerted by SWEET11b over sugars and cytokinins likely predetermines their synergy, resulting in adjustments to the grain's biochemistry and transcriptome[3].

Molecular mechanisms regulating carbohydrate and hormone allocation at the maternal-filial interface open new perspectives for improving the efficiency of the grain filling process in major cereals.

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MODULATION OF LEAF SURFACE FEATURES FOR IMPROVED WHEAT DROUGHT TOLERANCE

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Limitation of water availability significantly hampers crops productivity, and the duration of drought periods is increasing due to global warming. Water preservation during the dry periods, which relies on the ability to retain accumulated water reserves, is one of the essential plant drought adaptation mechanisms. To the large extend, water lost in plants occurs through leaf surfaces, mostly contributed from stomatal transpiration and cuticle diffusion. Reduction of this loss is a promising strategy toward improving crops drought tolerance, therefore, leaf cuticle and stomata became a subject of intensive investigations in relation to improve water use efficiency in cereals during the recent years. Our previous studies characterized biochemical features of cuticle in a range of Australian wheat cultivars and revealed gene networks controlling leaf surface features and drought tolerance [1]. Overexpression of wheat transcription factor *TaSHN1* affected composition of cuticle waxes, frequency of stomata, drought tolerance and performance of the transgenic wheat lines [2]. Here, we complemented these studies with the data on leaf surface analysis in Ukrainian wheat varieties with contrasting drought tolerance, focusing on stomata physiology and key molecular factors controlling stomata frequency. In particular, we characterized cultivar-specific expression and promoter structure of genes encoding EPF polypeptides and transcription factor MUTE, the key components controlling stomata biogenesis. Based on these results, a gene editing strategy for modulating stomata frequency in wheat with the prospect of improving wheat performance under water-restricted conditions will be presented.

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OBTAINING SUNFLOWER MAINTAINERS WHICH ARE RESISTANT TO SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA* WALR.)

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Sunflower (*Helianthus annuus* L.) is one of the most widespread oil crops in the world and for Ukraine is one of the main crops. Breeding programs are aimed at obtaining high-yielding hybrids with a complex of economic and valuable traits (resistance to herbicides, resistance to bioactive and abiotic factors). The creation of such a hybrid takes an average of 12 years. And considering that sunflower cultivation is based on cytoplasmic male sterility (*CMS*), which consists of three main components: sterile line (*S rfrf*), maintainer (*N rfrf*) and restorer line (*S/N RfRf*) [1]. Desirable economically valuable traits should be introduced into three components. When involving in breeding programs, methods which accelerate creating sunflower lines are relevant. Such methods include: molecular biological and biotechnological methods, assessment of the resistance of lines to pathogens on an artificial infectious background.

Sunflower broomrape (*Orobanche cumana* Wallr.) is a holoparasitic angiosperm that parasites on sunflower roots, which is widely distributed in sunflower growing areas. Sunflower lupus is classified into races, depending on virulence, and they are designated by letters. Currently, it is known about the presence of G and H races of broomrape on the territory of Ukraine. In general, when growing sunflower hybrids that are not resistant to broomrape, crop yield losses can range from 40 to 100% [5]. It has been established that the SCAR marker HRG01 is effective in identifying the dominant gene for restoring the fertility of sunflower pollen (Rf_1) [2,3]. That is why, the goal of our study was to conduct a two-stage analysis of the sunflower line, to identify maintainer lines resistant to sunflower lupus. At the first stage, we used the SCAR marker HRG01, and we selected maintainer lines. At the second stage, we used an infectious background to differentiate maintainer lines which are resistant to sunflower broomrape.

Thus, during the first stage, we analyzed 75 lines and found that all of them are sunflower maintainers, as they did not contain the dominant gene for restoring pollen fertility (Rf_1). Molecular analysis was carried out according to the standard method, where DNA was isolated from sunflower leaves using the STAB method, the nucleotide sequence of the primers to the HRG01 locus: F: 5`–TATGCATAATTAGTTATACCC–3`; R: 5`–ACATAAGGATTATGTACGGG–3` [3], PCR was performed in a thermal cycler according to the program: 1 cycle – initial denaturation at 94°C for 10 min; 35 cycles – 94°C for 45 s, 58°C for 45 s, 72°C for 60 s; 1 cycle – final elongation at 72°C for 6 min.

During the second stage, we used an artificial infection background to isolate lines that are resistant to broomrape. As a result, we found that among 75 lines, only 5 have resistance to these parasitic plants. Since nodules of broomrape were not formed on the root system of these lines.

So, as a result of a two-stage study, we selected 5 maintainer lines as resistant to sunflower broomrape. In the future, these lines will be used in the creation of broomrape resistant hybrids of the first generation (F_1) and as donors of resistance to this parasite plant for the improvement of other lines.

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PRIMING WITH *GAMMA*-AMINOBUTYRIC ACID ALLEVIATES OXIDATIVE STRESS DURING GERMINATION OF AGED WHEAT AND TRITICALE SEEDS

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Seed storage, especially under conditions of temperature and humidity fluctuation, leads to accelerated aging and loss of germination. To increase the speed and uniformity of seed germination, priming, a method based on controlled moistening and drying of grains, is increasingly being used (Waqas et al., 2019). Evidence was obtained that such a procedure can activate the antioxidant system, which mitigates the effect of oxidative stress accompanying the germination of old seeds. It is assumed that the effect of seed priming lasts for some time due to the presence of transcripts of protective proteins and the functioning of epigenetic mechanisms. It is known that the efficiency of hydropriming can be enhanced by the use of phytohormones and stress metabolites. Since senescent seeds are characterized by an imbalance between ROS generation and their neutralization, it is believed that the use of antioxidants as priming agents can mitigate the manifestation of oxidative stress and enhance the germination of old seeds (Deng et al., 2017).

In recent years, there has been increasing interest in the functions of gamma-aminobutyric acid (GABA) in plants. It is considered to be one of the most important stress metabolites, the direct protective effect of which has been linked to its participation in the maintenance of the reducing agent pool through the activation of the GABA shunt. Recently, experimental evidence has been obtained for the involvement of GABA in signaling processes involving ROS and calcium ions, leading to the activation of the enzymatic antioxidant system and the synthesis of secondary metabolites. The influence of GABA has been demonstrated to enhance seed germination in a range of cultivated plants subjected to conditions of drought, salinity and high temperature. However, the effect of GABA priming on the germination of seeds with low seed quality has not been investigated so far. The aim of the work was to study the effect of GABA treatment on germination of wheat and triticale seeds with low germination and the state of antioxidant system of seedlings.

Seeds of wheat cultivar ‘Scorpion’ and triticale cultivar ‘Raritet’ of the 2020 generation were used for the experiments. The seeds were stored indoors under uncontrolled conditions for four years, which resulted in a decrease in seed germination to approximately 35-45%. Treatment of seeds with GABA at optimal concentration (1 mM) for 3 h followed by drying for 24 h increased germination of wheat seeds by 18% and triticale seeds by 21% compared to the hydropriming procedure. Simultaneously, root and shoot weight of seedlings of both cereal species increased significantly. In wheat and triticale seedlings treated with GABA, lower levels of $O_2^{\cdot-}$ generation, hydrogen peroxide content and LPO product malonic dialdehyde were observed. An increase in the content of phenolic compounds was also observed in wheat seedlings treated with GABA, and in anthocyanins in triticale seedlings. In both cereals under the influence of GABA, an increase in catalase activity and a decrease in superoxide dismutase activity were recorded. The latter may be associated with a decrease in ROS generation and accumulation of low-molecular-weight antioxidants. Thus, priming of old seeds of two cereal species with GABA significantly increased their germination and improved seedling growth compared to conventional hydropriming. One of the components of the beneficial effect of GABA may be mitigation of the effects of oxidative stress during germination of aged seeds.

REPLACEMENT OF GROWTH REGULATORS *IN VITRO SOLANACEAE* CROPS

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Numerous studies have found that the genotype of the plant greatly affects the morphogenetic potential of explants *in vitro*. The genetic diversity of callus tissues allows them to be used for cellular breeding for resistance against adverse environmental factors, phytopathogens and increased productivity. It is also well known that each new variety of cultivated plants requires an individual selection of nutrient media compositions, growth regulators and optimization of cultivation technology.

The purpose of our research was to establish the possibility of initiation of a potassium crop of potato plants and tomato using industrial growth regulators, which are allowed for use in Ukraine, which includes substances with pronounced auxin-cytokinin activity.

The work used varieties of *Choriv* tomatoes and *Borivsky* and varieties of *Bozhadar* and *Povin*. Work with cultures was carried out *in vitro* culture according to conventional methods.

Results. In a series of experiments for stimulating tomato callusogenesis in MS medium instead of cytokinin and auxin, substitutes-solutions of ecostim (15-35 mg/l) and ecostim 1 (20-40 mg/l) in different concentrations were added. The same concentrations of auxin-cytokinin substitute (ACS) were added to the MS medium to stimulate the morphogenesis of tomato plants. A substitute for phytohormones was created in a well-known agarized medium according to Murashige-Skoog. For the cultivation of plants and potatoes *in vitro*, phytohormones have been replaced with ecostim and ecostim solutions that have found auxin-cytokinin activity. The cost of these substitutes is much lower than the price of commercial phytohormones. Artificial growth regulators and methods of cultivation of tissues and cells are the main factors. The role of artificial growth regulators in the processes of cell division in *in vitro* culture has now been studied quite deeply. However, a significant impact on the choice of a particular regulator is revealed by species, genotype, and epigenetic factors. Therefore, the approach to each particular culture and variety or hybrid remains empirical. There is also information about the interchangeable auxins and cytokinins. The same growth is observed with different ratios of these growth regulators. The decrease in auxin concentration was compensated by a tenfold increase in the concentration of cytokinin. It can be predicted that cytokinin stimulates the synthesis of endogenous auxin in cells, as well as that under the influence of cytokinin increases the absorption of auxin from the environment and weakens its inactivation. Our phytohormone substitutes are of vegetable origin-metabolites of endophytes, isolated from the roots of sea buckthorn and ginseng. And plant preparations, compared to synthetic, have a number of advantages. Being quite complex in biochemical composition, they contain a number of ingredients that have quite valuable properties and provide multilateral effect on the plant organism.

Conclusions. The determining factors of the nutrient medium that effectively regulate the activity of growth processes of plant cells cultivated *in vitro* are phytohormones. The analysis of the results suggests that optimal for the growth of the cell culture of Solanaceae and morphogenesis are variants with the use of cytokinin replacements of ecostim and ecostim 1 with a rate of consumption of 35.0 and 40.0 mg/l.

GENOME EDITING OF WAK GENES IN POTATO (*SOLANUM TUBEROSUM*)

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Potatoes are one of the main components of the modern human diet. However, a significant part of the harvest is regularly lost due to damage of leaves and tubers caused by various pathogens [Gebhardt, 2013]. Therefore, it is necessary to understand the mechanisms of natural resistance of potato plants in order to reduce economic losses at successive stages of cultivation and storage.

The plant cell wall is the first barrier to the penetration of phytopathogens, as well as the initial interface for interaction between microbes and the host cell. Therefore, strengthening the cell wall is one of the main defence mechanisms against biotrophic and necrotrophic pathogens [Bellincampi et al., 2014]. This study focused on genes of the WAK family, which can act as both positive and negative regulators of plant protection against biotic stress. Wall-associated receptor-like kinases (WAKs) are transmembrane proteins that link the cytoplasm with pectin carbohydrates in the extracellular matrix of the primary cell wall. In some plants, they can be targets for binding fungal signalling proteins, thereby enhancing fungal penetration and invasion of the host plant [Delteil et al., 2016]. Bioinformatic identification and expression analysis of selected StWAK and StWAK-like genes in two potato varieties (‘Désirée’ and ‘Botond’) were performed, three plasmid vectors for transformation and gene editing using CRISPR/Cas were constructed, genetic transformation using *Agrobacterium tumefaciens* was carried out, and six lines of potato variety Désirée with confirmed knockout of the StWAK12 gene were obtained. Further studies of the obtained mutant lines of potato plants for resistance to infection with *R. solanacearum* and *P. infestans* pathogens are planned.

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AGROBACTERIUM TUMEFACIENS AND *AGROBACTERIUM RHIZOGENES*-MEDIATED TRANSFORMATION FOR *CUCURBITA* PLANTS

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Agrobacterium tumefaciens and *Agrobacterium rhizogenes* are used for genetic transformation of plants. Both *Agrobacterium* species have the ability to infect host plants. The transformation system mediated by *A. rhizogenes* is known to have an advantage over *A.tumefaciens* in that Ri-transformed host plant cells spontaneously differentiate into Ri-transformed roots called "hairy roots", which serve as excellent systems for biotechnology research (Bahramnejad et al 2019).

The current study focuses on agrobacterium-mediated transformation studies by two strains – *A.rhizogenes* (strain A4) and *A.tumefaciens* (strain GV3101) of two types of explants - *Cucurbita pepo* var. Giromontia and *Cucurbita ficifolia* Bouche

Cucurbita plants are an important vegetable crop in the world and are used for food and medicinal purposes as an antioxidant, anti-inflammatory, diuretic and oncoprotective agent. The possibility of using pumpkin plants as a phytoremediator for a polluted environment has also been shown (Mierzejewska-Sinner et al., 2024). Improved transgenic approaches may enable more efficient development of plants that produce important pharmaceutical proteins and plants used for effective phytoremediation.

For transformation, we used *A.rhizogenes* with a vector construct containing the target gene *huINFa-2b* under control of the constitutive 35S promoter and selective kanamycin resistance gene (pCB124) and *A.tumefaciens* containing the same target gene *huINFa-2b* and the *bar* gene for plant resistance to the herbicide phosphinothricin (pCB125).

For more shoots we used proximal parts of 7-day cotyledons, cut across into two parts. Transformation by *A.rhizogenes* showed that regeneration in *C.pepo* was 57.4%, which is 42.6% more than in *C.ficifolia. A.tumefaciens* transformation showed a 30.7% higher morphogenic potential of *C.ficifolia* explants than *C.pepo* explants. Shoots were formed in an amount of 2.5 per explant only in *C.pepo* after the transformation of *A.rhizogenes*. In other cases, shoots formed on average one shoot per explant. Vacuum infiltration was used and increased the transformation efficiency by more than twofold in both cases. The shoots subcultivation showed that phosphynotricin as a selective agent was more suitable for selection transgenic plants, as the initial selection on ppt clearly identified plants capable of further growing on a selective medium. Whereas shoots died in greater numbers during selection on kanamycin, revealing the imperfection of such selection.

The transformation results showed differences, which manifested themselves in a greater frequency and efficiency of shoot formation when using *A.rhizogenes*. However, more final lines were found in shoots after subcultivation on phosphinothricin than after kanamycin selection. The phenotypic difference between transformed and native plants after *A.rhizogenes* transformation was expressed in the reduction of plant size and tissue compaction, which is expected due to the presence of agrobacterial *rol*-genes in the plant cell genome.

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TRANSIENT GENE EXPRESSION IN *OCIMUM BASILICUM* HAIRY ROOTS

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Transgenic plants produce proteins and secondary metabolites that are successfully used for various applications in pharmaceuticals, cosmetology and the food industry.

Hairy root (HR) culture induced by infecting plants with the bacteria *Agrobacterium rhizogenes* (now renamed *Rhizobium rhizogenes*) can be obtained from a wide range of plants and is a good alternative for plants under aseptic *in vitro* conditions. Hairy roots have been successfully exploited in various fields in biotechnology, including secondary metabolite research, recombinant protein production, bioremediation and others. *In vitro* culture allows the secretion of complex and diverse molecules without the influence of seasonal, geographical factors or climatic changes. This technology combines *in vitro* tissue culture with a recombinant DNA mechanism. HR cultures are preferred over other *in vitro* model systems due to their biochemical and genetic stability

However, obtaining stable transformation of both plants and cell cultures is limited due to the complex and lengthy route to obtain them and the levels of recombinant proteins are typically low.

Numerous studies in recent years have convincingly demonstrated the effectiveness of the transient expression method capable secreting high levels recombinant protein.

Here we proposed a method for initiating the expression of foreign proteins using transient expression technology in HR culture obtained from basil (*Ocīmum basilīcum L)* leaf explants. The aim of this study was to develop a transient expression technique in *O.basilīcum* HR culture to produce green fluorescent protein (GFP) grown under *in vitro* conditions.

A stably growing HR basil culture was obtained on solid and liquid B5 medium. Then the culture was infected with agrobacterium suspension containing a plasmid vector with the *gfp*-gene. The reporter *gfp*-gene was chosen because it's easily detectable under UV light. Roots were agroinfiltrated using a bacterial suspension applied on a filter. A cell culture infected with *Agrobacterium* that did not contain a reporter gene was used as a negative control. There was a 24-hour exposition with a bacterial suspension. The presence of GFP in roots was determined visually using a hand-held UV lamp. Green fluorescence indicating on GFP accumulation appeared in infiltrated areas 1 day after root infection and was detected for 5 days. Fluorescence under UV light was observed only on roots cultivated on solid agar medium. Liquid culture infiltrated with a bacterial suspension showed extensive bacterial contamination, precluding further cultivation. Completely darkened roots did not express GFP.

Thus, we demonstrated the ability of basil hairy root culture to express green fluorescent protein on agar medium. This study may provide an alternative method to potentially generate functional proteins in *in vitro* cell culture and will also promote further exploration of HR culture as a promising host for the production of recombinant proteins through transient gene expression.

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METABOLIC CHANGES IN TRANSGENIC *PETUNIA×HYBRIDA* AND *N. BENTHAMIANA* PLANTS, EXPRESSING HETEROLOGOUS *ZRNASE*II GENE

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Plant viral infections are widespread among crops and cause significant yield loss and decrease of its quality. Due to different environmental stresses, that weaken plants, nowadays the production of transgenic plants with increased resistance to viruses is of a great demand. Genetic engineering methods such as genetic transformation can achieve this goal and create virus tolerant plants. Ribonucleases (RNases) are supposed to be engaged in antiviral responses of plants, so heterologous RNase gene expression can be a tool for the production of cultivars with multiple resistance to viruses and viroids. However, possible metabolic changes in the biochemical composition of obtained transgenic plants are extremely important and require to be studied. ATR-FTIR spectroscopy is a convenient method for such analyses. The advantages of this method are speed, simplicity, reliability and reproducibility of results combined with maintaining the inherent living system structure of spatial compartments and macromolecules [1].

The goal of our work was to study biochemical changes in the transgenic *Petunia×hybrida* and *N. benthamiana* plants, expressing heterologous *ZRNase*II gene.

The transgenic *Petunia×hybrida* and *N. benthamiana* plants, expressing heterologous *ZRNaseII* gene, were maintained in the collection of ICBGE [2, 3]. ATR-FTIR spectroscopy (Nicolet FTIR IS50 Spectrometer, Thermo Fisher Scientific, USA) of the air-dried leaves had been performed to compare the biochemical content of wild-type and transgenic plants.

Various changes in the biochemical composition of the epidermal and cuticular layer of transgenic plants leaves were identified. The most pronounced biochemical changes in transgenic lines, true to both determined plant species, concerned to the content of nucleic acids, proteins, starch and lignin:

1. The nucleic acid content increased in transgenic lines (70-212% for *N. benthamiana*, 2-12% for *Petunia×hybrida*);
2. The protein content decreased in transgenic plants (by 2-8% for *N. benthamiana*, by 17-38% for *Petunia×hybrida*);
3. The starch and lignin content significantly decreased in transgenic lines (the starch content by 59-70% for *N. benthamiana* and by 35-41% for *Petunia×hybrida*, while the lignin content by 45-59% and by 66-72%, respectively).

The identified patterns are confirmed by changes in the same indicators normalized by cellulose content, which characterizes corresponding changes in the biochemical composition of epidermal plant cells. The reasons causing of these metabolic changes are not determined yet. It should be determined in future whether the changes were caused by *ZRNaseII* heterologous gene expression or by the procedure of genetic transformation by itself.

Our data revealed the shift in the metabolic profiles of transgenic *Petunia×hybrida* and *N. benthamiana* plants, expressing heterologous *ZRNase*II gene, compared to those non-transformed plants.

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INHERITANCE OF TRANSGENES BY MAIZE PLANTS OF UKRAINIAN BREEDING

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Maize (*Zea mays* L.) is one of the main grain crops in the world [1]. It is also a culture in which genetic engineering has been used extensively to improve its various properties. The commercial success of maize transformation is unmatched by any other crop [2]. Thus, the development of technologies for developing transgenic maize of Ukrainian breeding is relevant and timely.

The aim of the work was to establish the inheritance of transgenes by Ukrainian maize plants of the T_1 generation after *Agrobacterium*-mediated transformation. The methods of total DNA isolation, polymerase chain reaction (PCR), electrophoresis of DNA in agarose gel, and histochemical analysis of β-glucuronidase activity were used in the work. To determine whether there is a statistically significant difference between the expected frequencies and the observed frequencies of transgene distribution, Pearson's chi-squared test was done at the level of significance $p < 0.05$.

Three groups of T_1 generation maize plants (T_1($T_{0\ pCB271}$(KP7×PRZh5)× $Ò_{0\ pCB271}$(KP7×PRZh5)), T_1($T_{0\ pCB202}$(DK232) × $Ò_{0\ pCB271}$(KP7×PRZh5)), and T_1($T_{0\ pCB271}$(KP7×PRZh5) × $Ò_{0\ pBi2E}$(KP7×PRZh5))) were studied for the presence of transgenes by PCR. Plants of T_1 generation were produced by crossing regenerants (T_0 generation) obtained after *Agrobacterium*-mediated transformation using pCB202, pCB271 or pBi2E vectors. In each experimental group, one or both parent plants were selected after transformation with the vector pCB271, which contained a synthetic mutant reporter gene (*S65Tpgfp*) of green fluorescent protein (GFP) [3]. Therefore, we tried to study the GFP gene inheritance in T_1 generation maize plants. The DNA of 180 plants of T_1 generation was analyzed, and the GFP gene was detected in 96 of them (53.3%). The ratio of plants in the presence/absence of the *S65Tpgfp* transgene was close to 1:1 and significant according to the Pearson's chi-squared test. A group of plants in which one of the parental forms was obtained after transformation with the pCB202 vector, which contained the β-glucuronidase (*uid*A) reporter gene, was examined for the presence of enzyme activity. Expression of the β-glucuronidase gene was detected in 52.5% of the plants, which corresponds to a 1:1 split, characteristic of the heterozygous state of the functional gene in testcross, accordingly, single-locus insertion of the *uid*A gene into the genome of the DK232 mother plant.

Inheritance of transgenes of GFP and neomycin phosphotransferase II (*npt*II) in the next maize generation was shown using PCR analysis. Based on the results of the inheritance of reporter genes, the incorporation of one copy of the transgene into the plant genome has been statistically proven. In this way, the effectiveness of the protocol of *Agrobacterium*-mediated transformation of pre-cultivated immature embryos for obtaining transgenic maize plants of Ukrainian breeding has been established. The research is important for the future practical use in breeding high-yielding maize hybrids.

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ANALYSIS OF THE HUMAN *IFNA-2B* EXPRESSION IN TRANSPLASTOMIC TOBACCO

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Nowadays, plants are increasingly used as effective and cheap platforms for the production of recombinant proteins. The value of plant-based platforms lies in the ability of correct modification of human and animal proteins to gain their functional activity, the absence of contamination of with bacterial toxins and/or human pathogens. Pharmaceutical proteins can be synthesized in plants with genetically transformed plastome in higher concentrations compared to the transgenic due to the presence of extremely increased copy number of genes in each cell (several thousand copies). In addition, plastids are maternally inherited in most crops, which prevents pollen mediated spread of heterologous genes. In molecular farming the chloroplast transformation gives a unique advantage for the development of biopharmaceutical production [Daniell et al., 2016].

The goal of this work was to create biotechnological transplastomic tobacco plants for production of human interferon, the cytokine with antiviral activity; and to analyze the activity of obtained plant-based recombinant interferon against viruses.

Transplastomic tobacco plants were obtained by biolistic transformation with vectors containing the *ifn α-2b* and *aadA* selective genes together or in combination with other genes. The obtained plants were analyzed by the PCR and the hybridological method. The interferon activity was measured by microtitration method (Rubinstein et al. 1981) based on the ability of studied extracts to protect renal epithelial cells MA-104 from *Cercopithecus aethiops* (from cell collection of Zabolotny Institute of Microbiology and Virology, NAS of Ukraine) against cytopathic effect of vesicular stomatitis virus (VSV), Indiana strain (collection of Zabolotny Institute of Microbiology and Virology, NAS of Ukraine).

The integration of genes of interest into the plastome was confirmed by PCR analyses. T_1 generation of transplastomic plants demonstrated maternal inheritance of the selective *aadA* gene (coding for resistance to spectinomycin and streptomycin). Transplastomic clone of tobacco, with *aadA* and *ifn α-2b* genes, demonstrated high activity of human interferon α-2b (42,300 IU/g of raw mass). For comparison, the interferon activity in the leaves of the transgenic clone of tobacco was about 1000 IU/g of raw material. We also used constructs in which the interferon gene was fused with some other genes. It was previously demonstrated that some proteins can be unstable in transgenic chloroplasts and their stability can be increased by fusion with other genes. In particular, IFN-γ degraded quite severely in tobacco chloroplasts (< 0.2% of TSP), but after fusion with β-glucuronidase (GUS), its yield significantly increased (up to 7% of TSP) [Leelavathi and Reddy, 2003]. We observed opposite effects in our research. Co-expression in tobacco chloroplasts of *ifn α-2b* gene with *esxA* and $fbpB^{\Delta TMD}$ genes, encoding proteins, that induce the immune response to *Mycobacterium tuberculosis* Esat Ag 6, decreased the activity of human interferon α-2b. In plants with *ifn α-2b* and *gfp* fused genes interferon activity was almost absent.

Transplastomic tobacco plants producing high amounts of human interferon α-2b were obtained. In our research co-expression of *ifn α-2b* gene with other genes in tobacco chloroplasts reduced the activity of human interferon.

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DNA MARKERS TO DETECT POLYMORPHISMS IN STOMATAL BIOGENESIS GENES *EPF1* AND *EPF2*

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One of the most important problems today in the field of agriculture is the development of drought-tolerant varieties. Many factors influence the formation of drought tolerance in plants. However, in our work, we focus on the important genes that determine stomatal biogenesis. *EPF1* and *EPF2* are the key genes in stomatal biogenesis. The aim of the work was to develop DNA markers for genotyping wheat varieties to find valuable alleles of the stomatal biogenesis genes *EPF1* and *EPF2*.

The work analyzed a set of 67 bread wheat cultivars of Ukrainian origin, including two control genotypes. For molecular genetic analysis, the total DNA of plants was isolated from three seeds according to a CTAB method. The PCR was followed by electrophoresis in a 1.2% agarose gel.

The nucleotide promoter sequences of the *EPF1* and *EPF2* genes were used to develop four DNA markers, which aim to identify polymorphisms within this region. The EPF1-A1 DNA marker was designed to detect two SNPs (-1223 A→G; -1221 A→C) in the promoter region of the *EPF1* gene from subgenome A. The length of the expected amplified fragment is 380 bp. Its frequency for Ukrainian cultivars was quite high, at 0.88.

The EPF1-B1 DNA marker was created to detect variability in the promoter part of the *EPF1* gene from subgenome B. This system detects a 127-bp insertion at the position -1486...-1487 from the start of transcription. The expected amplicon size is 480 bp in the presence of an insertion, and 353 bp in the absence of an insertion. Among Ukrainian varieties, only 2 samples had this insertion (frequency 0.03). However, in 6 varieties, two amplification products were detected at the same time, since different genotypes are used during selection, it can be stated that these varieties are heterozygous for this feature.

EPF1-D1 is designed to detect the 11-bp GACCACTACTT insertion at position -1584...-1585 from the start of transcription in the promoter part of the *EPF1* gene from subgenome D. The size of the expected amplicon is 280 bp. The frequency of this polymorphism was 0.36 among Ukrainian common wheat varieties. The DNA marker EPF2-B1 was created in order to detect polymorphisms in the promoter region of the *EPF2* gene. This marker allowed us to determine the 297-bp insertion (at positions -963...-964). The size of the expected amplified fragment was 267 bp, indicating an insertion presence (frequency 0.37).

The results show that two SNPs (-1223 A→G; -1221 A→C) of the promoter region of the *EPF1* gene from subgenome A are most common among Ukrainian varieties. The lowest frequency of occurrence among Ukrainian varieties is the insertion in the promoter region of the *EPF1* gene from subgenome B is detected by the EPF1-B1 marker. Therefore, the developed DNA markers can be utilized for describing diverse wheat samples and screening breeding material for the identification of potential drought-tolerance donors.

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MARKER-ASSISTED SELECTION OF SWEET MAIZE WITH ANTHOCYANIN GRAIN COLOURATION

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Anthocyanins are valuable antioxidants and provide plants with bright colours, which make it reasonable to create analogues of well-known food products with an increased content of anthocyanins.

The aim of the work was to obtain an inbred of sweet maize with anthocyanin grain colouration through marker-associated selection for the *Sh*1 gene, the expression of which leads to inhibition of starch synthesis and a sharp increase in the level of free sugars and water-soluble saccharides.

The methodology consisted in crossing sweet inbred with yellow grain CE401 and the population Chornosteblova with purpule grain and screening in subsequent generations on grain colour and grain wrinkling in the phase of full ripeness. The last trait is a characteristic of sweet maize. In generation F_4(CE401× Chornosteblova), selection was carried out using the phi033 marker of the *Sh*1 gene [Psolova et al., 2020]. The content of anthocyanins in technically ripe grains, in particular, on the 21st day after self-pollination at 70% humidity, was determined by the method of differential spectrophotometry [Giusti, Wrolstad, 2001]. The sugar content was determined according to [Satarova et al., 2022].

The total content of anthocyanins in the purple grain of F_4(CE401× Chornosteblova) (2951.4±11.2 mg/kg of grain) was significantly higher than that of the yellow grain of CE401 (1174.5±6.6 mg/kg of grain). Under the colour intensification in F_4(CE401× Chornosteblova) there was a decrease in the content of pelargonidin by 1.59% due to the redistribution of this part to an increase in the content of cyanidin by 0.71%, peonidin by 0.47%, delphinidin by 0.39% and glycosylated forms by 0.02%. In particular, in F_4(CE401× Chornosteblova) compared to CE401, the part of delphinidin-3-glucoside decreased by 0.24%, instead, the part of pelargonidin-3-glucoside increased by 0.21%, cyanidiin-3-glucoside by 0.03% and of peonidin-3-glucoside by 0.02%. That is, anthocyanins were contained in both white and purple unripe grains, but both the total content and the content of individual analyzed kinds and forms of anthocyanins significantly increased with the appearance of intense purple grain colouration [Psolova et al., 2019]. According to the organoleptic evaluation the taste in the inbred with purple grain after the sixth self-pollination, that is, in the genotype F_7 (CE401×Chornosteblova) was at the level of the original sweet maize inbred CE401 and the standard - the hybrid Spokusa. The new line of sweet maize with purple grain was also at the level of CE401 and the standard in total sugar content at 21st day, but exceeded CE401 in monosaccharide content.

The inbred F_7(CE401×Chornosteblova), obtained by marker-associated selection, with high taste qualities, a high content of soluble sugars, monosugars, anthocyanins, and the original attractive purple grain colour is recommended for practical utilization in the selection of sweet maize hybrids for food purposes.

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SUPPORTING YOUNG PROGRAMMERS IN BIOINFORMATICS EDUCATION: A PATHWAY TO SCIENTIFIC INNOVATION

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In light of the contemporary challenges posed by the COVID-19 pandemic and current ongoing military conflict, the landscape of education has undergone significant changes, with remote learning becoming the norm for many students. However, the effectiveness of such distance education has been a subject of discussion among educators, students, and parents alike. Recognizing this, it is widely acknowledged that there is an urgent need to provide young minds with meaningful educational opportunities, promised for their future.

Project Goal is to attract the attention of young Ukrainian programmers interested in bioinformatics to the scientific problems of computer-mathematical analysis of DNA code and 3D analysis of protein structure. We are creating a Ukrainian-language web portal similar to the English-language platform (URL: https://rosalind.info/problems/locations/), which is extremely important to provide domestic youth with opportunities to participate in significant scientific and educational events remotely (see URL: https://ru.calameo.com/read/003168372d2ef28271af6).

Involvement of Academically Gifted Youth: Our project aims to engage academically gifted youth, particularly from the Junior Academy of Sciences of Ukraine (https://man.gov.ua/), in research activities in bioinformatics. By involving them in the development of educational content on bioinformatics, we hope to stimulate a culture of scientific interest and collaboration among young programmers (URL: https://www.calameo.com/read/0031683722fd980de5b9a).

Educational Content and Methodology: The Ukrainian-language educational content on bioinformatics will cover a wide range of topics, including an introduction to the Python programming language, programming tasks related to genetic data processing, and theoretical concepts in genetics and bioinformatics. Through practical examples and programming exercises, we will unravel the complexities of bioinformatics and empower young learners to explore this exciting field (URL: https://www.calameo.com/read/003168372e3e544a0d5d3) [1].

Exploration of Genetic Information: The tasks presented on our educational website elucidate the complex process of protein synthesis based on genetic information encoded within DNA. These tasks, ranging from recoding genetic information from DNA to RNA to synthesizing protein molecules based on RNA code, offer young programmers practical experience in understanding the fundamental processes of molecular biology [1].

The project aims to support young programmers in Ukraine in achieving heights in the field of bioinformatics. By providing access to high-quality educational content and promoting collaborative learning, we aim to cultivate the next generation of scientific innovators who will contribute to the development of bioinformatics research in Ukraine and beyond. Through their dedication and passion for learning, these young talents will play a key role in shaping future discoveries and innovations in bioinformatics science.

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CROP GENOME AND EPIGENOME DYNAMICS IN THE CONTEXT OF CLIMATE CHANGE

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Accelerating climate change and ongoing wars have reminded us of the importance of wheat to feed the global population. It has further highlighted the central role that crop breeding must take to contribute to alleviate these challenges. And yet, even though the emergency could not be clearer, all innovations in crop breeding are being blocked in Europe. In this presentation, I will present you how my group has developed a novel crop breeding method that is based on drug-induced genetic and epigenetic changes and how European legislators effectively prohibited its use.

Transposable elements are stress-responsive genetic elements that have been key drivers of domestication of important crops. Here, our aim is to accelerate this process by temporarily releasing transposable element mobility by using epigenetic drugs in combination with abiotic stresses. First, I will show you in Arabidopsis how controlled mobilization of transposable elements can change stress responsiveness of the plant and thereby contribute to rapid stress adaptation. We also found that treatments with epigenetic drugs could lead to stable epigenetic changes that can affect gene expression. I will then present recent data that we have obtained in key crops such as rice and wheat suggesting that induced transposable element mobilization could be a useful tool to accelerate crop breeding.

In conclusion, the presented epi/genetic breeding method could render plants more tolerant to climate change and reduce pesticide use, but not in Europe if legislation does not change.

EPIGENETIC REGULATION OF TRANSGENERATIONAL RESPONSE TO STRESS

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Most plants are adapted to their environments through generations of exposure to all elements. The adaptation process involves the best possible response to fluctuations in the environment based on genetic and epigenetic make-up of the organism. Many plant species have capacity to acclimate or adapt to certain stresses, allowing them to deal with the same or similar stress more efficiently, with fewer resources diverted from growth and development. However, plants can also acquire protection against stress across generations. Such response is known as intergenerational response to stress; typically plants loose most of the tolerance in the subsequent generation when propagated without stress. Occasionally, the protection lasts for more than one generation after stress exposure and such response is called transgenerational.

In our work, we generated 25 generations of Arabidopsis plants exposed to various stresses, including cold, heat and UVC. We tested phenotypic appearance in the progeny of stressed plants and noted some degree of stress tolerance. We then profiled genome and epigenome of the progeny, and found drastic changes, predominantly in single nucleotide polymorphisms. The progeny of stressed plants clearly clustered separately from the progeny of control plants. Methylome analysis revealed that the stressed progeny showed a lower global methylation level in the non-symmetrical CHH context than the control progeny. The progeny of stressed plants displayed higher frequency of methylation changes in the gene body and lower in the body of transposable elements (TEs). Gene Ontology analysis revealed that CG-DMRs were enriched in processes such as response to abiotic and biotic stimulus, cell organizations and biogenesis, and DNA or RNA metabolism.

Overall, our study showed that progenies derived from multigenerational stress displayed a notable adaption in context of phenotypic, genotypic and epigenotypic resilience.

EPIGENETICS REVEALS GOOD GENOMES

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The objectives of research was study of epigenetic aspects of asynchronous germination of a random sample of cereal seeds of the same variety and harvest, biological significances of this phenomenon in plant' individual and population stability .

The tasks of research were included:

1. Is the difference of the time of seed germination associated with their epigenetic differences?
2. Are epigenetic differences associated only with the degree of seed maturity, with different positions on a single epigenetic pathway or with a diversity of pathways?
3. Does the difference in resistance to abiotic and biotic factors depend on the epigenetic pathway?
4. Develop a quantitative measure to assess epigenetic diversity within a variety and compare it with the yield and ecological plasticity of the variety.
5. Assess the Spearman rank correlation between epigenetic diversity within a variety, productivity and ecological plasticity of the variety.

Research was carried out on eight of winter wheat genotypes. Seedlings were exposed at a dose of 5-20 kJ/m² using an OBM-150M installation (Ukraine). DNA methylation research was carried out through restriction analysis followed by ISSR - ITS-PCR. For restriction analysis two types of restrictase - isoschizomers were used: MspI and HpaII. Three types of DNA- markers were used: ISSR-5, ITS1 and ITS4.

The sensitivity of DNA was determined by the number of unstable chromosomal aberrations.

DNA methylation research was carried out through restriction analysis followed by ISSR-ITS-PCR. For restriction analysis two types of restrictase - isoschizomers were used: MspI and HpaII.

As an indicator of the difference between the amplicon' set the indicator D = epigenetic distance was used. It was calculated similarly to the estimation of the genetic distance according to Nei.

.It was demonstrated that both seedlings the same physiological age and the same chronologic age epigenetically are different. This is proof that seedlings in genetically homogeneous population ontogenesis takes place on different epigenetically pathways. Next, it was shown that of seedlings with different rate of germination have different damage of DNA in control, different stability to the UV-C irradiation and different adaptive capacity.

A study of the level and rate of development of phytopathogens contamination showed a significant difference in seedlings of different epigenetic pathways and the advantage of quickly germinating seedlings. Correlation analysis showed that wheat varieties with higher epigenetic diversity have higher productivity and environmental plasticity.

Totally, results of these research have show that development of new varieties of cultivated plants needs to focused attention not only on the search of "good" genes but also on the search of good genomes, which successfully cooperate with an environment and create the higher diversity of epigenetic pathway. Crops with higher diversity of epigenetic pathways have less intravarietal competition and produce higher yields with less management..

IONIZING RADIATION AFFECTS AGGREGATED PROTEINS AND AMYLOIDOGENESIS IN *PISUM SATIVUM* l.

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Introduction. Ionizing radiation is a harsh environmental factor that could induce senescence in living organisms [1;2]. We hypothesized that radiation-related senescence remodels proteome, particularly by triggering the accumulation of prion-like proteins in plant tissues. The object of this study, pea (*Pisum sativum* L.), is an agriculturally important legume. To our knowledge, no previous research has delved into the functional significance of amyloidogenic proteins in the physiology and biochemistry of this species, particularly under stress conditions such as acute irradiation.

Our work aimed to study the impact of ionizing radiation on protein conformation changes triggering amyloidogenesis in *Pisum sativum* L.

Materials and methods. Pea seeds were irradiated in the dose range 5–50 Gy of X-rays. Afterward, Fourier-transform infrared spectroscopy (FTIR) was used to investigate changes in the secondary structure of proteins in 3-day-old seedlings. Our focus was on the ratio between the amide I and II peaks. We conducted protein staining with Congo red to compare the presence of amyloids in the samples. Simultaneously, we analyzed the detergent-resistant protein fraction by ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS). To identify putative PrLPs, we utilized the PLAAC tool (Lancaster et al., 2014) [3]. Potential PrLPs and the differentially accumulated proteins were functionally annotated in MapMan Software (Forschungszentrum Jülich GmbH, Germany).

Results. Our findings revealed a reduced germination rate but higher plant height and faster appearance of reproductive organs in the group irradiated at a dose of 50 Gy compared to the control. Moreover, we observed increasing amyloid aggregates in the roots of seedlings grown from irradiated seeds. We identified 531 proteins in the detergent-resistant fraction extracted from roots, with 45 established as putative PrLPs. Notably, 29 proteins showed significant differential abundance between the irradiated and control groups. These proteins spanned various functional categories, including amino acid metabolism, carbohydrate metabolism, cytoskeleton organization, regulatory processes, protein biosynthesis, and RNA processing. Thus, our discovery proteomics approach provided in-depth data on novel aspects of plant stress biology.

Conclusions. Our data hinted that the accumulation of putative amyloids and PrLPs influences plant ontogenesis, probably through translation and RNA processing. The increased abundance of primary metabolism-related proteins indicates more intensive metabolic processes triggered in germinating pea seeds upon X-ray exposure. The functional role of detected putative amyloidogenic proteins should be validated in overexpression or knockout follow-up studies.

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BOOSTING OF BIOSYNTHETIC POTENTIAL OF PLANTS WITH EPIGENETIC MODIFIERS: IS IT POSSIBLE?

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Plants are irreplaceable source of various substances - both primary metabolites and secondary ones, which are widely used in most spheres of human life. So that, the attempts to increase the productivity of plants in order to use their biosynthetic potential more extensively are being carried out. The effect of different factors is realized (at least partially) through epigenetic modifications, which lead to significant changes in the state of chromatin and affect its availability in general and gene promoters, following by the changes of the gene transcriptional activity. For example, such intensification of DNA methylation leads to the weakening of gene expression and the acetylation of histone proteins is directly correlated with the accessibility of chromatin for transcription factors.

Therefore, it is quite logical to use the substances able to influence the epigenome directly in order to enhance the biosynthesis of secondary and primary metabolites. Such a role can be performed by the compounds that inhibit relevant enzymes, in particular DNA methyltransferases for DNA methylation and histone protein deacetylases. Since epigenome modifiers can effect gene activity, it is logical to assume that their usage may result in the expression of hitherto inactive genes and, consequently, the synthesis of new potentially promising metabolites. These modifiers may also cause the activation of expression of the functioning genes, that can lead to hyperaccumulation of their products. Such methods for regulating the activity of heterologous genes would be very useful, in particular, for intensifying the biosynthesis of biopharmaceutical recombinant proteins.

The usage of some of these modifiers in microorganism biotechnology demonstrated their significant effect on secondary metabolism processes in a number of bacteria and fungi species; so that the emergence of new secondary metabolites was reported.

The positive effects of such substances are shown also for plants. For instance, the significant intensification in the tashinone synthesis in the hairy root culture of *Salvia miltiorrhiza* under the effect of 5-azacytidine was recently reported (Yang et al., 2022). There are also some reports of the positive effect of histone deacetylase inhibitors on the production of some recombinant proteins (Rebelo et al., 2020).

However, the usage of such compounds in plant biotechnology is currently very limited and poorly studied. So we decided to fill this gap (at least partially) by means of the comprehensive analysis of the effects of epigenome-modifying substances on the biosynthetic potential of a number of plant species. The obtained results will broadly illustrate the response of different plant species and their primary and secondary metabolite production to the effect of epigenetic modifiers.

Our preliminary results deal with the inflluence of valproic acid as a powerful HDAC inhibitor on the regeneration processes of some plants of different families. The wide range of concentrations (0,005-10 μM) of valproic acid was shown to influence the regeneration process significantly. Thus, it demonstrated a dose-dependent phytohormone-like effect mimicking cytokinin or auxin activity in different cases.

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TRANSGENERATIONAL EFFECT OF OXALIC ACID AND SODIUM NITROPRUSSIDE AS ELICITORS IN WINTER WHEAT *T. AESTIVUM*

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The usage of biotic elicitor is a way to protect yields from plant pathogens and minimize influence on environment. It is known that induction of non-specific plant protection activates signal molecules and antioxidant system. However, there is still a question about the longevity of effect of biotic elicitors and epigenetic mechanisms.

Our previous research demonstrated that citric and succinic acid solutions stimulated metabolic processes during the whole vegetation period, from seed treatment to yield. It was also shown that oxalic acid and sodium nitroprusside (SNP, NO donor) acted as biotic elicitors to prevent damage of wheat fungal diseases in field. However, oxalic acid and SNP had positive effect on wheat plants in experiments with abiotic stresses (flooding and wounding).

The aim of our research is to investigate the epigenetic effect of biotic elicitors (oxalic acid and sodium nitroprusside, SNP, NO donor) in winter wheet plants.

Seeds from winter wheat cv Poliska 90 received from previous field experiments (untreated and treated with 0.1 mM oxalic acid and 0.5 mM sodium nitroprusside (SNP) were grown in water culture. In laboratory seedling at the stage of two leaves were sprayed by oxalic acid water solution 0.1 mM). The morphometric parameters of growth and hydrogen peroxide content in leaves were measured during the experimental period. The data were statistically processed.

It is shown the dynamics of hydrogen peroxide content in wheat leaves after oxalic acid treatment in different generations of plants. During the first day after spraying plants with oxalic acid water solution the hydrogen peroxide content decreased in plants without pre-treatment in previous generation in field experiment. However, in plants grown from seeds of pre-treated in previous generation plants this parameter increased. The differences were less during the third day. At fifth day there was a high level of hydrogen peroxide in leaves from variant with oxalic acid pre-treatment in previous generation**.**

It is shown the transgenerational effect of elicitor treatment with oxalic acid and SNP in winter wheat plants. The biotic elicitor oxalic acid is capable of an epigenetic influence on wheat that induces the reaction of plant antioxidant system during the repeated imitation of a phytopathogen attack at the early stages of ontogenesis. The action of the nitric oxide donor at the epigenetic level was less obvious compared to oxalic acid, which indicates the advantages of biotic elicitors for the formation of nonspecific immunity. It is shown that the transgenerational effect of biotic elicitors oxalic acid and SNP induced the system reaction of wheat plants during early stages of ontogenesis. The dynamics of endogenous peroxide during such induced reaction is similar to the latent period of phytopathogen.

THE EFFECT OF UV C AND KOJIC ACID ON *TRITICUM AESTIVUM* IMMUNITY

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The stress responses of important agricultural plants such as wheat is a common concern for humankind especially during the war times of war and climate changes. That difficulties have more possibilities for pathogen proliferation and spreading due to lack of control, high level of phytopatogen agression and their mutations. UV-C usage decreases risks however but there is an additional stress for plants via treatment.

The aim of our research is to increase efficacy and minimaze risks of UV-C treatment for plants. O It is shown in our previous work that kojic acid is a biotic elecitor, effective stimulates wheat immunity against fungal phytopathogens, protect plant tissues against damages of necrosis and regulates the antioxidant system activity enzyms during the abiotic and biotic stresses.

The wheat plants cv."Podolyanka" were grown during 14 days. Seedling were irradiated with 15kDj/m2 UV C radiation by Philips TUV 30W lamp and sprayed by 0.1 mM kojic acid water solution before the irradiation. The contamination were evaluated and phytopathogen Mucor sp. was identified. During the experiments the ehdogenous content of hydrogen peroxide and morphometric parameters of leaves and roots were measured. The pathogens and degree of their growth and contamination were identified by visual diagnostic and microscope technics. The data were statistically processed.

It is detected the infection of Mucor sp. from natural environment It is shown that combination of kojic acid pre-treatment and UV- C irradition influenced plant growth and hydrogen peroxide level in wheat leaves. During the first day after the irradition the reaction of antioxidant system was more significant in plants with combined treatment. But after 7 and 9 days the levels of hydrogen peroxide in leaves stabilized in treated wheat plants almost at the control level. Conclusions.The impact of kojic acid before UV-irradiation protect the growth and development of wheat plants tissues, stimulate the tolerance and the wheat non-specific immunity. The processes of plant reparation were accelerated and therefore the damage and risks for wheat plants were minimazed.

EPIGENETIC AND GENETIC FACTORS IN THE FORMATION OF LONG-TERM METABOLIC CHANGES UNDER ACUTE STRESS

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The objectives of the study was assessment of the involvement of X-ray and UV-C-induced epigenetic rearrangements and genomic instability in induction plant long-term protective reactions.

In a series of experiments using both X-ray and UV-C radiation exposure a parallel study of several pharmacological characteristics of the *Matricaria chamomilla* L. genotype group was carried out.

Research was carried out on 8 genotypes of chamomile. Dry seeds were exposed using the RUM-17 X-ray installation (Russia) at a dose of 5-15 Gy, with a dose rate of 1.42 cGy/s. UV-C exposure was conducted at a dose of 5-20 kJ/m² using an OBM-150 M installation (Ukraine). DNA methylation research was carried out through restriction analysis followed by ISSR-ITS-PCR. For restriction analysis two types of restrictase-isoschizomers were used: MspI and HpaII. Three types of markers were used for PCR: ISSR-5, ITS1 and ITS4.

As an indicator of the difference between the set of electrophoregrams of control and exposed samples the indicator D = epigenetic distance (hereinafter - ED) was used. It was calculated similarly to the estimation of the genetic distance according to Nei[1].

The study of rearrangements of the primary structure of DNA under conditions of different doses of X-ray and UV-C exposure was carried by PCR using 8 ISSR and 10 RAPD primers.

Two indicators were used to characterize dose-depend changes in amplicon spectra using ISSR and RAPD DNA markers' analysis of both control and irradiated variants. The indicator "% of atypical amplicons" was calculated as the ratio of the number of amplicons which were different from the control to their total number. To assess the significance of the difference in average indicators for variants of the experiment, the non-parametric Van der Waerden X-test was used. In the cluster analysis of changes within the amplicon spectra as an integral group the Jacquard similarity index was used. Extraction of flavonoids and phenols was carried out by traditional methods (Djeridane et al. 2006) which was also used in the previous works of the authors with SF-46 (Shimatzu UV-1280) spectrophotometer [2,3].

Results. It was shown that DNA methylation was switched to the *de novo* mode in plants of all studied genotypes of *M. chamomilla* under both types of irradiation. That indicates changes in the epigenetic program of the plant organism. Comparison of the epigenetic pattern between control and irradiated samples, based on the difference in DNA methylation patterns in terms of a statistical indicator, shows that there is no unambiguous relationship between the epigenetic changes and increasing yield of antioxidant synthesis. This is additional evidence of the diversity of metabolic rearrangements and adaptive strategies of the plant organism under radiation exposure even within one species[1].

The objectives of the next study of our investigation was assessment of the involvement of X-ray and UV-C-induced genomic instability in induction plant long-term protective reactions.

The results of the study suggest that genomic instability is a link between the direct effects of UV-C exposure and stimulation of metabolic rearrangements at the final stages of ontogeny[2,3]. A hypothetical scheme for the transformation of primary X-ray and UV-C DNA damage into long-term maintenance of genomic instability signs and metabolise transformation has been proposed.

ECOLOGICAL ADAPTATIONS AND FUNCTIONAL ROLES OF ANTARCTIC ENDOPHYTIC BACTERIA WITHIN THEIR PLANT HOSTS

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Deschampsia antarctica Desv. (Poaceae) and *Colobantus quitensis* (Kunth) Bartl. (Caryophyllaceae) are the only two vascular plants that have colonized the Antarctic continent, which is usually exposed to extreme environmental conditions. Endophytic bacteria might influence the plant's epigenetic memory of stress responses as well as could prime the plant for better tolerance to future stressors. Epigenetic modifications induced by endophytes could enhance the plant's fitness by promoting growth or other beneficial traits. Knowledge of endophytes, their diversity and factors affecting their patterns and assembly in host plants, is fundamental to our understanding of plant-microbial interactions and how plant microbiomes affect stress tolerance. In this work, we aimed to characterize 12 endophytic bacterial strains associated with these plants describing their ecological adaptations and functional roles including plant growth-promoting activity, nutritional strategies and environmental tolerance to varying pH and salt levels.

Endophytic bacterial cultures were isolated from the inner part of roots and leaves of *D.antarctica* and *C.quitensis* sampled during the 25th Ukrainian Antarctic Expedition (January-April 2020) and identified by the 16S rRNA molecular approach and followed phylogenetic analysis using GenBank database and Blast software. Plant growth-promoting activity such as Nitrogen-fixing activity, biosurfactants, siderophores, HCN, ammonia and indol-3-acetic acid production as well as phosphates solubilization were tested by generally accepted methods. Bacterial ability to thrive in oligotrophic environments were tested in diluted nutrient broth media (NB, HiMedia Ltd.) with the concentration range 30-300 mg/L of total Carbon. Environmental tolerance to varying pH (3-11) and salt levels (3%-25%) were tested in NB medium at 20ºC in planktonic and biofilm growth modes.

Firstly, some bacteria were identified as species belonging to relatively unexplored groups like *Hafnia* sp. and *Agreia* sp. with the potential for a variety of applications in agricultural and pharmaceutical studies. Most bacteria were able to use atmospheric N_2 as a source of nitrogen (at least for their own growth) and may be able to provide the host plant with an extra source as well. Additionally, all studied bacteria were able to produce ammonia which could also be used as a nitrogen supplement for plant growth. Besides, bacteria can enhance plant nutrient uptake by solubilizing immobilized phosphates. Most of identified endophytic bacteria were able to dissolve immobilized mineral phosphates, suggesting that during initial colonization, bacteria can increase the availability of phosphates to plants. Less frequent was the trait to produce IAA, which was observed only in four strains, and HCN production – only in two. According to the growth rate studied bacteria are not halophilic, but halotolerant microbes instead. The majority of studied strains could be characterized as oligotrophic and slightly to moderate halotolerant bacteria.

Overall, this study highlights the functional diversity of Antarctic endophytic bacteria and offers hypotheses on how these bacteria assist their plant hosts' growth and resilience in extreme environments.

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THE ROLE OF ENDOGENOUS AND RHIZOSPHERE LIGHT IN THE FUNCTIONING OF PLANT ORGANISM, POPULATIONS, BIOCENOSES AND ECOSYSTEMS

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Algae, many of which are obligate photoautotrophs, exist not only in the upper layers of the soil but also occur at depths of up to several meters. At the same time, they are capable of active vegetation. On average, 1 g of the top layer (about 2 cm deep) of soil contains 10^3 to 10^6 cells of different types of algae. The area of direct light penetration through the soil is limited to a few millimeters. The latter indicates that their photosynthetic apparatus must receive light quanta even in very small quantities and accumulate them. Feyer and Frank hypothesized that deep algae can use the "invisible" ultraviolet part of the spectrum for photosynthesis. Along with chlorophyll a, which is present in all plant organisms, many species contain chlorophyll c with three absorption maxima. In photosynthetic organisms, there are at least 3 varieties of it. The maximum absorption peaks of chlorophylls - c_1 (λ = 444, 577, 626 nm), c_2 (λ = 447, 579, 629 nm) and c_3 (λ = 454, 583, 630 nm) are located in the blue, green and red spectrum, respectively. Their main function, as well as pigments in algae and pigment-forming microorganisms, is the accumulation and consistent transfer of light energy, which, through luminescent radiation, reaches the reaction center of chlorophyll a. Algae have a wide variety of pigments - phycobilins, which have luminescence parameters in the absorption regions of chlorophyll a. Chlorophyll d, along with chlorophyll f, is synthesized in small quantities by many cyanobacteria during their growth under near-infrared light (λ = 710 - 750 nm).

A mixed research group has shown the ability of at least 22 species of higher plants belonging to 5 divisions of the plant kingdom to conduct low-intensity light in the spectral range up to the rhizosphere. The presence in cells of proteins with light-sensitive complexes - cryptochromes, which are capable of perceiving light of a certain quality (quanta of ultraviolet, blue and green spectral ranges) and intensity, provide indirect changes in the regulation of gene transcription, which causes certain changes in the physiological and morphological responses of plants. Thus, spatial conformational changes in the peptide components of cryptochromes can be initiated even by two quanta of the blue spectral range. The latter leads to changes in gene transcription and regulation of physiological reactions of the plant organism. Thus, plant structures contain endogenous protein photo-sensory systems activated by radiation of a certain quality and intensity, which, due to transformations of their spatial position, lead to indirect or direct changes in gene expression.The latter initiates the processes of certain morphological and physiological changes in the plant organism.Thus, plants have structures and mechanisms that provide light to their underground parts, and light-sensitive root hair cells are the last link in the transmission of quanta to soil algae and light-sensitive microorganisms.The intake of light of a certain quality and intensity into the rhizosphere as photo-signals can contribute to certain changes at both the organismal and group levels. It is highly likely that light signals entering the rhizosphere affect the structures of water-soil capillaries, which act as an oscillator and initiate the reorganization of biological processes at the population, biocenotic, and even ecosystem levels.

PHYSIOLOGICAL RESPONSES OF WILLOW "ZHYTOMYRSKA-1" TO EXCESSIVE LEVELS OF UV-B RADIATION

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Willow plantations represent a promising resource for various industries, such as biofuel and pharmaceutical production. However, contemporary climate changes, notably the significant depletion of the stratospheric ozone layer, lead to increased penetration of solar ultraviolet radiation, particularly UV-B and UV-C types, into the lower layers of the atmosphere. This influx of UV radiation can induce substantial biochemical and physiological alterations in plant organisms.

This study amed to assess the impact of UV-B radiation on the nitrogen balance index and the relative concentrations of chlorophyll, anthocyanins, and flavonoids in the leaves of highly productive willow clones.

Firstly, willow plants were cultivated in vitro to obtain the uniform experimental sample. Then, the plants were transferred into pots and adapted to soil conditions for a month. The plants were divided into four groups: a control group of six plants and three experimental groups, each consisting of five plants exposed to UV-B radiation at doses of 5, 10, and 15 kJ/m^2, respectively. Over the subsequent two months following irradiation, the relative content of anthocyanins, flavonoids, chlorophyll, and the nitrogen balance index in the leaves of the studied plants were analyzed using a portable multiple wavelength pigment meter "Opti-Sciences MPM-100".

Statistically significant discrepancies in the relative chlorophyll content in the leaves of irradiated plants compared to the control were observed only on July 31 and August 7 in willows exposed to a 5 kJ/m^2 dose of radiation. Regarding the nitrogen balance index and the relative content of flavonoids, differences from the control were noticeable on August 7 in plants subjected to a 15 kJ/m^2 dose of radiation. Interestingly, from July 31 to September 29, all groups of irradiated plants exhibited a decreased relative content of anthocyanins compared to the control. Additionally, leaf curling was observed in two plants exposed to a 10 kJ/m^2 dose of radiation. However, willows subjected to 5 and 15 kJ/m^2 doses did not display visible morphological abnormalities.

Therefore, applied doses of UV-B radiation (5 - 15 kJ/m2) did not clearly impact the physiological parameters of willow "Zhytomyrska-1", particularly the relative content of chlorophyll, flavonoids, and the nitrogen balance index. However, the reduced levels of relative anthocyanin content suggest a post-radiation recovery process due to damage to primary metabolic pathway components. These findings may indicate the high resilience of willow "Zhytomyrska-1" to UV-B radiation.

TOOLS AND STRATEGIES FOR ENGINEERING ANTHOCYANIN AND BETALAIN BIOSYNTHETIC PATHWAYS IN HETEROLOGOUS HOSTS

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Anthocyanins and betalains are natural plant pigments that contribute to the vibrant array of colors found in flowers and fruits across various species. Both classes of compound offer health benefits when consumed through natural sources like red beet, red cabbage, cherries, and blueberries. Natural foods rich in these pigments are however limited, and expanding the variety of fruits and vegetables containing high anthocyanin or betalain content would enhance consumer choices and nutrition. This can be done by engineering biosynthetic pathways to produce these compounds in fruits or vegetables that do not naturally produce them or do not produce them in the consumed parts of the plants. Engineering of anthocyanin and betalain biosynthetic pathways could also be used to produce fruits or vegetables with high pigment content to use as a source for production of natural food colorants. Engineering of anthocyanin biosynthetic pathways can be achieved in plants by tissue-specific expression of transcription factors. This strategy is however limited to species that already contain the biosynthetic pathway to enable expression in tissues or organs where the compounds are not naturally produced. Consequently, only the native pigment produced by the species is generated using this method. To produce novel anthocyanins and potentially increase the color range available in species of interest, more complex engineering strategies are required. This includes introducing genes that are not naturally present in this species, suppressing the expression of some genes while increasing the expression of others. One of the critical factors is to be able to simultaneously induce expression of all genes of the pathways in a specific tissue. This can be done using tissue-specific promoters or by using natural or synthetic transcription factors. We will give some examples of strategies that we have used for engineering betalain and anthocyanin biosynthetic pathways in species such as tomato, *Nicotiana benthamiana* and yeast.

RECENT ADVANCES IN POLYPHENOLS BIOTECHNOLOGY

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Plant phenolic compounds are ubiquitous calls of specialized metabolites providing plant organism with essential functions in defense, structural reinforcements, signaling and interactions with other organisms. Typically, phenolics are classified structurally by containing one or more phenolic rings substituted with at least one free or substituted hydroxyl group. However, biosynthetic origin is limited to shikimic acid derivatives, and the natural diversity goes beyond this simplified definition, including compounds without a hydroxyl group or polymerized structures. Conversely, some other compounds with a phenolic part originate from polyketide or terpenoid pathways. Polyphenols, phenolics with several hydroxyl groups are known in pharmacology and nutrition as important contributors to preventive properties of herbs and foodstuff. The demand for high quality and safe polyphenol products points to biotechnology as means to achieve improved, cleaner or safer bioactive substances. However, biotechnological approach goes far beyond the utilitarian goals but is also essential for understanding the mechanisms that orchestrate complex metabolic networks resulting in final, yet dynamic composition of natural products.

In this presentation, some examples of using plant cell, tissue and organ culture for obtaining desired phenolic content as well as for discovery and manipulating biosynthetic pathways. The role of stress response is often listed as the most important factor to induce and boost polyphenol content. However, the picture is not always very clear, and some ambiguity still exists, opening great perspectives for further studies, especially employing recent advancements in gene editing and systems biology. Some current examples of polyphenols as plant biotechnological products will be also presented.

RECENT ADVANCES IN PAPAVERACEAE BIOTECHNOLOGY

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The Papaveraceae plants are known for their diverse group of pharmacologically active alkaloids. Within this plant family, species belonging to the Fumarioideae subfamily are rich in isoquinoline alkaloids (IQAs). The presence of IQAs in the latex influences its color, ranging from yellow to vivid red. These compounds are known, among others, for their antiviral, antiparasitic and antimicrobial properties. Some IQAs, like berberine and coptisine, are commercially produced using plant tissue cultures. However, our understanding of many other such compounds, abundantly produced in various medicinal poppy family plants, remains limited. In our research, we employed plant tissue and organ cultures to monitor the biosynthesis and extraction of IQAs. We tested their effectiveness against several *Candida* species, and pathogenic bacteria, including those forming biofilms. Additionally, we developed an innovative *Chelidonium majus* L. cell-support system using bacterial bionanocellulose. This system aims to elicit a response from partially immobilized plant cells, thereby enhancing their antimicrobial properties. Our research focuses primarily on *C. majus*, a herb traditionally used to treat skin infections. However, we have also examined several other species from genera such as *Corydalis*, *Glaucium*, and *Fumaria*. These advancements underscore the significant potential of *in vitro* cultures for producing pharmacologically important isoquinoline alkaloids and pave the way for future biotechnological applications using Papaveraceae plants. Furthermore, the role of other specialized metabolites, such as polyphenolic compounds, should not be overlooked, as they may significantly modulate the antimicrobial activity observed in these plants.

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ELICITED CALLUS CULTURES FROM APPLE PEEL: A UNIQUE RESERVOIR OF ANTIOXIDANTS

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The Annurca apple is a cultivar native to Southern Italy and listed as Protected Geographical Indication (PGI) product from the European Council [Commission Regulation (EC) No.417/2006)] due to its high nutritional content. This apple is enriched in polyphenols (hydroxycinnamic acids, dihydrochalcones, catechin, epicatechin and procyanidins) and terpenes, in particular triterpenic acids with health-promoting properties (Laezza et al. 2024). Although these specialized metabolites (SMs) are in high demand in the pharmaceutical, cosmetic and food markets, their production depends on the seasonality of plants. Furthermore, their production implies excessive use of water and land. Plant cell cultures (PCCs) are a "ready-to use" technology that allows SMs to be synthetized year-round and consistently without impacting the environment (Krasteva et al. 2021). Our work aimed to develop PCCs from the Annurca apple peel under sterile conditions, thus using a fruit tissue containing a wide variety of natural compounds. This is particularly important considering that the apple peel is treated with pesticides and therefore a waste product for both people and industries. After analysing the quantity of specific compounds found in peel-derived calli by HPLC, the use of yeast extract (YE) as elicitor was investigated. This technique is employed to trigger signals in plant cells thus inducing the accumulation of a greater amount of bioactive compounds. Two different concentrations of YE were tested: 300 and 500 mg L^{-1}. Elicitation of peel-derived calli with YE 500 mg L^{-1} resulted in an augmented production of phloridzin (dihydrochalcone) and ursolic acid (triterpenic acid). Metabolic results were confirmed by qRT-PCR as the expression level of genes directly related to the production of phloridzin (*MdDH* and *MdUGT88F1*) and ursolic acid (*MdOSC1* and *MdCYP716A175*) was found to be enhanced. To demonstrate the beneficial potential of elicited calli their extract was examined against foodborne pathogens by using the disk diffusion method to test bacterial growth. Interestingly, the outcomes revealed that the extract had effect against *S. aureus* and *B. cereus.* Our study demonstrated the capability of PCCs to be a valid alternative to the plant biosynthesis of natural compounds. The production of an elicited extract rich in phloridzin and ursolic acid from Annurca peel-derived calli may represent a promising technology with different use.

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CONSERVATION AND STUDY OF PLANT SPECIES USING *IN VITRO* BANK

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A lot of wild-growing plant species are still poorly studied but are of significant practical interest as a source of valuable secondary metabolites for pharmacology, agriculture etc. Many of them are endemic or/and are included in the lists of endangered or protected species. The use of botanical gardens or industrial field plantations for their preservation and practical utilization requires large areas, depends on climate and weather factors, and is vulnerable to pests and diseases. *In vitro* conservation can avoid these limitations. It allows large-scale cultivation in controlled aseptic conditions on the appropriate culture media; makes it possible to direct *in vitro* morphogenesis to the desired pattern; saves the working space etc. *In vitro* banks can be a basis for conservation of endangered/endemic or any other species of interest and their use in biotechnology.

Seed bank and *in vitro* collection of the world flora have been initiated in 1993. In the course of the work during these thirty years aseptic plants and cell cultures of near two thousands species were initiated from seeds and tested for their abilities to be cultured *in vitro* on a number of standard or modified culture media [1]. Studies on seed longevity were carried out on the basis of the seed bank which is stored at +4^{o}C. It allowed determining plant families and genera whose seeds are able to preserve their viability and to be used for *in vitro* culture initiation over decades of conservation. In addition to the standard protocols of *in vitro* cultivation the methods of slow growth on the media containing mannitol and abscisic acid were tested on some representatives of Caryophyllaceae, Scrophulariaceae and others (total 17 plant families). For the majority of the studied species the concentrations of mannitol and ABA were determined that allowed to decrease significantly the rates of plant growth *in vitro* without interfering their viability and to increase subcultivation intervals that is an important factor in maintaining large-scale collections of aseptic plant material. Methods of rapid propagation of endemic and endangered plant species were used to initiate and maintain *in vitro* cultures of *Juno* Tratt. (Iridaceae) and *Crambe* L. (Brassicaceae), *Fittonia albivenis* (Lindl. ex Veitch) Brummitt (Acanthaceae), Antarctic flora representatives (*Deschampsia antarctica* E.Desv. (Poaceae), near twenty species of Antarctic mosses), and many others. The extracts of aseptic plants and cell lines were studied for secondary metabolites [2]. A thorough research of the content of various biologically-active substances in different *in vitro* plants and cell cultures was carried out. Antidepressive substances hypericin and hyperforin (from *Hypericum*), rosmarinic acid (from *Salvia*), antimicrobial meroterpene bakuchiol (from *Psoralea*), and others are among them. Plant material of *in vitro* bank was used to initiate hairy root cultures *via Agrobacterium rhizogenes*-mediated genetic transformation (*Achillea lingulata* Waldst. & Kit., *Artemisia tilesii* Ledeb (Asteraceaea), *Nigella arvensis* L. (Ranunculaceae), etc) and for the search of efficient host species for *Agrobacterium*-mediated transient expression.

The bank is included to the List of the Objects of National Scientific Dignity of Ukraine and has an appropriate financial support.

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GELATINASE ACTIVITY IN THE PLANT EXTRACTS OF DIFFERENT FAMILIES

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Collagenases and gelatinases are enzymes of metalloproteinase class that can destroy collagen. They are widely used in various industries, in particular, in leather dyeing. Moreover, their medicinal properties are being actively studied, e.g. they can be use for fibrosis treatment, to improve drug delivery ways, in oreder to treat Dupuytren's contractures, and non-surgical treatment for Peyronie's disease. In addition, the bacterial collagenases are used for wound healing. The enzymatic debridement is a frequently used technique for removing necrotic tissue from wounds and the collagenase-based Santyl ointment has been recently approved by the FDA.

Plants do not synthesise collagen and therefore were thought to have no collagenases or gelatinases. However, this has been shown to be not the case; at least two plant species have been identified as capable of breaking collagen down: *Zingiber officinale* (1) and *Ficus carica* (2). Plant collagenases mainly break down collagen in its native form. These enzymes can play an active role, e.g. to protect against pests such as nematodes. The collagenolytic activity of plants can be activated by a variety of stresses, so that this can serve as an important defence mechanism against environmental changes.

The aim of our work is to find new plants that can produce collagenases and gelatinases. We have used *in vitro* plants of the Germplasm Collection of the Institute of Cell Biology and Genetic Engineering. So far, we have analysed 39 plant species of 17 families. The presence of gelatinase activity was detected by two methods: via digestion of gelatin films and via enzyme electrophoresis.

Some gelatinase activity was detected in nearly 20% of the studied species, in particular, the most noticeable activity was observed in the shoots of *Teucrium scorodonia* (*Lamiaceae*) and *Portulaca fluvialis*. It is currently unknown how specific their activity is, and this requires further analysis. The presence of gelatinase activity was noted not to depend on the taxonomic position of the studied plants.

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VARIABILITY IN B-D-GLUCAN CONTENT FOR NEXT USES OF OAT SEEDS IN THE FOOD INDUSTRY

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Oat grains (*Avena sativa* L.), referred to as functional foods, are good source of substances with high biological and/or functional activity. β-D-glucan, the cell wall homopolysaccharide composed of glucose units linked true both β-(1-3) and β-(1-4) glycosidic linkages in various ratios, is one of such ingredients. It is observed only in selected *Poales* and in oat grain its average level is 3-5%. Using the β-Glucan Assay Kit (Mixed Linkage) (Megazyme, Bray, Ireland) the content of this polysaccharide in mature grains of *Avena sativa* was 1.85-6.12%. Among different *Avena* species, the content of β-D-glucan decreases as follows: *A. sativa* var. *nuda* > *strigosa* > *byzantina* > *sativa* > *sterilis* > *abyssinica* >*ludoviciana* > *murphyi*. Genotype and environment (fertilisation with N and N+Se, precipitation, soil quality, type of cultivation, biotic stress) influence the content. Good natural sources of this polysaccharides can be used in the food industry. The β-D-glucan hydrogels are successfully used in bread, ketchup, and kombucha lemonade preparation to increase nutritional value, time of storage, and final taste. Oat bran (5-15%) is applicable to prepare mixed flour to increase the content of β-D-glucan and total dietary fibre and to decrease the content of starch.

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CALLUS CULTURES OF *CUCUMIS MELO*

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Cucumis melo is an economically important fruit crop in many areas of the world. Despite its significance, the yields and quality of melons are increasingly threatened by diseases. This study evaluates various hormone combinations on different explants to create an efficient micropropagation system, which could support further molecular biology research on viroid-callus interactions *in vitro*.

Leaf, root, and stem explants of *C. melo* were used for callus induction. The explants were incubated in Murashige and Skoog (MS) medium, supplemented with various combinations of growth regulators including IAA, BAP, TDZ, KIN, NAA, 2,4-D and IBA, at concentrations ranging from 0.1 mg/L to 3.0 mg/L. Cultivation was conducted at a temperature of 23±2 °C in complete darkness. We monitored several parameters including callus formation, necrotization, callus structure and color, as well as plant regeneration.

Optimal combinations of growth regulators were identified for each type of explant. A combination of 0.1 mg/L NAA and 3.0 mg/L BAP was most effective for root-derived calli. For leaf and stem explants, the combination of 0.1 mg/L NAA + 3.0 mg/L BAP and 1.0 mg/L NAA + 0,5 mg/L BAP proved effective. These combinations successfully induced robust callus formation without signs of necrosis, crucial for the long-term maintenance of callus tissue.

The study provides insights into the optimal conditions for *C. melo* callus cultivation, supporting its use in micropropagation and genetic studies. Future research will focus on the long-term monitoring of these cultures, introducing viroids into *in vitro* conditions, and analyzing the molecular effects on viroid-infected callus.

This work was founded by APVV-22-0067 from the Slovak Research and Development Agency, project "Viroids - unique subviral plant pathogens, their diversity and host interactions".

PRODUCTIVITY, ANTIARRHYTHMIC AND HYPOTENSIVE ACTIVITY OF ALKALOIDS OF *RAUWOLFIA SERPENTINA* TISSUE CULTURE

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Rauwolfia serpentina is a medicinal tropical plant widely used in the traditional medicine of India and China due to its antiarrhythmic, hypotensive, sedative, and psychotropic activities. Therefore, this species is a promising source of raw material for development of antiarrhythmic and hypotensive drugs. Considering the scarcity of natural resources, the strain K-27M of *R. serpentina* tissue culture has been established at the IMBG of the NAS of Ukraine.

The aim was to investigate the productivity of the strain and the content of indole alkaloids, as well as to evaluate the antiarrhythmic and hypotensive effects of various fractions of biomass extracts of the *R. serpentina* K-27M strain.

The strain was cultured at 27–28°C in the dark, in 370 mL glass jars on the 10C solid agar medium without growth regulators. The content of indole alkaloids in dry tissue, qualitative and quantitative composition of fractions was determined using HPLC-MS on the Agilent 1260 Infinity II system with C18 column. The hypotensive activity was evaluated using the rat thoracic smooth muscle *in vitro* and by assessing the effect of intravenous administration on blood pressure *in vivo*. The antiarrhythmic activity was studied in rats with models of adrenaline-induced arrhythmias as well as on the isolated guinea pig hearts with ischemia and reperfusion-induced arrhythmias.

The maximum wet biomass yield was at 69th day of subculture and amounted to 670-690g/L medium of wet biomass or 40-43g/L of dry biomass. The maximum content of indole alkaloids was observed from 88th to 108th days of subculture. The dry biomass contained 4.0% of total indole alkaloids, including 1.64% of ajmaline-like alkaloids, 0.79% of ajmaline, 0.34% of vomilenine, and 0.006% each of yohimbine and reserpine.

Five fractions were obtained from the extracts of dry (fractions 1,2,3) and wet biomass (fractions 4,5) of K-27M strain. Fraction 1 contained ajmaline and acetylajmaline (total alkaloid content (TAC) was 2.2% of dry biomass); fraction 2 consisted of ajmaline, acetylajmaline and raucafricine (TAC 6.4%); fraction 3 included mainly ajmaline and raucafricine (TAC 29.0%). Fraction 4 was dominated by vomilenine, methylajmalicine, ajmalicine and raufloridine (TAC 65.0%); fraction 5 contained acetylajmaline (TAC 47.4%).

The fractions 4 and 5 had a pronounced antiarrhythmic effect in rats. In contrast, depleted of indole alkaloid fractions 1 and 2 showed a weak proarrhythmic effect. The antiarrhythmic effect of fraction 5 on the isolated guinea pig hearts was not inferior to the control sample of ajmaline in terms of the activity intensity. Fractions 1 and 2 exerted a constrictor effect on the rings of the thoracic aorta of rats. The fractions 4 and 5 had a vasorelaxing effect *in vitro* and transitory (15-30s) hypotensive effect *in vivo*.

Thus, the studied K-27M strain of *R. serpentina* tissue culture is a promising producer of indole alkaloids. The physiological mode of action depended on the content and composition of alkaloids in the fractions. The wet biomass extracts have demonstrated significant antiarrhythmic and transitory hypotensive effects.

ANTIVIRAL POTENTIAL OF *UNGERNIA VICTORIS* TISSUE CULTURE BIOMASS-BASED BIOLOGICALLY ACTIVE COMPOUNDS

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Ungernia victoris Vved. ex Artjushenko is a valuable rare medicinal plant with a spectrum of biologically active compounds. The pharmacological value of *U. victoris* natural sources is associated with the presence of alkaloids, coumarins, essential oils, polysaccharide complexes, etc. Besides secondary metabolites, of particular interest are carbohydrate-binding proteins — lectins for which antiviral, antibacterial, immunomodulatory and antitumor activity are known.

Viral infections remain a major cause of morbidity and mortality worldwide. Natural pharmacotherapy is promising for prevention and additional therapy in the treatment of viral diseases and has such advantages as a wide spectrum of action, low toxicity, and lower side effects. In this regard, the aim of our study is identifying the components of *U. victoris* extracts with a pronounced antiviral activity.

Extracts were obtained from highly productive UV-2 strain of *U. victoris* tissue culture, which was grown on a specially developed solid hormone-free medium according to [1,2]. Secondary metabolites were extracted with ethanol at 40% saturation, then evaporated and the precipitate was dissolved in water. Extracts were tested for antiviral activity under *in vitro* conditions against strain of influenza virus A/FM/1/47(H1N1), swine transmissible gastroenteritis coronavirus and herpes simplex virus type 2 (HSV-2). To detect lectin-like substances, extracts were prepared from dry cell biomass in a 0.15 M NaCl solution for 2 hours at room temperature with subsequent centrifuged. Lectin activity and carbohydrate specificity were determined in the supernatant and sediment of the extracts by the generally accepted method in a series of double dilutions in a 96-well immunological plate. The measure of lectin activity was the highest dilution at which hemagglutination (with mouse red cells) was observed.

Antiviral activity of ethanol-extracted and water-dissolved secondary metabolites was shown for all three model viruses *in vitro* and have low cytotoxicity for cells. For example, it was shown that the extract of *U. victoris* inhibited the reproduction of the herpes virus up to a dilution of 1:6400.

As for lectin-like substances, the carbohydrate specificity for complex polymeric molecules (hyaluronic acid>heparin>mucin) was shown. It is known that mucin contains sialic acids, which are a component of the receptors of common animal viruses.

Thus, the studied UV-2 strain of *U. victoris* tissue culture is a promising producer of antiviral compounds, that require further research.

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EFFECT OF SALINITY OF GROWING MEDIUM TO COMPOSITION OF FATTY ACIDS OF LIPIDS OF *MEDICAGO SATIVA* IN SYMBIOSIS WITH RHIZOBIA AND UNDER INFECTION BY PHYTOPLASMA IN MICRO-VEGETATION EXPERIMENT

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A comprehensive study of the two components of the infectious process - the pathogen and host cells in the dynamics of their interaction in the case of plant phytoplasmosis is possible using the co-cultivation of phytoplasmas and plants under sterile conditions. The present work aimed to study the effect of salinity to the composition of total plant lipids under conditions of symbiosis of *Medicago sativa* with nodule bacteria *Rhizobium meliloti* of different sensitivity to salt and additional infection with phytopathogenic acholeplasma.

The effectiveness of strains of different *R. meliloti* genotypes with different salt tolerance was studied under salt stress conditions: an effective production strain *425a* (as a standard), *S19* (with increased tolerance) and *S157* (a salt-sensitive strain). The seeds of *M. sativa* (variety Sinyukha) were used in the study; plants were cultivated on Krasilnikov-Korenyako medium in a micro-vegetation experiment. To create conditions of salt stress NaCl 50 mM was added to the agar medium. *Acholeplasma laidlawii var.granulum 118* and *R. meliloti 425a* were received from the National Collection of microorganisms of Ukraine. Salt-sensitive rhizobia strain *S157* and salt-tolerant strain *S19* were kindly provided by Dr. Zatovska T. The fatty acid composition of cellular lipids was studied by gas chromatography-mass spectrometry on an Agilent 6890N/5973 inert instrument (Agilent Technologies, USA). We are sincerely grateful to Dr. Kharkhota M.A for invaluable collaboration in the chromatographic researches.

Under standard conditions of experiment the C16, C17, C18, C18:2, C18:3, C22, and C24 fatty acids were found in the total lipids of *M. sativa* plants, both intact and infected with acholeplasma. Changes in the composition of lipids in infected plants occurred: compared to sterile plants, the amount of saturated acids significantly increased (1.6-2.7 times) in them and unsaturated acids decreased (1.8 times). Under saline conditions, with *A.laidlawii var.granulum 118,* an increase in the intensity of phytoplasmosis symptoms and acceleration of death of infected plants are observed. Under the influence of two stress factors (salinity and infection), the amount of saturated fatty acids increases significantly (1.6 times), but the unsaturated fatty acid index decreases even more. In the variants of the experiment with acholeplasma infection and rhizobia strains *425a* and *S19*, under the influence of salinity, the content of C16 increased by 1.5 and 1.2 times, respectively. At the same time, the content of C18:2 and C18:3 significantly decreased: for *425a* - by 2.5 and 4.7 times, and for *S157* - by 1.7 and 1.9 times, respectively. In the variant with acholeplasma and *S19,* the tendency in changing of fatty acids content of lipids also was observed but it was less significant. When the medium was salinized, the content of C16 and C18:2 acids increased (by 10%), and the content of C18:3 acid, on the contrary, decreased by 20%. These results demonstrate that salinity is a strong stressor for alfalfa plants, which increases the damaging effects of phytopathogens and changes the fatty acid composition of lipids in sterile and infected plants. The use of tolerant to salinity cultures of nodule bacteria improves the physiological status of *M. sativa* plants and helps to decrease the symptoms of phytoplasmosis.

UNIQUE COLLECTION OF HAIRY ROOTS OF MEDICINAL PLANTS

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The collection of hairy roots of medicinal plants was created via *Rhizobium* (*Agrobacterium*) *rhizogenes* – mediated transformation for the research of the various methods of their usage as a source of biologically active compounds. Such collection may be invaluable not only for the human- and animal-centered pharmacology, but for the study in plant physiology as well (differences in structure, morphology, growth rate of hairy root lines).

Seeds surface sterilization was used to introduce plants *in vitro*. The obtained plants were cultivated on the surface of half strength Murashige and Skoog (1/2 MS) solidified medium at +24°C and 16h illumination. Leaves of 10-14-day-old seedlings were used as explants. For transformation, the explants were cocultivated with *A. rhizogenes* suspension for 30 min and then were transferred to the selective media to induce hairy root formation. The initiated roots were subcultivated on 1/2 MS solidified medium at +24°C and 16-hour illumination. The presence of bacterial *rol* genes as well as target *ifn-α2b* and *esxA-fbp*BΔTMD genes was proved by PCR analysis of genome DNA on Mastercycler personal 5332 amplifier (Eppendorf) using primers specific for these genes**.**

The collection consists of more than 80 lines of hairy roots of such medicinal plant species, as *Cichorium intybus, Althaea officinalis, Tragopogon porrifolius, Ruta graveolens, Bidens pilosa, Linaria maroccana, Calendula officinalis, Pyrethrum corymbosum, Lactuca sativa*, and *Artemisia spp.* (*A. tilesii, A. vulgaris, A. annua, A. absinthium, A. dracunculus, A. ludoviciana*). The most recent additions include the replenishment of collection with 15 new lines of *A. tilesii* hairy roots obtained via transformation using *A. rhizogenes* wild A4 strain.

Studies have revealed significant dissimilarities in morphology and growth rate among different lines of hairy roots. Such variations may be related to the indeterminacy of the incorporation of transferred genes of *A. rhizogenes* and their influence on the activity of the plant's own genes.

The transgenic roots, which had the human *ifn-α2b* gene, synthesized this protein, and the root extracts showed interferon-like antiviral activity against vesicular stomatitis virus. The hairy root extracts also demonstrated antioxidant, reducing and anti-inflammatory activity. It should be noted that this activity of extracts from transgenic roots was higher than the corresponding activity of extracts from roots of control plants. Study of possible elicitors has begun to find the ways of subsequent boosting of biosynthesis.

Transgenic roots of medicinal plants of various species, characterized by a high level of biological activity, can be used as producers of valuable compounds for their use in medicine.

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EFFECT OF X-RAY IRRADIATION ON THE CONTENT OF ROSEMARY ACID, PHENOLS AND FLAVONOIDS IN THE EXTRACTS OF *IN VITRO SALVIA OFFICIALS* PLANTS

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Salvia officials is an object of scientific interest due to the significant content of secondary metabolites – polyphenols and terpenoids, which exhibit antioxidant, astringent, anti-inflammatory, antiviral, hypoglycemic, neuroprotective, analgesic, antimicrobial and a number of other important medicinal properties. In official medicine the sage leaves obtained from the cultivated plants are used as medicinal plant raw material. Rosmarinic acid, flavone derivatives, tannins, mono-, di- and triterpenoids, as well as polysaccharides, carboxylic acids and vitamins etc. accumulate in sage leaves and flowers. Rosmarinic acid is strong antioxidant that prevents cell damage caused by free radicals and thus reduces risk of cancer and atherosclerosis. It is also used as food preservative.

This paper reports the results of the analysis of the content of flavonoids, phenolic compounds and rosmarinic acid in *in vitro* sage plants, which were effected by X-ray irradiation in order to identify the radiation doses able to stimulate the biosynthesis of pharmaceutically valuable substances. Irradiation of medicinal plant *in vitro* culture may be technologically simple and cheap way of increasing the pharmacological value of raw material in order to produce a wide range of medicals, which makes *in vitro* medicinal plants cultivation economically promising.

The sage seeds of Shans variety were donated by the Research station of medicinal plants of the Institute of Agroecology and Nature Management of the National Academy of Agrarian Sciences of Ukraine. Plants were grown *in vitro* on hormone-free Murashige-Skoog medium supplemented with 30 g/l sucrose. Irradiation was carried out on the RUM-17 X-ray device (National Cancer Institute) in 5 Gy, 10 Gy, 15 Gy and 30 Gy at dose rate of 1.42 cGy/s. The samples for analysis of the secondary metabolite content were taken in three and six weeks after irradiation. The plants were freeze-dried and extracted with 70% ethanol. The content of phenols in the extracts was measured in terms of ferulic acid. The content of rosmarinic acid was measured *by HPLC.*

According to the results of analysis of the received dose dependence on the content of the phenolic compounds in medicinal sage plants grown *in vitro,* we assume the content of the phenolic compounds in the irradiated samples to be twice as high in case of 10 Gy radiation compared to the control non-irradiated version. The content of phenols in the extracts of the plants irradiated at doses of 5, 15 and 30 Gy exceeded the level in the control samples by 60-70% in one week after irradiation. In 3 weeks after irradiation, no stimulation effect was observed. The slight stimulation (about 15%) in six weeks after irradiation was observed only for the plants irradiated by the doses of 15 and 30 Gy.

The greatest flavonoids yield increase in the extracts from irradiated plants compared to the control ones was observed for 5 Gy both after one week and after three week cultivation. In addition, in 3 weeks the significant increase was also observed for 15 Gy radiation dose. In six weeks after irradiation, the slight increase was observed for the variants irradiated with the doses of 15 Gy and 30 Gy.

The rosmarinic acid yield in the ethanol extracts shows the significant difference between control and experimental variants in one week after irradiation. Moreover, the four-time usage of irradiation in dose of 10 Gy led to the increasing of rosmarinic acid content comparing to the control ones. No effect was preserved in three and six weeks after the exposure.

Thus, we assume the ionizing radiation can be potentially used in order to modify the accumulation levels of the products of secondary metabolism for *in vitro* medicinal plants.

THE EFFECT OF X-RAY IRRADIATION ON THE CONTENT OF ESSENTIAL OILS IN PLANTS *MATRICARIA CHAMOMILLA L.*

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Matricaria chamomilla L. is a widely known and quite popular medicinal plant in medicine and cosmetology. The main pharmaceutically valuable part of the plant is the inflorescence, they contain free organic acids, vitamins, essential oil, phenols, polyphenol compounds, flavonoids. It is a powerful natural antioxidant, thanks to the combination of flavonoids and vitamin C, has wound-healing and anti-inflammatory, soothing and regenerating properties, fights aging and is a natural absorbent that removes toxins and products of cell decay.

For several years in a row, we have been working on the biotechnology the pharmaceutically valuable substances content in the raw material of medicinal chamomile increasing using pre-sowing X-ray irradiation in doses that lead to an increase in the phenols, flavonoids and essential oil content in the extracts.

There are presents the results of research on the content of essential oil in chamomile plants grown from seeds that were subjected to pre-sowing X-ray irradiation. In order to test the effect of irradiation of the same seed in different natural and climatic conditions, plants were grown in the Lubny Research Station of Medicinal Plants of the IANM of the National Academy of Agrarian Sciences of Ukraine fields and at the M. M. Hryshko National Botanical Garden (NBG) of the National Academy of Sciences of Ukraine the experimental plots. Irradiation was carried out on the RUM-17 X-ray device (National Cancer Institute) in doses of 5 Gy, 10 Gy, 15 Gy and 20 Gy, 1,42 cGy/s dose rate. The research used chamomile seeds of medicinal varieties Perlyna Lisostepu and Goral, as well as introductory seeds provided by the Department of Medical Botany of the M.M. Hryshko NBG of the National Academy of Sciences of Ukraine. Inflorescences of chamomile were collected and dried according to pharmacopoeial recommendations. The essential oil was extracted by steam distillation (essential oil distiller, Ukraine), the essential oil was washed with 96% ethanol. The experiment was carried out in triplicate.

As a result of studies of extracts from chamomile flowers obtained from plants grown from seeds irradiated with various doses of X-rays, dose dependences of essential oil content were obtained. Its analysis gives reasons to testify the essential oil in extracts from the inflorescences of chamomile content increasing of the variety Perlyna Lisostepu for doses of pre-sowing irradiation of 15 Gy, grown on experimental plots at the Lubny Research Station of Medicinal Plants. In the extracts from chamomile inflorescences of varieties Perlyna Lisostepu and Goral, as well as introductory seeds, the content of essential oil increased less clearly, but such an increase was observed - for the applied doses of 10, 15 and 20 Gy.

The obtained results indicate the X-ray irradiation using effectiveness to stimulate the synthesis and accumulation of medicinal plants not only of well-known antioxidants, but also of important polyfunctional compounds, the antioxidant properties of which have not been studied yet. Such small doses using makes it possible to increase both the yield of inflorescences, which is a pharmaceutically valuable raw material, and the specific content of medicinal compounds in the extracts.

STRIGOLACTONES AND OTHER COMPOUNDS IN THE MOSS *PHYSCOMITRIUM PATENS*

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Strigolactones (SLs) are plant hormones that were first identified as root-exudate products, exogenously indicating the vicinity of a host plant to parasitic plants such as Striga. The perception of SLs relies on a receptor called D14. The *D14* gene belongs to the same family as *KAI2*. KAI2 functions in plant responses to exogenous karrikins (KAR) which like SL are butenolides. MAX2 is an F-box protein that transduces both SL and KAR signals. KAI2 is also likely the receptor of an uncharacterized hormone termed KAI2 -ligand (KL). Outside flowering plants little is known about the role of SL and KL as a hormone or their evolutionary origins. However, both SL and KL pathways are found in the moss *Physcomitrium patens* (Lopez-Obando et al., 2021).

It was shown that (Proust et al., 2011) wild-type *P. patens* produces and releases SLs into the medium where they control branching of protonema filaments and plants' extension. Since they control the size of neighbor plants, SLs can be qualified as allelopathic compounds. Three homologues to the *KAI2* receptor gene (*PpKAI2L-G,J,M*) were previously reported as best candidates for SL perception (Lopez-Obando et al., 2021).

The first aim of the presented work was to estimate the possibility of production of SL by the triple *Ppkai2l-gjm* mutant (Lopez-Obando et al., 2021). So we followed the size evolution of a receiver plant surrounded by various donor plants during 5 weeks. In our experiment we used the SL synthesis mutant *Ppccd8* as receiver. WT, *Ppccd8*, *Ppkai2l-gjm*, or OEPpCCD8 (a line over-expressing the *PpCCD8* gene) were used as donor. The obtained results indicate that the size of the receiver was significantly the same when we used *Ppkai2l-gjm* mutant or OEPpCCD8 as donor, and significantly smaller when *Ppccd8* or WT were used as donor. So, it looks like *Ppkai2l-gjm* produces similar amounts of metabolites as the over-expressor OEPpCCD8, presumably SLs.

The UV irradiation is convenient (no hazardous chemical to handle) and efficient to do mutagenesis in *P. patens*. The purpose of the reported experiments is to set up the appropriate tools for a screen for KL synthesis mutants, that will lead to eye-visible phenotypic identification. The working hypothesis is that synthesis mutants will show a similar phenotype to KL perception and/or signaling mutants i.e that of the *Ppmax2* mutants (Lopez-Obando et al., 2021). We used two genotypes: WT to estimate the efficient UV dose and to establish the protocol, and the *Ppccd8* mutant as most appropriate. It was established that 60 seconds is the optimal irradiation length of protoplasts for mutagenesis. Mutations in the *APT* gene confer resistance to 2 Fluoro-Adenine, 2-FA, a toxic compound for cells. The number of 2-FA resistant colonies reflects the frequency of UV-induced mutations in one gene (Trouiller et al, 2006). After sequencing, we confirmed three *Ppapt* mutants, further showing that the mutagenesis conditions were appropriate.

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RESOLVING THE STATUS OF THE DUCKWEED GENUS LEMNA, SECTION ALATAE, BASED ON SEQUENCE AND KARYOTYPE VARIABILITY

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Recent breakthroughs have unveiled the multiple potential applications of duckweeds as fast-growing, high biomass accumulating aquatic plants. This has sparked a renewed interest in duckweed genetics, molecular evolution, and diversity (Acosta et al., 2021). Duckweeds, an ancient group of monocot plants divided into five genera, with extremely reduced morphology, have their phylogenetic status and species differentiation, especially within the genus Lemna, still under debate (Bog et al., 2019). Targeted applied strategies are required to exploit duckweed's full potential. This requires deep knowledge of duckweed's genetic and physiological diversity.

In our study, we employed a novel and comprehensive approach, integrating flow cytometry, in situ hybridization with genomic DNA (GISH), and chloroplast and nuclear DNA markers. The combination of these methods was instrumental in resolving the status of the duckweed species *Lemna aequinoctialis* and its relations to *Le. perpusilla* and *Le. tenera.*

Measuring the genome size revealed a high level of variability among the duckweed clones, as well as a few groups of triploid and tetraploid clones. The studied duckweed clones were genotyped using direct sequencing of the chloroplast spacers *atpH-atpF* and *psbK–psbI*. The results allowed us to distinguish the *Le. tenera* clones from those assigned to the other two species. However, clones of *Le. aequinoctialis* and *Le. perpusilla* did not form distinct groups with strong bootstrap support, but rather clusters with low support. To circumvent chloroplast barcoding's lack of resolution, we amplified and sequenced the ITS1-5.8SrDNA-ITS2 region. Bioinformatics and phylogenetic analysis revealed compelling differences between ITS from *Le. aequinoctialis*, *Le. perpusilla*, and *Le. tenera* clones. The presumed clones of *Le. tenera* and *Le. perpusilla* formed separate clusters. The *Le. aequinoctialis* clones formed a homogeneous cluster with strong support. Inside this cluster, three clones formed a subcluster. Barcoding, ITS polymorphism and genome size revealed, in addition to the three species, potential hybrids between *Le. aequinoctialis* and *Le. perpusilla*.

For cytogenetic studies, we prepared metaphase chromosomes of selected clones with contrasting characteristics, counted their chromosome numbers and performed genomic *in situ* hybridization (GISH) with the nuclear DNA of the diploid species as probes. Diploid clones of *Le. aequinoctialis, Le. perpusilla* and *Le. tenera* revealed 42, the presumed triploids 63 and presumed tetraploids >80 chromosomes. GISH results support the assumption that clone Bog0001 represents an allotetraploid of *Le. perpusilla* and *Le. aequinoctialis*.

Based on our comprehensive analysis, we conclude that *Le. aequinoctialis* and *Le. perpusilla* are distinct but closely related species, capable of forming allotriploid and allotetraploid hybrids. The high variability between *Le. aequinoctialis* accessions suggest a separation into two (sub)species. These data contribute to the understanding of the phylogenetic status and species differentiation within the genus Lemna and have implications for the broader field of biotechnology, plant genetics and molecular evolution.

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INFLUENCE OF ABIOTIC FACTORS ON THE CONTENT OF PHENOLIC COMPOUNDS AND FLAVONOIDS IN *DESCHAMPSIA ANTARCTICA* TISSUE CULTURE

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Deschampsia antarctica É. Desv. is an extremophile plant of Antarctica and may be promising in terms of studying the biological activity of its secondary metabolites. Previously, we introduced *D. antarctica* plants to *in vitro* culture, obtained tissue culture and regenerated plants that allow to produce the required amount of plant material and experimenting with it in controlled laboratory conditions. It is known that abiotic factors can change and in a certain way significantly increase the content of biologically active substances (BAS) in plant cellular biomass. Therefore, we aimed to study the influence of growth conditions (darkness/light and temperature 18°/26°C) on the content of BAS in *D. antarctica* morphogenic tissue culture, obtained from *in vitro* plants of two genotypes (DAR12, G/D11-1/3).

The total content of phenolic compounds and flavonoids in the biomass of the tissue culture of *D. antarctica*, when growing in light at an intensity of 6500 lux and increased temperature up to 26°C, decreased in 1,1–2 times. In calli, culturing in the darkness, regardless of temperature, the level of phenolic compounds (from 21.4 to10.5 mg/g of dry weight for DAR12 and 20.8 to 6.8–7.4 mg/g of dry weight for G/D11-1/3) as well as flavonoids (from 8.6 to 3.6–3.8 mg/g of dry weight for DAR12 and 11.9 to 2.4–2.6 mg/g for G/D11-1/3) decreased. It can be assumed that a sharp BAS decrease in the darkness, regardless of temperature, is caused by the lack of photosynthetic activity of cells in such conditions. It is known that biosynthesis of these BAS requires the presence of aromatic amino acids, the synthesis of which depends on the presence of compounds formed in photosynthesis process.

Qualitative analysis carried out by the HPLC method showed that the antioxidant and antitumor compound tricin was detected in most of the examined calli, as well as in the *in vitro* initial plants. Its content in plants DAR12 was 0.15 mg/g of dry weight, while in plants G/D11-1/3 it was three times lower – 0.05 mg/g of dry weight. The content of tricin in the calli was lower than that in the initial plants. When cultures were cultivated in the light, the content of this compound was significantly higher than that in the darkness.

Thus, it was found that the highest level of phenolic compounds and flavonoids accumulation in the morphogenic tissue culture of *D. antarctica* were observed when it has been grown in the light intensity of 6500 lux and at the temperature of 18°C. The tricin found in the samples of *D. antarctica* provides a basis for its further biochemical studies as a potential source of BAS for the purpose of their possible applying in pharmacy as antioxidant and antitumor agent.

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OPTIMIZATION OF THE TECHNOLOGY OF OBTAINING THE SYNTHETIC SEEDS FOR THE PLANTS WITH DIFFERENT TYPES OF STEM

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Russian aggression in Ukraine has posed new challenges for research groups and companies working with *in vitro* plants in order to preserve valuable species and plant clones. Reliability of Ukraine's power supply has been endangered by Russian attacks on the energy system, thus increasing the costs for cultivation of large-scaled plant *in vitro* culture (for instance, it does for the Embryo Plasma Collection, maintained for many years at the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine).

In order to optimize the number of plants in cultivation rooms by taking them out of active vegetation, and thus to reduce the load on the power system, the researchers can from synthetic seeds from some plants. The synthetic seeds are space-efficient, can be stored at room temperature, and do not require lighting or additional heating. The synthetic seed consists of two main components: the plant explant which simulates the zygotic embryo and the gel capsule which simulates the endosperm and seed-coat of natural seed. Unlike cryopreservation, these synthetic seeds do not require special equipment or liquid nitrogen for long-term storage, and the method of their production is relatively inexpensive and simple. What is more, the synthetic seeds do not need special procedures for breaking their dormancy and germination.

The prevalent explants for synthetic seed production are somatic embryos. However, using the vegetative buds is much simpler because it does not require induction of somatic embryogenesis. Location of vegetative buds varies depending on the stem type. The Embryo Plasma Collection includes plants with different stems types, such as shortened stems (rosettes) and modified stems (bulbs). These types require additional efforts when developing the technology for creating synthetic seeds due to their shape, size, and the presence of reserve compounds. For example, forming a thick capsule around a large rosette explant is difficult. If the explant is a part of stem that serves for storage (like a bulb), these synthetic seeds germinate quickly and are not stored for long. The published data do not report the creation of synthetic seeds from such explants.

Our work aims to optimize the technology for creation and storage of the synthetic seeds for plants with different of stem types as we use nodal segments of *Hypericum perforatum*, bulbs of *Allium oschaninii*, and rosettes of *Rumex hydrolapathum* as model plants.

The work has not been completed yet, though we can report some preliminary results. For instance, the optimal concentration of alginate gel for forming stable gel capsules is 2-3%. The optimal incubation time for the capsules in 100mM $CaCl_2$, which enables the obtaining of the shape-stable capsules, is 15 minutes. Using the double capsules made of 3% alginate gel was proved optimal for *A. oschaninii* microbulbs and *R. hydrolapathum* rosettes. This method enables total covering of the large explants with the gel, preventing them from drying out and premature germination. We found no published references to the technology of encapsulating such explants with double capsules.

According to the published data, the storage of seeds and their germination may be effected by the sucrose content and the concentration of macronutrients in gel. However, we did not find any statistically significant differences in germination of the synthetic seeds of *H. perforatum* and *A. oschaninii* encapsulated in gel with different concentrations of sucrose and macronutrients.

Our further research will focus on the analysis of the influence of the effective concentrations of growth inhibitors on the explant ability to retain viability for at least several months.

PLANT-PRODUCED SARS-COV-2 NUCLEOPROTEIN (N) AND CHIMERIC RECEPTOR-BINDING DOMAIN (RBD) AS DIAGNOSTIC REAGENTS

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Serological testing is done to evaluate the humoral response to viral exposure and pathogen protection. During the current SARS-CoV-2 pandemic, the rapid development of efficient and sensitive serological tests for monitoring the dynamics of the disease as well as the immune response after illness or vaccination was critical. In this regard, low-cost and fast production of immunogenic antigens is essential for the rapid development of diagnostic serological kits. For this research, the nucleoprotein (N) of SARS-CoV-2 and chimeric hepatitis E virus capsid protein bearing receptor-binding domain (RBD) of SARS-CoV-2 were expressed in and extracted from *Nicotiana benthamiana* plants, purified through immobilized metal-anion chromatography (IMAC), and used to develop a serological ELISA. The antigens were tested with hospitalized (n=84) and negative pre-pandemic (n=8) patient sera sample lots and batch reproducibility was examined. First, we tested the serum samples with a commercial ELISA assay coated with S1 (RBD) and S2. The results from commercial ELISA confirmed that 48 (57%) of the tested samples were positive for anti-S Ab. For the iELISA, the optimal antigen coating concentration was determined to be 5µg/mL N and HEV-RBD. The used serum dilution was 1:40, and 1:10,000 for secondary anti-human-IgG. We determined the optimal relation between the positive and the negative samples (P/N) in the different coating antigen concentrations. Furthermore, iELISA coated with HEV-RBD showed that 43 (51%) of the tested samples were positive for anti-RBD Ab. Notably, a comparison (ROC analysis) between iELISA (HEV-RBD) and a validated, high-sensitivity commercial ELISA kit showed a sensitivity of 89.58% and a specificity of 94,44% at cut-off = 0,175. The agreement between the two tests is 91.7%, with a *kappa* index of 0.83. Moreover, the in-house ELISA based on plant-derived nucleoprotein for testing anti-N antibodies showed that 36 (43%) of the tested samples were positive for anti-N Ab as the cut-off for positivity was determined as 3xSD (standard deviation).

From this study, it was concluded that the chimeric HEV-RBD and N can be expressed, extracted, and purified and can be used to reliably detect responses to SARS-CoV-2 in sera.

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