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

11th International Meeting

Recent Advances in Plant Biotechnology

11th International Meeting on Recent Advances in Plant Biotechnology

RAPB 2026

2-3 June, 2026

Kyiv, Ukraine

Dear colleagues,

Welcome to the 11th International Meeting on Recent Advances in Plant Biotechnology (RAPB 2026).

RAPB 2026 serves as an international platform for researchers and professionals to engage in discussions on recent advances and emerging trends in plant biotechnology, plant genetic engineering, and related fields. The meeting encourages the exchange of ideas, presentation of original research, and the development of new scientific collaborations.

We invite you to join the conference, explore the scientific program, and submit your abstracts and research contributions. RAPB 2026 will be held in a hybrid format, allowing participation both on-site in Kyiv, Ukraine, and online.

Welcome to the RAPB 2026!

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Organizing committee is whole-heartedly grateful:

- for the all Speakers, who generously shared their knowledge and inspiration with us
- for the all Participants, whose interest and high engagement were very rewarding
- for The International Society for Plant Molecular Farming and the Recrop COST Action for their invaluable support
- for the Armed Forces of Ukraine as we owe them every hour of this Meeting and every second of our lives

TUESDAY, JUNE 2, 2026 (OFFLINE AND ON-LINE)

00:00–00:00 - Keynote Speakers

PLENARY SESSION.
Moderator - Mykola Kuchuk

08:50–09:00 — Mykola Kuchuk (Ukraine), Welcome speech
09:00–09:01 - Nationwide Minute of Silence
09:01–09:25 — Julian Ma (United Kingdom), Plant Molecular Farming – developing a roadmap for a brighter future
09:25–10:10 — Yuri Gleba (Ukraine/Germany), **Plant Biotechnology for manufacturing novel foods and a new platform for agriculture**

SESSION MF. MOLECULAR FARMING
Moderator - Kateryna Lystvan

10:10–10:50 – Mykola Borysyuk (Nikolai Borisjuk) (Ukraine), **Duckweed: applications in academic research and biotechnology**
10:50–11:25 – Felix Krujatz (Germany), **Microalgae as production host of biomolecules**

11:25 – 11:40 — Coffee break

11:40–12:00 – Yinuo Liu (United Kingdom), Context-dependent Pattern-triggered Immunity Control of Agrobacterium-mediated Transient Expression Efficiency
11:50–12:15 – Olga Ovcharenko (Ukraine), Towards molecular farming of antimicrobial peptides in plants. Production in models and edible plants.
12:15–12:35 – Yana Sindarovska (Ukraine), Transient gene expression: a method for the production of plant-made recombinant proteins in semi-open and closed environmental systems
12:35–12:55 – Olena Kishchenko (Ukraine/Germany), Immunization of common carp by duckweed biomass expressing recombinant koi herpesvirus antigens induces the production of the neutralizing antibodies
12:55–13:20 – Yulia Fridman (Israel), Milking the Meadow: Engineering a Cow-Free Dairy Aisle in the Field
13:20–13:30 – Presentation of Awards

13:30 – 14:00 — Coffee break & Graffiti Wall Whiteboarding

SESSION PSM. BIOTECHNOLOGY FOR PLANT STRESS MECHANISMS AND ADAPTATION

14:00–14:10 – Sotirios (Akis) Fragkostefanakis (Germany) ,RECROP COST Action: building climate resilient agriculture
14:10–14:50 – Vasileios Fotopoulos (Cyprus), Next-generation chemical priming as a green strategy for the development of climate-smart crops

14:50–15:30 – Ülo Niinemets (Estonia), **Plant stress in the future: stress responses, acclimation and implications for selection of improved and novel crops**
15:30–16:10 – KC Bansal (India), **Gene Discovery and Crop Engineering to Enhance Climate Resilience and Nutritional Quality**

16:10 – 16:30 — Coffee break

16:30–16:50 – Harzli Ines (Turkey), Fungal symbionts of coexisting orchids facilitate seed germination in co-occurring orchids in Türkiye
16:50–17:10 – Olena Bublyk (Ukraine), In silico analysis of post-transcriptional regulation of stress-associated DaDREB2B gene expression in *Deschampsia antarctica*
17:10–17:30 – Daria Kozikova (Spain), Organoleptic properties of grapes of Tempranillo and Cabernet Sauvignon as affected by mycorrhizal symbiosis and abiotic stressors linked with climate change
17:30–17:50 – Csaba Eva (Hungary), Endodermis suberization influences root based inorganic carbon uptake, that increases growth and osmotic stress tolerance
17:50–18:10 – Olexandra Kravets (Ukraine), Biotechnological using of remote effects of pre-sowing irradiation of plant seeds

18:10 – 18:30 — Coffee break

18:30–18:50 – Iryna Zhuk (Ukraine), Hydrogen Peroxide in Wheat Immunity: System-Level Redox Responses in Plant–Fungal Interactions
18:50–19:10 – Benjamin Jeremías (Argentina) , Nanoclay Based Agroinputs
19:10–19:30 – Yulia Rymar (Ukraine), Stomatal Morphology and Gas Exchange Dynamics in Triticum Species under Water Deficit
19:30–19:50 – Serhii Litvinov (Ukraine), Morphometric and biochemical transgenerational effects of irradiation of pea seeds

WEDNESDAY, JUNE 3, 2026 (ON-LINE)

PLENARY SESSION.

09:00–09:01 - Nationwide Minute of Silence
09:01–09:45 - Mohsen Hesami (Canada), **Application of Artificial Intelligence in Plant Tissue Culture and Plant Biotechnology**

SESSION GE. GENETIC ENGINEERING.

09:45–10:30 — Goetz Hensel (Germany), **CRISPRing barley for a sustainable beer production**
10:35–11:15 — Etienne Bucher (Switzerlad), **Epigenetics and mobile elements: Hidden treasures for crop breeding?**
11:15–12:00 — Kathleen Hefferon (USA), **Biofortification of crops**
12:00–12:20 – Daniel Jánoška (Slovakia), Variability of nutritional and functional properties of cereals in relation to year and tillage system

12:20–12:40 – Hamza Sohail (China), Cucurbit Root Transformation System: A Tool for Studying Functional Genes
12:40–13:00 – Bogdan Morgun (Ukraine), Genetic engineering of wheat for drought tolerance

13:00-13:20 – Virtual Coffee Break & Graffiti Wall Whiteboarding

SESSION GE. SECONDARY METABOLISM AND BIODIVERSITY

13:20–14:00 – Paul Fraser (United Kingdom), **Engineering industrial and nutritional isoprenoids in Solanaceae platforms**
14:00–14:25 – Anna Szakiel (Poland), The effect of selected elicitors on triterpenoid production in *Calendula officinalis* plants and hairy root culture.
14:25–14:45 – Anton Stepanenko (Ukraine/Germany), Small but diverse: hidden genetic complexity in duckweed (*Wolffia*)
14:45–15:05 – Iva Doycheva (Bulgaria), Influence of cryopreservation and melatonin priming on the seed germination and dynamics of the Bulgarian endemic *Papaver degenii*
15:05–15:25 – Valeria Belokurova (Ukraine), *In vitro* banks as a constituent of the system of plant biodiversity conservation and use in biotechnological research.
15:25–15:45 – Kateryna Lystvan (Ukraine), *In vitro* bank of ICBGE: how we (could) use it
15:45–16:05 – Yevhenii Stohnii (Ukraine), Plants as sources of proteases with fibrinogenolytic activity
16:05–16:45 – Diego Orzaez (Spain), **Targeted engineering of plant-made volatiles for sustainable crop protection**
16:45–17:05 – Rostislav Blume (Ukraine), Identification of aae15/16 genes in the pangenome of *Camelina* for metabolic engineering

PLANT MOLECULAR FARMING – DEVELOPING A ROADMAP FOR A BRIGHTER FUTURE.

Sutinee Soopairin*, Mathew Paul**, Suthira Taychakhoonavudh* and Julian Ma**

* - Department of Social and Administrative Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.
** - Institute for Infection and Immunity, School of Health and Medical Sciences, City St. George's University of London, London, UK

Plant molecular farming (PMF) has been a promising manufacturing platform for over three decades, but has yet to make a significant commercial breakthrough. The closure of large and important PMF companies in recent years was a major blow to confidence and interest in plant manufacturing technologies, leaving PMF in a potentially existential crisis.

The purpose of this study was to assess attitudes towards PMF around the world, from within and without the field and to identify common themes to inform a forward strategy for PMF. To this end, we have conducted two interview studies. The first was conducted using detailed semi-structured interviews with 63 people involved in recombinant protein production, including senior academics, early career researchers, CEO/CSOs from PMF companies, representatives from the mainstream pharmaceutical industry, government, major funders and private investors. In order to include the public voice, the second study involved shorter semi-structured interviews with 44 members of the public.

This talk will discuss the key findings, highlighting areas where there are differences in opinions between different stakeholders, such as the key advantages of plant manufacturing platforms, selection of target products and potential contribution to global health. The future of PMF still needs excellent science, but it also requires strengthening collaboration, improving communication, and demonstrating credible success stories to build global interest and confidence toward PMF.

PLANT BIOTECHNOLOGY FOR MANUFACTURING NOVEL FOODS AND A NEW PLATFORM FOR AGRICULTURE

Yuri Gleba,

Nambawan Spain SL, Spain, and Nomad Bioscience GmbH, Germany

In 2026, NAMBAWAN Spain, a technology spin-off Nomad Bioscience, started industrial manufacturing of Thaumatin II, an intensely sweet protein. The protein is produced in bioengineered plants using plant molecular farming. Thaumatin II is the sweetest known natural substance, over 10,000 times sweeter than sucrose on a weight basis. It is non-caloric and non-glycemic. We position it as a universal natural co-sweetener that improves the taste and has the lowest sweetness cost, thus being an excellent competitor in the $150 billion-per-year sweetener market. Thaumatin II has been approved by multiple regulatory authorities as both a sweetener and flavor modifier. In USA it has a GRAS status by FDA and is already being sold as a blend with other sweet protein, Brazzein III. Thaumatin II is a highly promising product that offers multiple health and environmental benefits over sugar, other artificial and natural sweeteners.

Traditional and modern plant breeding is severely limited because of long times, usually more than a decade, required to develop and register a new variety, and associated high costs & low throughput. Current changes in climate along with planet overpopulation & destabilization result in pathogen pandemics in humans, animals & plants as well as abiotic stresses in plants – all of those require fast responses from us. We propose to reprogram plants for agronomic performance using RNA viral transfection methods that can be implemented on an industrial scale, with many crop plants. The process doesn’t involve genetic modification of plant genome and thus is limited to a single plant generation. The process is broadly applicable, fast, tuneable and versatile, and it can be used throughout crop cultivation circle. RNA-based plant reprogramming may be especially useful in plant pathogen pandemics and for rapid improvement of orphan crops.

APPLICATION OF ARTIFICIAL INTELLIGENCE IN PLANT TISSUE CULTURE AND PLANT BIOTECHNOLOGY

Mohsen Hesami

Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

The integration of Artificial Intelligence (AI) into plant tissue culture and plant biotechnology offers transformative opportunities for accelerating research, enhancing precision, and optimizing production systems. AI-driven tools, including machine learning and optimization algorithms, enable predicting and optimizing *in vitro* culture systems. Additionally, AI facilitates the analysis of complex omics datasets, guiding genetic engineering, metabolic pathway optimization, and trait selection with high accuracy.

This presentation highlights recent advances in the application of AI for plant tissue culture, plant biotechnology, and synthetic biology. Challenges, such as data standardization, algorithm interpretability, and scalability, are also addressed. Leveraging AI has the potential to reduce labor, improve reproducibility, and accelerate innovation in crop improvement, conservation, and sustainable bioproduction.

DUCKWEED: APPLICATIONS IN ACADEMIC RESEARCH AND BIOTECHNOLOGY

Anton Stepanenko[1,2], Olena Kishchenko[1,2], Olha Lakhneko[1,3], Maksym Danchenko[3], Yuzhen Zhou[4], Guimin Chen[4], Veit Schubert[2], Todd Michael[5], Eric Lam[6], Ingo Schubert[2], Anton Peterson[1,2], Anatoli Giritch[7], Mykola Kuchuk[1], Mykola Borysyuk[1]

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Duckweed is a common name for a group of tiny monocotyledonous aquatic plants in the Lemnaceae family represented by 35 species classified into five genera with worldwide distribution. They exhibit the fastest among flowering plant growth rates efficiently accumulating green biomass rich in protein, starch and other nutrients. These features, together with predominantly vegetative propagation and simple *in vitro* cultivation, made duckweeds an attractive object for academic studies and various biotechnological applications. Our research mostly focuses on greater duckweed, *Spirodela polyrhiza*, a species with the largest, 5-8 mm in diameter, size of fronds and the smallest genome size compared to other duckweed species. The survey of *S. polyrhiza* genome revealed an unusually low for eukaryotes representation of rDNA, tandemly repeated copies of 18S-5.8S-25S and 5S rRNA genes, encoding corresponding ribosomal RNA species crucial for biogenesis of ribosomes. Taking advantage of this specific feature of the *Spirodela* genome, we deciphered the first for a flowering plant detailed molecular organization of the rDNA loci at nucleotide level [1]. We have shown that the GC-rich rDNA repeats of 35S and 5S rDNA genes are surrounded by highly AT-enriched sequences with possible regulatory functions. A combination of high resolution in situ hybridization, extra-long DNA reads and conventional sequencing allowed us the assembling of complete loci sequences of 5S rDNA located respectively on ChrSp6 and ChrSp13. Both loci are composed of head-to-tail repeat units with identical sequences of the 5S rRNA gene intertwined with locus-specific intergenic spacers. Our study also demonstrated haplotype specificity of the loci arrangements, different evolutionary dynamics and possible differential regulation of the two 5S rDNA loci. Duckweed is also well recognized as a promising platform for biotechnological applications, especially for wastewater remediation and production of recombinant proteins. Our study of duckweed responses to different sources of Nitrogen and elevated concentrations of Manganese [2] revealed a range of differentially expressed genes and proteins, related to RedOx processes and oxidative stress, pathogenesis and defense response, and heavy metal transportation. The obtained results shed new light on plant responses to heavy metal stress, revealing the major pathways involved in the response processes and paving ways for further investigations and application of these gene networks for engineering crops with improved stress tolerance. We also demonstrated *Agrobacterium*-mediated high-yield transient expression of a reporter protein in *Spirodela polyrhiza*. Aseptically cultivated duckweed fronds yielded reporter protein accumulation of >1 mg/g fresh biomass, when the protein was expressed from a deconstructed potato virus X-based vector, usually applied for transient expression in terrestrial dicotyledonous plants. The vector was efficiently replicated and support cell-to-cell movement of the replicons in monocotyledonous duckweeds. The demonstrated expression efficiency places duckweed among the most efficient host organisms for plant-based transient expression of recombinant proteins, with the additional benefits of easy scaling-up and full containment.

1. Stepanenko A, Schubert V, Chen G, Kishchenko O, Michael T, Lam E, Hrmova M, Schubert I, Borisjuk N. The genome sequence assembly of the 5S rDNA loci informs their haplotype specificity and evolution in the greater duckweed *Spirodela polyrhiza*. Communications Biology, 2026 9:516. https://doi.org/10.1038/s42003-026-09598-8

2. Kishchenko O, Stepanenko A, Straub T, Zhou Y, Neuhäuser B, Borisjuk N. Ammonium uptake, mediated by ammonium transporters, mitigates manganese toxicity in duckweed, *Spirodela polyrhiza*. Plants (Basel). 2023 Jan 3;12(1):208. doi: 10.3390/plants12010208.

MICROALGAE AS PRODUCTION HOST OF BIOMOLECULES

F. Krujatz[1,2], Y. Ihadjadene[1], S. Streif [1]

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Microalgae are emerging as highly promising biotechnological production hosts due to their exceptional photosynthetic efficiency, sustainable resource utilization, and broad product spectrum. In particular, microalgae offer significant potential for the production of bulk commodities such as single-cell proteins (SCP), bulk chemicals, lipids, specialty food ingredients, and high-value biopharmaceuticals. Compared with conventional microbial production systems using agricultural feedstocks, microalgae offer superior land-use efficiency by directly converting solar energy and carbon dioxide into biomass and valuable metabolites. Consequently, phototrophic microorganisms are increasingly regarded as key production platforms for a future sustainable and circular bioeconomy. Recent progress in genetic engineering and synthetic biology has significantly expanded the molecular toolbox available for industrially relevant microalgal species. At the same time, high-throughput screening and phenotyping technologies accelerate the identification and optimization of robust industrial strains suitable for large-scale cultivation under dynamic environmental conditions.

In parallel to molecular developments, efficient cultivation technologies in photobioreactors play a crucial role in enabling economically viable production processes. The optimization of light distribution, gas transfer, nutrient utilization, and process scalability remains one of the central technical challenges in industrial microalgal biotechnology. Furthermore, the integration of renewable energy systems, direct air capture technologies for carbon dioxide supply, and sustainable nutrient recycling concepts are becoming increasingly important for future large-scale production platforms.

In this context, digitalization and data-driven technologies are gaining substantial relevance for process optimization and quality assurance. Advanced sensor technologies, online monitoring systems, digital twins, and AI-supported process control enable improved cultivation stability, real-time product monitoring, and predictive process management. These technologies are expected to significantly enhance process robustness, product quality, and economic competitiveness.

The presentation provides an overview of current trends in microalgal product development, recent advances in molecular and bioprocess engineering, the major technological challenges for industrial production, and emerging data-driven solutions for process and product monitoring in phototrophic biotechnology.

CONTEXT-DEPENDENT PATTERN-TRIGGERED IMMUNITY CONTROL OF AGROBACTERIUM-MEDIATED TRANSIENT EXPRESSION EFFICIENCY

Yinuo Nora Liu, Nattapong Sanguankiattichai, Renier A. L. van der Hoorn

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Agroinfiltration is widely used for transient protein expression, and is increasingly adopted as a low-cost, sustainable alternative to mammalian protein production platforms. However, inconsistent protein yield remains a challenge, limiting broader application. Previous data demonstrate that plant immunity decreases *Agrobacterium*-mediated transient expression efficiency. Depletion of pattern recognition receptors which recognise *Agrobacterium* and facilitate early immunity, such as CORE in *N. benthamiana* and EFR in *A. thaliana*, enhances transient expression efficiency[1, 2]. Furthermore, agroinfiltration of many plant species fails to induce cell death characteristic of late-stage host immune response, but still results in undetectable protein production, pointing to the possible suppression of transient expression by non-specific, early immunity.

These lines of evidence led us to test whether pattern-triggered immunity (PTI) mounted during early *Agrobacterium* infection is a major barrier to transient expression efficiency. We artificially induced PTI responses against *Agrobacterium* in *N. benthamiana* and assessed effects on bacterial virulence gene expression and transient protein expression. Unexpectedly, PTI triggered by the flg22 elicitor peptide had no effect on *Agrobacterium* virulence or GFP expression, indicating that acute PTI activation alone is insufficient to suppress *Agrobacterium*-mediated transient expression. In contrast, sustained PTI generated by pre-expression of pattern recognition receptors that recognise

Agrobacterium caused a significant decrease in bacterial virulence and protein yield, demonstrating that persistent immune activation reduces transient expression success.

To investigate why elicitor-triggered PTI is tolerated whereas sustained PTI is not, we explored whether *Agrobacterium* is able to suppress early PTI responses such as reactive oxygen species (ROS) bursts. Strikingly, we observed that *Agrobacterium* suppresses flg22-triggered ROS bursts in a concentration-dependent manner. Together, these results show that *Agrobacterium* actively suppresses elicitor-triggered PTI responses, while sustained immune activation remains a barrier to efficient transient expression. Our data show that PTI conditionally inhibits *Agrobacterium*-mediated transient expression in a non-binary, context-dependent manner, providing mechanistic insight into variability in transient expression efficiency caused by plant immunity.

1. Dodds I, Chen C, Buscaill P, van der Hoorn RAL. Depletion of the NbCORE receptor drastically improves agroinfiltration productivity in older Nicotiana benthamiana plants. Plant Biotechnol J. 2023 Jun;21(6):1103-1105. doi: 10.1111/pbi.14037. Epub 2023 Mar 14. PMID: 36917445; PMCID: PMC10214749.
2. Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell. 2006 May 19;125(4):749-60. doi: 10.1016/j.cell.2006.03.037. PMID: 16713565.

TOWARDS MOLECULAR FARMING OF ANTIMICROBIAL PEPTIDES IN PLANTS. PRODUCTION IN MODELS AND EDIBLE PLANTS.

Ovcharenko Olga[1], Shcherbak Nataliia[1], Dzuh Maryna[1], Vasylenko Maksym[1], Luchakivska Julia[1], Lystvan Kateryna[1], Balko Oleksandr[2], Balko Olga[2], Rudas Volodymyr[1], Kuchuk Mykola[1]

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The problem of increased antibiotic resistance of pathogenic bacterial strains is a serious challenge of modern microbiology, biotechnology, human and veterinary medicine. Bacteriocins are peptides naturally produced by some strains of bacteria that are able to eliminate closely related bacterial strains. High antimicrobial activity at pico- and nanomolar concentrations, combined with stability at wide pH and temperature ranges makes these peptides attractable especially for prophylactic use as potential alternative to antibiotics in both food and feed. Narrower spectrum of activity compared to antibiotics allows to use them as a remedy to prevent dysbiosys after antibiotic treatment. Among the promising representatives of bacteriocins are salmocins, that exhibit antimicrobial activity against a wide range of bacteria of the genus *Salmonella*, and colicins, inhibiting *Escherichia coli* growth. Plants can be potential producers of bacteriocins, since they are characterized by a number of advantages. Schulz et al (2015) and Hahn-Löbmann et al (2019) demonstrated successful bacteriocins synthesis in tobacco plants. Plant-produced colicins have been recognized as GRAS (Generally Recognized as Safe) antimicrobial food processing aids by FDA. The production of bacteriocins in edible plants can allow us to avoid the necessity of purification of these peptides especially for the veterinary purposes.

Vectors carrying salmocine (*SalE1b*) and colicin M (*cma*) genes were kindly provided by Nomad Bioscience GmbH or created in our laboratory.

We carried out *Agrobacterium*-mediated genetic transformation of tobacco (*Nicotiana tabacum*) of the Samsun variety with the genetic construct pNMD35541 with the salmocin gene *SalE1b*. The construct used a vector with the magnICON system, which carries the genes for salmocin biosynthesis and elements of the tobacco mosaic virus under the control of an ethanol-inducible promoter. The obtained transgenic plants were adapted ex vitro and grown in a greenhouse. After induction with ethanol, the expression of the salmocin biosynthesis gene occurred in the plants. Plant extracts showed antimicrobial activity against *Salmonella enterica* subsp. enterica serovar Ebony NCTC 6017 and *E. coli*. The transgenic lines differed in the level of antimicrobial activity. Some selected transgenic lines demonstrated high antimicrobial activity of extracts, which amounted to more than 600 thousand AU/g of leaf FW. The antimicrobial extracts from transgenic plants that produce salmocin demonstrated antimicrobial activity both on *Salmonella enterica* and *Escherichia coli*.

A new vector for expression of salmocin under the constitutive promotor was designed and successfully tested by transient expression in *Nicotiana bethamiana* and stable genetic transformation of *Nicotiana tabacum*.

Transgenic plants of a number of edible crops such as lettuce (*Lactuca sativa*), mizuna (*Brassica rapa subsp. nipposinica var. laciniata*), carrots (*Daucus carota*) and kale *(Brassica oleracea)* were obtained via *Agrobacterium*-mediated transformation with colicin M gene.

A high amount of plant biomass of transgenic kale expressing colicine M was grown in a greenhouse. Leaves of kale were dried at 40°C and grinded to powder. The obtained powder retained antibacterial activity for at least 4 years. Addition of such powder to wheat grains as a model of compound feed, demonstrated protective effect against *E. coli* growth on such feeds. Adding of 2.0 % of such dried plant biomass with colicin activity of about 30,000 AU/kg of dry weight biomass provided compound feed antimicrobial protection by inhibiting the growth of *Escherichia coli.*

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TRANSIENT GENE EXPRESSION: A METHOD FOR THE PRODUCTION OF PLANT-MADE RECOMBINANT PROTEINS IN SEMI-OPEN AND CLOSED ENVIRONMENTAL SYSTEMS.

Yana Sindarovska, Mykola Kuchuk

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Transient gene expression (TGE) is considered to be a suitable method for using plants as scalable biofactories for producing valuable recombinant proteins. TGE is an efficient method allowing reach high levels of target recombinant proteins (up to 4 mg/g of fresh weight, FW) within one-two weeks after plant infection. However, TGE can be influenced by many factors, and optimized protocols may differ depending on the target protein. Here, we summarize our achievements in the production of some recombinant proteins in plants: jellyfish GFP (reporter protein), human interferon alpha 2b (antiviral biopharmaceutical protein), bacterial colicin M (antibacterial protein), and others.

Agrobacterium-mediated and *Agrobacterium*-free TGE methods were used for the production of target recombinant proteins in semi-open (greenhouse) and closed (in vitro) systems. Target transgenes were incorporated into the genetic constructs with different backbones: simple 35S- constructs, and improved constructs based on genome elements of potato virus X (PVX-based) and tomamoviruses (TVCV-based) [1–3]. Spectrophotometric, biochemical, immunological, and microbiological methods were used to confirm the production of target recombinant proteins in plants and to assess their bioactivity.

Nicotiana benthamiana is a dominant producer of recombinant proteins in plants through TGE. Using a PVX-based genetic vector and GFP as a reporter, we developed a new method for obtaining target recombinant proteins in *Agrobacterium*-free *N. benthamiana* plants through long-term TGE in closed (in vitro) systems. Ther results showed that plants expressed active GFP at high levels (up to 2 mg/g of FW) and can be multiply by micropropagation [1]. This approach was also tested with colicin M protein, and the results showed that its production was comparable in plants grown in vitro and in greenhouse. Using the PVX-based and TVCV-based genetic vectors and GFP, we discovered a new edible host (grown in greenhouse) for TGE, namely sweet basil (*Ocimum basilicum*). High levels of active GFP (up to 1–3 mg/g of FW) were detected after using of both improved viral-based constructs, but no GFP expression was observed when simple 35S-GFP construct was used [2]. Improved PVX-based genetic construct was used to increase production of recombinant interferon alpha 2b 80 times compared to simple 35S-INF construct in *N. benthamiana* plants grown in greenhouse. The same genetic construct (PVX-INF) was used to produce recombinant interferon alpha 2b in *O. basilicum* plants [3]. However, PVX-based genetic construct was not so efficient for the production of bioactive colicin M in *N. benthamiana* plants grown in greenhouse: the levels of colicin M were comparable (0.1–0.3 mg/g of FW) regardless of which construct the transgene was incorporated PVX-Col or 35S-Col. Data about other recombinant proteins will be also presented. Our results show that different recombinant proteins can be efficiently obtained in both semi-open and closed environmental systems, but the yield of recombinant proteins vary depending on their nature, genetic construct and many other factors.

1. Sindarovska Y, Kuchuk M. Long-term potato virus X (PVX)-based transient expression of recombinant GFP protein in *Nicotiana benthamiana* culture in vitro. *Plants*. 2021; 10(10):2187. https://doi.org/10.3390/plants10102187
2. Sindarovska Y, Kuchuk M. Viral-based expression cassettes ensure high level production of recombinant green fluorescent protein (GFP) in sweet basil (*Ocimum basilicum*) plants. *Plant Cell Tiss. Organ Cult*. 2023; 154:121–130. https://doi.org/10.1007/s11240-023-02516-4
3. Sindarovska Y, Kuchuk M. Construction of viral-based expression vectors for high-level production of human interferon alpha 2b in plants. *Appl. Microbiol. Biotechnol*. 2024; 108:229. https://doi.org/10.1007/s00253-024-13069-7

IMMUNIZATION OF COMMON CARP BY DUCKWEED BIOMASS EXPRESSING RECOMBINANT KOI HERPESVIRUS ANTIGENS INDUCES THE PRODUCTION OF THE NEUTRALIZING ANTIBODIES

Olena Kishchenko[1,2,] *, Anton Peterson[1,2], Mikolaj Adamek[3], Verena Jung Schroers[3], Veronika Piackova[4], Tomas Korytar[5], Anatoli Giritch[6], Ingo Schubert[1] and Manuela Nagel[1,7]

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Vaccination as the most effective method of preventing disease outbreaks reduces antimicrobial usage in aquaculture, thus having a positive impact on human health, environment and food security. Our study explores duckweed as a platform for subunit vaccine production and as vehicle for their oral administration to fish. Recombinant proteins, which are analogs of the koi herpesvirus antigens, were accumulated at high level in duckweed biomass using *Agrobacterium*-mediated transient expression. Immunization of common carp via intraperitoneally injection with a fine powder of lyophilized duckweed biomass containing these antigens induced specific antibodies production that was proved by the serum neutralization test. In order to estimate efficiency of oral delivery of recombinant proteins from freeze-dried duckweed, common carp was intubated with either untransformed duckweed or duckweed expressing recombinant green fluorescent protein (GFP), or GFP fused to cholera non-toxic subunit B (CTB::GFP). The GFP fluorescence was detectable in intestinal mucosa and submucosa only in case of CTB::GFP-harboring freeze-dried biomass, showing that CTB is essential as transmucosal carrier.

Thus, transient expression of recombinant antigenic proteins in duckweed is a promising tool for developing oral vaccines for veterinary application.

MILKING THE MEADOW: ENGINEERING A COW-FREE DAIRY AISLE IN THE FIELD

Arie Michal, Lusthaus Orly, Lifshits Sveta, Pinkert Yuval, Sahar Michal, Fridman Yulia

NewMoo Foods Inc, Israel

What if the next dairy farm didn't need a single cow? Our global food system is stuck in a massive paradox: the world's hunger for high-quality protein is skyrocketing, but relying on livestock to meet that demand is destroying the planet. While precision fermentation seemed like a great alternative early on, it keeps hitting a wall. Massive steel bioreactors are incredibly expensive to scale, and they struggle to handle the heavy lifting required to make complex proteins at a global scale.

That is why we decided to turn to nature's original, solar-powered factory: plants. By leveraging the natural scalability of agriculture, we are bypassing the limitations of the lab and turning crops into high-efficiency producers of dairy proteins. Think of it as a critical bridge connecting traditional farming with cutting-edge food science.

In this talk, I want to take you behind the scenes of our journey. We will break down the real-world technical headaches of getting plants to express fully functional caseins, moving step-by-step from raw gene preparation to harvesting seeds in the field. But we aren't just talking about theory here. I will share the data from our fields and showcase NewMoo's hybrid mozzarella on actual pizza - proving you can get the exact same melt, stretch, and flavor profile as the bovine original without the animal. Join me for a candid look at the hurdles we've cleared, the lessons we've learned, and why the real future of sustainable dairy is rooted in the soil.

PLANT-BASED EXPRESSION OF RECOMBINANT ANTIMICROBIAL PEPTIDES

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Plants are considered promising platforms for the production of recombinant pharmaceutical and biologically active proteins due to their scalability, biosafety advantages and relatively low production costs. Antimicrobial peptides (AMPs) are small bioactive molecules possessing broad-spectrum antimicrobial activity and significant potential for pharmaceutical and agricultural applications. In addition to their direct antimicrobial properties, AMPs are involved in stress-response and defense mechanisms in many organisms, making them attractive candidates for recombinant production in plant systems.

The aim of this work was to develop transgenic plants expressing recombinant AMPs and to evaluate their suitability as plant-based production systems. Candidate peptides were selected using computational screening approaches based on physicochemical properties important for heterologous expression in plants, including charge, hydrophobicity and predicted membrane affinity. Structural modeling and comparative analysis of peptide properties were performed to identify candidates potentially compatible with stable plant expression systems. The analyzed panel included eleven AMPs representing different biological origins and design strategies: amphibian-derived peptides Dermaseptin-B1 (AP00293) from *Phyllomedusa bicolor* and Buforin II (AP00308) from *Bufo bufo gargarizans*; plant defensin-like peptides AP00479 from *Phytolacca americana* and AP01330 from *Vitis vinifera*; the bacterial peptide S-subtilin (AP00206) from *Bacillus subtilis*; three modified variants, including mod AP00293+AP00308, mod AP01330 and mod AP00206; and three synthetic peptides, APs.SM1b, APs.SM2a and APs.Ai.

To optimize antimicrobial potential and compatibility with plant expression systems, rational design strategies were applied during peptide selection and modification. Modified AMP variants were designed to alter net charge, hydrophobicity and predicted membrane interaction properties associated with antimicrobial activity. The developed workflow combined bioinformatic screening, recombinant vector construction and stable plant transformation into a unified platform for evaluation of recombinant antimicrobial peptides in plant systems.

Genetic constructs for plant transformation were generated using Modular Cloning (MoClo/Golden Gate) methodology. Each construct contained the AMP coding sequence, the *bar* selectable marker gene and the fluorescent reporter protein ZsGreen, enabling rapid identification of transformed tissues and regenerated plants. Stable transformation of *Nicotiana tabacum* was performed using *Agrobacterium tumefaciens*-mediated transformation followed by selection on phosphinothricin-containing medium.

Multiple independent transgenic tobacco lines were successfully regenerated. Integration of AMP transgenes into the genome of regenerated plants was confirmed by PCR analysis using gene-specific primers. ZsGreen fluorescence enabled rapid screening of transformed tissues during early developmental stages and facilitated selection of putative transformants. Additional confirmation of transformation efficiency was obtained through herbicide resistance screening associated with expression of the *bar* selectable marker gene. Importantly, regenerated plants developed normally and did not exhibit visible phytotoxic effects associated with AMP expression.

The obtained results support the potential application of transgenic plants as platforms for recombinant production of antimicrobial peptides and demonstrate the applicability of plant molecular farming approaches for production of biologically active compounds.

PRODUCTION AND ANTIBACTERIAL ACTIVITY ASSESSMENT OF SALMOCIN IN TRANSGENIC TOBACCO PLANTS

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Salmonella remains one of the leading causes of foodborne infections, causing substantial economic losses in livestock production and posing a serious threat to food safety. Moreover, *Salmonella* is becoming an increasing challenge in the context of antibiotic resistance, highlighting the need for alternative approaches to pathogen control. According to the European Centre for Disease Prevention and Control, resistance of *Salmonella* to fluoroquinolones (one of the key classes of antibiotics used for the treatment of salmonellosis) is increasing in most countries. Salmocins are antibacterial proteins produced by bacteria (bacteriocins) that selectively kill pathogenic *Salmonella* serotypes. Like other bacteriocins, salmocin is considered a potential alternative to conventional antibiotics, at least for prophylactic purposes, and its use may help prevent the spread of bacterial infections. Salmocins have previously been produced in plants using transient expression systems (Schneider et al., 2018; Hahn-Löbmann et al., 2019). Their functionality was confirmed, and the recombinant bacteriocins were recognized by the U.S. Food and Drug Administration as GRAS and approved for use as antibacterial agents GRAS Notices (fda.gov). The aim of our work was to generate transgenic tobacco plants accumulating recombinant salmocin using both inducible and constitutive expression systems.

Vectors for ethanol-inducible salmocin expression in plants were kindly provided by Nomad Bioscience GmbH. For constitutive salmocin expression, a vector with the salmocin E1b gene under the control of the CsVMV promoter using Golden Gate cloning technology was constructed. In addition to the target gene, the vector also contained the reporter gene *ZsGreen*. Transgenic tobacco plants were generated using *Agrobacterium*-mediated genetic transformation. The transgenic nature of the obtained plants was confirmed by PCR using primers specific to the salmocin gene. The antibacterial activity of extracts from transgenic plants was evaluated using the soft-agar overlay method against *E. coli* strain XL1-blue and *Salmonella enterica*. In plants carrying the ethanol-inducible expression system, the tobacco leaves were pre-incubated in chambers containing 4% ethanol and maintained for 3 days to allow accumulation of the recombinant product.

Both the inducible and constitutive expression system enabled the production of plant extracts exhibiting high antibacterial activity against *Salmonella enterica*, which was retained even after 32-fold dilution. Transgenic tobacco plants carrying the ethanol-inducible system showed the ability to accumulate recombinant salmocin at a specific time point following ethanol induction, which is convenient for the subsequent isolation and purification of the recombinant protein.

The presence of the reporter protein ZsGreen in the vector with constitutive salmocin expression allowed us to selectively screen transgenic tobacco plants with high levels of recombinant protein expression by assessing fluorescence intensity under blue light illumination. Such a vector design could facilitate the development of transgenic lines in recalcitrant crop species by providing rapid visual selection of successfully transformed and highly expressing events.

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EFFECT OF TOBACCO MOSAIC VIRUS ON THE CONTENT OF HETEROLOGOUS GFP PROTEIN IN *NICOTIANA TABACUM* AND *NICOTIANA BENTHAMIANA* TRANSGENIC PLANTS

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Plant viruses are known as a serious stress factor reducing the yield of cultivated plants and its quality. Nowadays molecular farming is a promising area for scalable, safe and relatively cheap production of pharmaceutical proteins. Although the recombinant virus-like particles are sometimes used for intensive transient production of proteins, less is known about the influence of wild-type viruses on transgenic plants expressing recombinant proteins.

Transgenic plants *Nicotiana benthamiana* and *Nicotiana tabacum* with GFP superexpression were created according to standard *Agrobacterium*-protocol (Horsch, et al, 1985). Wild-type and transgenic plants of lines with different level of GFP accumulation were microclonally propagated in vitro and acclimated in greenhouse. One-month-old plants were infected with Tobacco mosaic virus (TMV). GFP content was detected on spectrophotometer Fluostar Omega. ELISA and RT-PCR were conducted to proof the systemic viral infection.

Transgenic *N. benthamiana* and *N. tabacum* lines with the brightest fluorescence of GFP regenerated slower compared to those with moderate brightness. Transgenic lines were selected both on BASTA and visually. GFP content varied from neglectable to superexpression that masked red chlorophyll fluorescence under 488 nm light. Lines with moderate and the highest GFP accumulation were selected for further experiments.

After inoculation of *N. benthamiana*, no necrosis was detected, in contrast to *N. tabacum*. The first signs of TMV were detected in inoculated plants in 2 weeks after inoculation. The systemic viral spread was determined in upper leaves of infected and control plants 3 weeks post inoculation. In all inoculated *N. benthamiana* plants, both transgenic and wild type, bright signs of systemic viral infection were observed after week 3, in contrast to non inoculated plants. Mosaic, growth retardation, leaf wrinkling and lack of flowers were the common symptoms. GFP content was measured 4 weeks post inoculation. Measurement of GFP revealed decrease of GFP content in both *N. benthamiana* and *N. tabacum* transgenic lines. ELISA and RT-PCR proved TMV infection in all infected *N. benthamiana* plants. TMV particles were not detected in *N. tabacum*. We can propose that plant defence mechanisms blocked not only viral spread both with transgene expression in *N. tabacum*. Thus plant viruses can affect yield in molecular farming by biomass decrease and low content of protein of interest, and maybe sometimes by blocking of transgene expression by the plant as a consequence of infection.

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GENE DISCOVERY AND CROP ENGINEERING TO ENHANCE CLIMATE RESILIENCE AND NUTRITIONAL QUALITY

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Climate change poses a significant threat to global food security by adversely impacting crop productivity. Rising temperatures, erratic rainfall patterns and drought stress necessitate the development of climate-resilient crops. Advances in gene discovery, functional genomics, and crop engineering technologies have opened new avenues for accelerating crop improvement through the use of plant genetic resources (PGRs) for strengthening food and nutritional security in this era of climate change. The integration of pan-genomics, genome-wide association studies, and multi-omics approaches has facilitated the discovery of candidate target genes governing climate adaptation for the development of improved crops by precision breeding and CRISPR-based genome engineering.

Recent developments in CRISPR-based genome editing, including base editing and prime editing, have enabled precise modification of genes for improving traits related to crop resilience. In addition, genome engineering approaches are contributing to the development of climate-smart agricultural systems that support sustainable production under resource-constrained environments.

However, major emphasis is needed on mining the natural genetic variation locked in genebanks, for identifying suitable genomic regions and target genes for accelerating the development of climate-resilient and high-yielding crop varieties. There is an urgent need to characterise and enhance the utilisation of the genebank accessions through field phenotyping and pangenomics to capture structural variations, significantly broadening the pool of potential editing targets.

The presentation will highlight the need for enhanced use of PGRs for gene discovery followed by recent advances in crop engineering aimed at improving climate resilience and nutritional quality of crops. The wealth of plant genetic resources conserved in global genebanks, and the recent fast expanding genomics and genome engineering innovations hold considerable promise for achieving sustainable agriculture, nutritional security, and long-term climate resilience.

FUNGAL SYMBIONTS OF COEXISTING ORCHIDS FACILITATE SEED GERMINATION IN CO-OCCURRING ORCHIDS IN TÜRKIYE

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Interactions with mycorrhizal fungi are increasingly recognized as key ecological factors shaping orchid distribution and local abundance. While some orchid species associate with multiple fungal partners, others display a high degree of specificity in their mycorrhizal relationships. Moreover, orchids co-occurring within the same habitat may interact with distinct fungal communities, potentially reducing competition and promoting stable coexistence. However, the direct influence of mycorrhizal partner identity and origin on orchid seed germination remains poorly understood.

In this study, we investigated how the identity and origin of fungal associates affect seed germination in *Spiranthes spiralis* and *Serapias orientalis* using in situ symbiotic germination experiments. Four mycorrhizal fungal isolates belonging to the families Tulasnellaceae and Ceratobasidiaceae were successfully isolated and cultured from *S. spiralis*, *S. orientalis*, and two co-occurring orchid species, *Neotinea tridentata* and *Orchis provincialis*. Although all isolates induced seed embryo swelling, only a Ceratobasidiaceae isolate obtained from *N. tridentata* (NT2) promoted protocorm formation and subsequent seedling development in *S. spiralis*. Similarly, a Tulasnellaceae isolate from *O. provincialis* (OP3) supported seed germination and seedling formation in *S. orientalis*, whereas the remaining isolates failed to promote germination beyond the swelling stage.

These results demonstrate the selective nature of orchid–fungal associations and suggest that fungal partners of co-occurring orchids may facilitate seed germination and early development. Such interactions have important implications for orchid ecology, conservation, and propagation strategies.

IN SILICO ANALYSIS OF POST-TRANSCRIPTIONAL REGULATION OF STRESS-ASSOCIATED *DADREB2B* GENE EXPRESSION IN *DESCHAMPSIA ANTARCTICA*

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Deschampsia antarctica Desv. (Poaceae) is the only grass species adapted to survive in Antarctica under conditions of low temperatures, salinity, limited water availability, and intense UV radiation. The mechanisms underlying its adaptation to abiotic stresses are of considerable scientific interest. Due to its high tolerance, this species is regarded as a potential source of genetic material for improving stress resistance in crops. One of the key transcription factors (TFs) involved in plant responses to drought, salinity, and cold stress is DREB2B (Dehydration-Responsive Element Binding factor) from the AP2/ERF family. An important role in its activation is played by post-transcriptional regulation through alternative splicing with replacement of non-functional transcripts by functional ones under stress conditions [1,2].

The aim of this study was to investigate the features of post-transcriptional regulation of the *DaDREB2B* gene in *D. antarctica* and to characterize its alternative transcripts and corresponding protein isoforms using bioinformatics approaches.

Based on analysis of NCBI SRA datasets (genomic: SRX465632; transcriptomic: SRX5305101–SRX5305108), and using orthologous grass genes as references, two variants of the *DaDREB2B* gene having 93.3% identity were predicted, likely representing alleles. These sequences were deposited in GenBank under accession numbers BK068403 and BK068404. Alternative splicing was examined by comparing genomic and transcriptomic sequences.

The *DaDREB2B* gene consists of four exons and three introns and can produce four alternatively spliced transcripts. The two longest variants (*dadreb2b.1a* and *dadreb2b.1b*) include all exons and differ by three nucleotides due to an alternative splice site at the 3′ end of intron 2, an uncommon feature among grasses. The intermediate-length transcript (*dadreb2b.2*) lacks the third exon, whereas the shortest variant (*dadreb2b.3*) lacks both the second and third exons.

In most grasses, alternative splicing of *DREB2B* produces two [1] or three [2] transcript variants, one or two of which encode functional proteins. In contrast, in the *dreb2b.2*-type transcript, exclusion of the third exon causes a frameshift and premature translation termination [1,2]. In *D. antarctica*, however, the *dadreb2b.2* transcript contains, besides a short non-functional open reading frame (ORF), an alternative ORF with a non-canonical start codon at the beginning of the second exon. This ORF encodes a protein with an altered N-terminal region but retains the DNA-binding AP2/ERF domain, a nuclear localization signal, and a transcriptional activation domain. Such an alternative ORF was identified only in a few closely related species, including *Deschampsia cespitosa*, *Lolium rigidum*, and *Lolium perenne* [3].

In silico expression analysis showed that in various tissues of both laboratory-grown and wild plants, the predominant splice variant is *dadreb2b.2*, while other forms are represented by only a few sequence reads [3]. Unlike other grasses, where stress increases the proportion of transcripts encoding functional proteins [1,2], no such shift was observed in *D. antarctica* under natural stress conditions. This suggests potential functionality of the DaDREB2B.2 protein, which may compensate for the active isoforms DaDREB2B.1 and DaDREB2B.3 under stress exposure.

These findings provide a basis for further investigation of abiotic stress response mechanisms in grasses and may contribute to the genetic improvement of crops through transgenic approaches.

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ORGANOLEPTIC PROPERTIES OF GRAPES OF TEMPRANILLO AND CABERNET SAUVIGNON AS AFFECTED BY MYCORRHIZAL SYMBIOSIS AND ABIOTIC STRESSORS LINKED WITH CLIMATE CHANGE

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Climate change-induced temperature and drought stresses significantly impact grape aroma quality [1, 2]. Grapevine is considered as a vulnerable crop to future predicted climatic conditions. Several strategies have been proposed to increase grapevine resilience to stressors related to climate change, especially warming and water deficit, including the application of arbuscular mycorrhizal fungi (AMF). These fungi associate symbiotically with grapevine roots and improve growth, water and nutrient status and fruit quality, especially in plants undergoing stressful conditions [3]. Our objective was to evaluate whether the association of two grapevine varieties, Tempranillo (TEM) and Cabernet Sauvignon (CS), with AMF can modulate the profile of both amino acids and volatile compounds of fruits ripened under different climate change scenarios. Plants of TEM and CS grafted onto R110 rootstock were inoculated with a consortium of five AMF and four plant growth-promoting rhizobacteria (PGPRs) (+M) or with only PGPRs (-M). The plants were grown in greenhouses under two ambient conditions: current CO_2 concentration and ambient temperature (ACAT), or elevated CO_2 and temperature (700 ppm and ambient + 4˚ C, ECET). At veraison, two water availability treatments were applied: full irrigation (WW) or cyclic drought (D). For both varieties, each ambient condition, water availability and inoculation treatment were arranged in a factorial design. Elevated CO_2 and temperature (ECET) decreased the concentration of aroma-precursor amino acids in -M plants of TEM only. By contrast, the combination of elevated CO_2 and temperature with drought (ECET × D) resulted in the accumulation of these compounds in TEM and CS, with a more pronounced effect in +M plants. The most abundant amino acids in the fruits of both varieties were those with sweet and bitter flavours. Drought, either alone (D) or in combination with high CO_2 and temperature (ECET × D), induced the accumulation of sweet-tasting amino acids in +M TEM grapes, while reducing bitter-tasting ones. In CS, levels of sweet amino acids only increased and levels of bitter-tasting only decreased in -M plants subjected to D under ACAT. The most prevalent volatiles in both TEM and CS varieties and under all environmental conditions were aldehydes, followed by alcohols. Environmental stresses, whether acting alone (ECET or D) or together (ECET × D), reduced the concentration of total volatile compounds in non-inoculated TEM grapes. However, in +M plants, the levels of volatiles remained constant across the different environmental conditions. The concentration of total volatiles in CS was unaffected by the environmental stress factors. The effect of environmental conditions on amino acid and volatile profiles of both -M and +M plants will be discussed in light of the RNAseq analyses.

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ENDODERMIS SUBERIZATION INFLUENCES ROOT BASED INORGANIC CARBON UPTAKE, THAT INCREASES GROWTH AND OSMOTIC STRESS TOLERANCE

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Root-based inorganic carbon uptake is suggested by higher growth of various plants treated with low concentration bicarbonate solutions. This carbon source may still be accessible during osmotic stress conditions when plant response includes decreased stomatal conductance. Our aim was to study the mechanism of root-based inorganic carbon uptake of Arabidopsis (*Arabidopsis thaliana* ecotype Columbia) and barley (*Hordeum vulgare* 'Golden Promise') as well as its effect on growth and osmotic stress tolerance.

We found, that it is possible to optimize growth-promoting inorganic carbon treatment for both of the studied plants, working best at pH=5.6 and by including 2 mM $NaHCO_3$ to the hydroponic solution. The treatment improved growth parameters (shoot and root growth) with more pronounced effects in Arabidopsis (up to 50% increase), compared to barley (around 20% increase). As result of the inorganic carbon treatment, transpiration rate increased in both species and net photosynthesis rate increased in barley. Accompanying inorganic carbon treatment improved growth parameters of osmotically stressed Arabidopsis and some photosynthetic parameters of barley as indicated by fluorescence induction and gas exchange parameters. Fluxomic analysis following ^{13}C labelling proved the fixation of the supplied inorganic carbon in both species, though in barley we only detected in aspartic acid (marker or phoshoenolpyruvate carboxylase, PEPC, mediated fixation), while in Arabidopsis it was present in both aspartic acid and sucrose (marker of Calvin-cycle-based carbon fixation). Transcriptome sequencing also indicated, that barley fixed the inorganic carbon, taken up by the roots close to the point of entry within the roots by PEPC, while in Arabidopsis it was most likely transported up to the shoots in inorganic form and fixed by the Calvin-cycle. Transcriptome analysis further uncovered the regulation of growth promotion in the two species (trehalose-6-phosphate signaling in Arabidopsis, decreased ethylene signaling in barley) and that increased N and S-assimilation also made the higher growth possible. As for the original uptake and transport mechanism of inorganic carbon, transcriptome analysis and experiments with mutants showed the possible role of anion channels, and also the effect of decreased Caspary endodermis suberization in facilitating the diffusion of the inorganic carbon into the xylem and subsequently, into the shoots of Arabidopsis, an effect which was not observed in barley. The enhanced suberin biosynthesis Arabidopsis mutant took up and fixed less carbon from the hydroponic solution, as indicated by total carbon content and $\delta^{13}C$ ratio.

To conclude, the root-based inorganic carbon uptake and fixation mechanism of Arabidopsis was possibly more effective and energetically feasible, compared to barley which resulted in higher growth promotion and mitigation of the effects of osmotic stress. It was accompanied by the more robust trehalose-based growth regulation. The advantages of Arabidopsis may have stemmed from higher carbonic anhydrase expression in the shoots and decreasing of endodermis suberization facilitating carbon entry into the xylem. The results open new avenues for improving productivity and osmotic stress tolerance of plants. More details can be found in Gamarra Reinoso et al. (2024, 2026).

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BIOTECHNOLOGICAL USING OF REMOTE EFFECTS OF PRE-SOWING IRRADIATION OF PLANT SEEDS

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Background. The experience of studying plant reactions to stress indicates the possibility of reorienting metabolism in necessary for practice direction. Irradiation to shift metabolic processes towards the formation of secondary metabolites that have antioxidant, anti-carcinogenic, immunomodulatory and anti-inflammatory effects.

The aim of the work. To develop a technology for improving the quality of medicinal raw materials with various types of low dose irradiation.

Methods. Spectrophotometry, steam distillation, liquid chromatography, ISSR-RABD- ITS –markers-PCR, cluster analysis.

Results:

- The type of irradiation and the range of low doses that do not reduce the yield of medicinal raw materials and at the same time induce an increase accumulation of target metabolites have been determined; this range is 5-15 Gy and 5-20 kJ/m^2 for chamomile, St. John's wart, sage, milk thistle, and digitalis.
- Key markers of oxidative stress were studding; cultivars with high and stable productivity of pharmaceutical raw materials were selecting. A correlation was revealing between the control flavonoid content in different varieties and the increase one after irradiation.
- Pre-sowing X-ray irradiation stimulates the accumulation of essential oil and chamazulene in the inflorescences of chamomile and yarrow;
- Relationship between epigenetic polymorphism of varieties and the efficiency accumulation of the pharmaceutical substances was investigating;
-Transgenerational transmission of the effects of pre-sowing irradiation was established;
-For the first time in world we investigated the mechanisms connection primary radiation DNA damage and remote changes in plant metabolism [1,2]. The study had reveal the involvement of X-ray and UV-C-induced genomic instability in long –term metabolism rearrangement.

The important advantage for the practice is that dry seeds are irradiating, but the necessary substances are obtaining in pharmaceutical raw materials at the end of ontogenesis.

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HYDROGEN PEROXIDE IN WHEAT IMMUNITY: SYSTEM-LEVEL REDOX RESPONSES IN PLANT–FUNGAL INTERACTIONS

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Hydrogen peroxide (H_2O_2) is a key component of reactive oxygen species (ROS) metabolism in plants and is widely implicated in innate immune responses. In classical plant immunity frameworks, H_2O_2 is associated with early defense signaling, oxidative burst formation, and regulation of downstream stress-responsive pathways. At the same time, it is an intrinsic product of cellular redox metabolism, making its interpretation strongly dependent on physiological context, tissue state, and pathogen lifestyle (1)

In plant–fungal pathogen interactions, particularly in wheat responses to necrotrophic and hemibiotrophic fungi, accumulation of H_2O_2 is a recurrent feature of early infection stages. However, its functional role is not uniform across interaction types. In biotrophic interactions, controlled ROS production is often associated with localized defense activation and restriction of pathogen growth. In contrast, during necrotrophic infections, excessive or misregulated ROS accumulation may contribute to host cell death processes that can facilitate pathogen colonization. This functional duality highlights hydrogen peroxide as a context-dependent component of plant immune responses rather than a universally protective signal.

Importantly, ROS dynamics are tightly interconnected with broader metabolic networks, including redox homeostasis, energy metabolism, and secondary metabolite biosynthesis. Secondary metabolites such as phenylpropanoids, flavonoids, and other defense-related compounds are frequently co-regulated with oxidative stress responses, suggesting that redox perturbations are integrated into metabolic reprogramming during pathogen challenge. This coupling is particularly relevant for understanding how plants balance defense activation with metabolic cost under stress conditions.

From a biotechnological perspective, the dual role of H_2O_2 in plant immunity has practical implications. Modulating ROS balance may influence not only disease resistance but also the biosynthesis of valuable secondary metabolites, which are of interest for crop improvement and metabolic engineering. Understanding how ROS dynamics interact with pathogen lifestyle (biotrophic versus necrotrophic) may therefore inform strategies for optimizing both disease resistance and metabolic output in agriculturally important crops.

In this study, publicly available transcriptomic datasets from the Gene Expression Omnibus (GEO), including wheat–fungal pathogen interactions (2,3), were used to explore system-level transcriptional responses under infection conditions. Rather than focusing on predefined ROS-associated gene modules, a global perspective on transcriptional variation and functional coupling between redox and metabolic processes was considered. Our results indicate that pathogen exposure is associated with broad restructuring of transcriptional variability, including coordinated shifts in genes involved in redox regulation, primary metabolism, and secondary metabolite biosynthesis. These patterns suggest that oxidative responses are embedded within global metabolic reprogramming rather than representing a single hydrogen peroxide-centered signaling axis.

Overall, hydrogen peroxide should be viewed as a multifunctional component of plant redox biology, whose role in immunity emerges from its integration within broader metabolic and regulatory networks rather than from isolated signaling activity.

1. Alam, P. et al. (2025) Reactive oxygen species: balancing agents in plants *Frontiers in Plant Science* https://doi.org/10.3389/fpls.2025.1713590

2. Gene Expression Omnibus (GEO): GSE13660 Wheat–Fusarium crown rot related transcriptomic dataset https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13660

3. Gene Expression Omnibus (GEO): GSE74671 Wheat–fungal pathogen interaction transcriptomic dataset https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74671

NANOCLAY BASED AGROINPUTS

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The intensification of global agriculture, driven by increasing food demand and increasingly adverse environmental conditions, necessitates a transition toward more efficient and sustainable production systems. Modern crops are frequently exposed to fluctuating climatic stressors that impair physiological performance and yield. In this context, Salicylic Acid (SA) has emerged as a crucial signaling molecule capable of regulating plant growth and mediating resilience against environmental challenges. However, the practical application of free SA is often hindered by its chemical instability, rapid degradation under UV radiation, and narrow therapeutic window, which lead to inconsistent results in the field. To address these limitations, nanotechnology offers innovative solutions through the development of specialized nanocarriers designed to stabilize and optimize the delivery of bioactive compounds.

In this work, we evaluated the biostimulatory potential of a nanocarrier based on Mg/Al-Layered Double Hydroxides (LDH) functionalized with SA (LDH-SA). The nanohybrid was synthesized using a sustainable, fully aqueous reconstruction method, achieving a loading capacity of 9.6% (w/w) and a characteristic sustained-release profile. To bridge the gap between laboratory scale findings and practical horticultural settings, the performance of LDH-SA was assessed in cv. Micro-Tom plants grown under greenhouse conditions. This model allowed for the evaluation of the nanocarrier's impact throughout the entire developmental program, including vegetative growth and fruit quality parameters. The experimental design focused on foliar applications performed every 8 days between day 20 and day 60 of the plant's life cycle. This approach allowed for the evaluation of the nanocarrier's impact throughout the vegetative and early reproductive stages, monitoring its influence on development and fruit quality parameters.

Results demonstrated that the LDH framework effectively acts as a protective matrix, shielding SA from environmental degradation and ensuring its prolonged residency on the leaf surface. Compared to free SA applications, LDH-SA treatments resulted in more consistent biostimulatory effects, reflected in improved biomass accumulation and maintained photosynthetic efficiency under greenhouse conditions. The controlled release kinetics provided by the LDH nanocarrier prevented the transient peaks in endogenous SA levels typically associated with conventional aqueous sprays, leading to a more balanced and sustained physiological response.

In conclusion, LDH-SA represents a robust and scalable nanoinput for sustainable agriculture. By enhancing the stability and bioavailability of SA, this nanocarrier provides a reliable tool for improving crop performance. These findings in MicroTom establish a solid foundation for expanding this technology to intensive strategic crops, such as soybean and peanut, where stabilizing agroinput delivery is essential for maintaining productivity under contemporary climatic pressures.

STOMATAL MORPHOLOGY AND GAS EXCHANGE DYNAMICS IN TRITICUM SPECIES UNDER WATER DEFICIT

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Bread wheat (*Triticum aestivum* L.) is staple crop and, consequently, one of the most widely cultivated cereals worldwide. However, its productivity is increasingly threatened by drought. In this context, leaf structures such as stomata are of particular interest. Stomata, despite covering a minimal leaf area, regulate nearly 90% of transpiration, making them key targets for enhancing drought tolerance.

This study aimed to investigate the morphological characteristics of the stomatal apparatus in leaves of species of the genus *Triticum*: *T. aestivum* (cv. Zymoyarka), *T. spelta L.*, and *T. dicoccum (Schuebl.) Schrank*, and evaluate their relationship with stomatal conductance, and CO_2/H_2O gas exchange rates under both optimal moisture conditions and the influence of short-term soil drought.

The study evaluated bread wheat (*T. aestivum* cv. Zymoyarka) and its relatives: spelt (*T. spelta* var. *album*) and emmer (*T. dicoccum* var. *aeruginosum*). Plants were grown in pots under a polyethylene-covered shelter under natural temperature and light conditions. Drought stress was induced at the anthesis (flowering) stage by reducing soil moisture to 30% of field capacity (FC) for 7 days, monitored daily by the gravimetric method, while the control group was maintained at 70% FC. Stomatal traits of the flag leaf of control plants at the flowering stage were determined. Gas exchange rates were measured in both control and stressed plants on the seventh day of the drought treatment (30% FC).

Results showed that emmer wheat exhibited the highest stomatal density on both leaf surfaces. However, its guard cell size and pore aperture area were the smallest compared to spelt and bread wheat. Consequently, while the total stomatal pore area on the adaxial side of emmer was comparable to cv. Zymoyarka and spelt, it was the highest on the abaxial surface.

Under optimal conditions (70% FC), emmer exhibited higher stomatal conductance and transpiration rates, while photosynthetic intensity remained similar across all genotypes. However, short-term drought (30% FC for 7 days) induced a more pronounced reduction in stomatal conductance, transpiration, and photosynthesis in emmer (by 60%, 40%, and 34%, respectively) compared to bread wheat (38%, 35%, and 21%) and spelt (42%, 28%, and 28%). Notably, intrinsic water-use efficiency (WUEi) increased by 6% in bread wheat and 11% in emmer. Correlation analysis revealed that higher stomatal density in emmer contributes to intensive gas exchange under optimal conditions but leads to a somewhat lower WUEi under high light. Conversely, under short-term soil drought, a greater number of smaller stomata in emmer was associated with a significant decrease in stomatal conductance but an increase in water use efficiency and photorespiration activity.

In conclusion, the unique stomatal morphology of *T. dicoccum* influences on stomatal conductance and gas exchange. These traits represent vital adaptive mechanisms for maintaining productivity under limited water supply.

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MORPHOMETRIC AND BIOCHEMICAL TRANSGENERATIONAL EFFECTS OF IRRADIATION OF PEA SEEDS

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Introduction. One of the most promising areas of contemporary radiobiological research is the study of the transgenerational inheritance of the effects of radiation exposure. We have previously demonstrated that irradiation of pea dry seeds at a dose of 50 Gy, considerably lower than the sublethal dose, has a significant impact on the proteome of seedling roots [1]. This study **aimed** to identify and compare the effects of ionising radiation on pea seeds (F0) across two successive generations of offspring F1 and F2.

Materials and methods. Pea (*Pisum sativum* L. cv. Aronis) seeds were irradiated with X-rays at dose 50 Gy. Morphometric analysis of the plants at the vegetative and ripening stages and Fourier-transform infrared spectroscopy (FTIR) of the ground seeds were then used to investigate the biochemical changes. The ATR-FTIR analysis was performed using a Nicolet FTIR IS50 (Thermo Fisher Scientific, USA) spectrophotometer within the spectral range of 400–4000 cm^{-1}.

Results and discussion. A dose of 50 Gy stimulated the growth of plants in the F0 generation. This was followed by a decrease in growth relative to the generation control and F0 control in the F1 and F2 generations. In each subsequent generation, the stem length of plants grown from irradiated seeds decreased. Meanwhile, the 50 Gy dose had no significant impact on plant yield in the F0, F1 and F2 generations. However, the test irradiation had a pronounced stressful effect on seedlings from the irradiated F0 generation, with a 17% decrease in germination energy and shorter roots (by 19%). Transgenerational biochemical changes in composition were observed in the offspring of plants grown from irradiated seeds. Compared with those derived from non-irradiated seeds, the content of proteins (by 5%), starch (by 8%) and polysaccharides (by 16%) was significantly lower in the seeds of the first generation (F1), whilst the content of cellulose and nucleic acids was 18% and 6% higher, respectively. In the second generation (F2) the protein content increased by 10%, and the starch and polysaccharide content increased by 11% and 15%, respectively, compared with the control, the nucleic acid content increased by 7%. Generally, the content of the main biochemical substances returned to the F0 control level in the F2 generation, except for cellulose, which remained 36% higher than the F1 control overall.

Conclusions. The obtained data indicate that changes to the parameters of pea plants (a decrease in plant growth and an increase in cellulose content in the seeds) induced by acute ionizing radiation persist in two successive generations of their offspring.

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COMPARISON OF WATER STRESS TOLERANCE POTENTIAL OF *NIGELLA* L. SPECIES

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Plants of the genus *Nigella* L. are known for their medicinal and technical properties. The cultivation of this crop is spreading in Ukraine, which requires the selection of species and adaptation to growing conditions. Modern climate changes exacerbate the problem of water supply to plants during cultivation, which directly affects plant productivity and the quality of plant raw materials. Literature data confirm a reliable dependence of fruit, seed, above-ground biomass productivity, essential and fatty oil content and their components (nigellone, monounsaturated and polyunsaturated fatty acids) in *Nigella sativa* L. plants on the level of water supply [1]. Among the three most common species of the genus *Nigella* in culture, the effect of water stress has been mainly studied on *N. sativa* plants. Phenolic compounds, particularly flavonoids, play an important role in plant growth and their response to stress. Phenolic compounds, particularly flavonoids, play an important role in plant growth and their response to stress[2]. Markers of plant stress under drought conditions also include the state of the stomatal system of leaves, which affects the process of photosynthesis, growth, and plant productivity. The aim of our study was to compare the water stress tolerance indicators of the following species: *N. sativa*, *N. arvensis* L. and *N. damascena* L. The experimental plots were located on the territory of the M.M. Gryshko National Botanical Garden. Methods for determining the water regime of plants (Relative water content, leaf wilting rate according to Arlando, water deficit, moisture), the impression method for anatomical study of leaf blade epidermis structure, as well as biochemical methods for determining the total phenols content and total flavonoid content, were used.

It was found that *N. arvensis* plants had the highest leaf wilting rate – after 7 hours, the moisture loss was 28.4%. *N. damascene* had the lowest loss during the same period – 19.1%. The highest moisture (83.2%) and water deficit (25.3%) are also characteristic of *N. arvensis*. The lowest moisture (77.2%) and water deficit (22%) were found in *N. damascene*. The relative water content was highest in *N. damascene* – 71.8%. *N. sativa* had average values for all parameters. The observed difference in water regime indicators demonstrates the greatest drought stress resistance in *N. damascene* plants. It can be assumed that better resistance to stress conditions does not contribute to the synthesis of greater amounts of polyphenols and flavonoids. *N. damascene* contains the least of these compounds compared to *N. sativa* and *N. arvensis*. The characteristics of the water regime of the three species are determined by the anatomical structure of the stomatal apparatus. In the more stress-resistant *N. damascene*, the adaxial surface of the leaf has the lowest stomatal density (35.6 pcs/mm2) and stomatal index (0.13), whereas in *N. arvensis* – 93.1 pcs/mm2 and 0.22, respectively. Based on the combination of water regime markers, stomatal apparatus structure, and biochemical composition, Nigella species can be ranked in increasing drought resistance: *N. arvensis*→ *N. sativa*→ *N. damascene*.

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MISSILE-INDUCED HEAVY METAL CONTAMINATION OF AGRICULTURAL SOILS AND LEAD-INDUCED MORPHOPHYSIOLOGICAL RESPONSES OF *TRITICUM AESTIVUM* L.

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Heavy metal (HMs) contamination from military activity poses a serious threat to agricultural ecosystems and plant productivity. Missile strikes can release toxic elements, including Pb, Zn, Cu, and Cr, into soils, where they may persist and adversely affect plant physiological processes. Lead (Pb) is considered one of the most hazardous pollutants because of its persistence and strong phytotoxicity. Wheat (*Triticum aestivum* L.) is highly sensitive to heavy metal stress and serves as an effective model for evaluating phytotoxic effects in contaminated agroecosystems (Lamhamdi et.al., 2011). This study aimed to assess HM contamination in agricultural soils affected by missile strikes and to investigate the morpho-anatomical responses of wheat seedlings to lead-induced stress.

Materials and Methods. Soil samples were collected from missile impact craters and nearby control plots in the Dnipropetrovsk region of Ukraine. HMs concentrations were determined by energy-dispersive X-ray fluorescence spectroscopy (ED-XRF). Pollution intensity was assessed using the geoaccumulation index (Igeo) and enrichment factor (EF) (Müller, 1981). Winter wheat cv. 'Podolyanka' was grown in a hydroponic system. Pb was used as a model pollutant in the form of $Pb(NO_3)_2$ at concentrations of 0.005, 0.025, 0.05, 0.075, and 0.5 mM (Pb^{2+}). Morphophysiological parameters, stress tolerance indices, phytotoxicity index, and root anatomical characteristics were analysed in 14-day-old seedlings.

Results. Soils collected from explosion craters showed elevated concentrations of HMs, particularly Pb, Zn, Cu, and Cr. Pb concentrations reached 243 mg kg^{-1}, and EF values (6.5–10.4) and Igeo values (2.12–2.80) indicated significant anthropogenic contamination. Pb exposure induced concentration-dependent changes in wheat seedlings. Low Pb concentrations (0.005–0.025 mM) slightly stimulated growth, indicating a possible hormetic response. Higher concentrations significantly inhibited shoot and root growth, reduced fresh and dry biomass accumulation, and decreased stress tolerance indices. At 0.5 mM Pb, root length decreased by 93.8%, root fresh weight by 77.1%, and root dry weight by 60.2% compared with the control. Strong negative correlations were observed between Pb concentration and morphophysiological parameters ($r = -0.92$ to -0.94, $p < 0.05$).

Anatomical analysis revealed substantial reorganisation of root tissues under Pb stress. Exposure to 0.5 mM Pb reduced the stele-to-xylem area ratio, increased cortical porosity, and enhanced suberisation and lignification of the endodermis. Vessel diameter and xylem area decreased markedly, indicating reduced hydraulic efficiency.

Conclusions. Missile strikes contribute to persistent HM contamination of agricultural soils, creating long-term risks to crop productivity. Lead-induced stress in wheat seedlings is characterised by severe morphophysiological inhibition and extensive anatomical reorganisation of root tissues. Structural modification of the root system appears to be an adaptive response to Pb toxicity, but it also limits hydraulic conductivity and plant growth.

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DROUGHT-INDUCED PHYSIOLOGICAL DYSFUNCTION DEFINES KEY SIGNALING STAGES DURING GRAIN FILLING IN OAT CULTIVARS

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Drought is one of the most limiting abiotic stresses affecting cereal productivity and global food security. Water deficit disrupts morphological, physiological and molecular processes, compromising nutrient balance, growth and yield. Oat (*Avena sativa* L.) is a nutritionally valuable cereal rich in proteins, lipids and antioxidants, but it is particularly vulnerable to hot and dry conditions, showing increased grain abortion and reduced grain filling under drought (Allwood et al., 2021). In previous work, untargeted metabolomics was used to characterise drought-induced metabolic shifts at anthesis in two Mediterranean oat cultivars with contrasting drought responses, the susceptible Flega and the tolerant Patones (Girija et al., 2024).

The aim of this study was to evaluate physiological parameters in order to identify critical periods during grain filling under drought in Flega and Patones, providing a basis for future molecular studies focused on drought-related signalling pathways.

Adult plants of Flega and Patones cultivars, grown outdoors in large containers under Mediterranean climatic conditions, were evaluated under control and drought treatments in a time-course experiment from flag leaf emergence to grain filling, corresponding to Zadoks growth stages 45 to 92 (Zadoks et al., 1974). Physiological measurements were conducted on flag leaves using a LI-600 porometer/fluorometer (LI-COR Biosciences). Recorded parameters included stomatal conductance (gs), electron transport rate (ETR) and photosystem II efficiency (ΦPSII).

Drought induced a rapid decline in gs in both cultivars, although the reduction was earlier and more pronounced in Flega, starting at Zadoks stage 59 and remaining at very low levels throughout grain filling. In contrast, Patones maintained higher gs under drought, indicating a more controlled stomatal regulation. ΦPSII progressively decreased in both genotypes, with Flega showing an early and sustained impairment of PSII photochemistry, whereas Patones preserved higher ΦPSII values. ETR was also strongly affected by drought, particularly in Flega, which exhibited a sharp early decline, while Patones showed a more gradual reduction and maintained higher ETR throughout the experimental period. Control plants remained stable across all parameters. The greatest physiological differences between cultivars were observed around anthesis, corresponding to Zadoks stages 61 to 69.

These physiological patterns complement previously described metabolic differences and highlight anthesis and early grain filling as critical reproductive stages shaping drought resilience in oat. By linking photosynthetic performance with developmental timing under water deficit, this work contributes to a better understanding of how reproductive processes respond to extreme climatic conditions. Such insights are essential for the reproductive enhancement of crop resilience, supporting targeted molecular and breeding strategies aimed at safeguarding yield stability under increasingly frequent drought events.

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THE REGENERATION CAPACITY COMPARISON OF THREE TOBACCO CULTIVARS DURING THE AUTUMN-WINTER AND SPRING-SUMMER PERIODS

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Nicotiana tabacum is one of the most widely used model organisms in plant biotechnology and molecular biology. Its extensive use in research is due to rapid growth, high regenerative capacity, ease of cultivation, and high sensitivity to *Agrobacterium*-mediated transformation. The relevance of this study stems from the need for year-round use of tobacco crops in biotechnology research, including during the fall and winter, when natural conditions can affect the intensity of growth processes and the regenerative capacity of plants.

The aim of the study was to investigate the regenerative capacity of the Wisconsin, Petit Havana, and Samsun varieties of *Nicotiana tabacum* during the fall-winter period and to compare these results with their regeneration rates during the spring-summer period.

Leaf discs measuring 1×1 cm, obtained from the upper and middle leaves of *Nicotiana tabacum* plants, were cultured on MSR nutrient medium (Murashige and Skoog medium for regeneration). Regeneration was evaluated after two months of cultivation during the autumn-winter (November-February) and spring-summer (March-April) periods. For each cultivar, regeneration frequency (the percentage of explants that formed regenerants) and regeneration efficiency (the number of regenerants per explant) were determined.

As a result of the study, the regeneration frequency for all cultivars of Nicotiana tabacum was 100%. However, differences in regeneration efficiency were observed depending on the cultivar and cultivation period. During the autumn–winter period, regeneration efficiency was similar among all studied cultivars and ranged from 6,8 to 7,7 ± 0,8 regenerants per leaf disc, with the highest value recorded for the Wisconsin cultivar. In the spring-summer period, regeneration efficiency increased significantly. It reached 28,8 ± 1,9 regenerants per leaf disc for Petit Havana, 20 ± 1,7 for Samsun, and more than 34 ± 1,2 for Wisconsin.

Thus, the cultivation season significantly affects regeneration efficiency in different cultivars of *Nicotiana tabacum*. Despite the stable regeneration frequency observed throughout all studied periods, regeneration efficiency during the autumn–winter period was considerably lower compared to the spring–summer period. The Wisconsin cultivar demonstrated the highest regeneration efficiency in both cultivation periods.

PROTECTIVE EFFECTS OF PINE BARK EXTRACT UNDER SALT STRESS CONDITIONS

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Pine bark is a rich natural source of phenolic compounds with strong antioxidant potential. Pine bark is used medicinally to produce dietary supplements. Pine bark extract contains numerous phenolic compounds, catechins, taxifolin, and phenolic acids. Recent studies have shown that pine bark extracts have strong restorative, antimutagenic, and antioxidant properties. [1,2]. Eldar pine (Pinus eldarica) is one of the most common coniferous tree species on the Absheron Peninsula. In this study, we aimed to examine the effects of pine bark extract solutions on the morphological and physiological parameters of wheat under salt stress. Pine bark extract (Pinus eldarica) was obtained by alkaline extraction. Dried bark was first crushed in a crusher. Extraction was performed with a 2% NaOH solution at 70-80°C with constant stirring [3]. UV-Vis spectra of the extract were analyzed on a Varian Cary 50 Scan spectrometer (USA) in the 300-800 nm range. The elemental composition of the extract was analyzed using an Agilent Technologies ICP-MS 7700 mass spectrometer (USA). Field experiments were conducted on plots with moderately saline soils. The effect of pine bark extract on the yield of photosynthetic pigments was measured using a Multiscan Go spectrophotometer (Germany). The "Tale 38" wheat variety was used as a model plant for the experiments. To determine the effectiveness of the extract, seeds were treated with pine bark extract solutions at concentrations of 0.1%, 0.01%, and 0.001% before sowing. During field trials, the morphological and physiological parameters of seedlings were studied, including growth, germination, the amount of photosynthetic pigments, and malondialdehyde, a product of lipid peroxidation.

The experimental results showed that seeds treated with 0.1% and 0.01% pine bark extract solutions before sowing had a 5-7% higher germination rate than seeds not treated with the extract. Salt stress inhibited seedling growth by 7-8% compared to the control. A significant positive difference in growth was observed in seedlings obtained from seeds treated with a 0.1% pine bark extract solution. These seedlings were 6-7% taller than seedlings from untreated control samples subjected to salt stress. Salt stress is known to cause lipid peroxidation, damage to membrane integrity, and chlorophyll degradation. The effect of the extract on the levels of photosynthetic pigments and malondialdehyde was studied. Salt stress in plants led to a decrease in the chlorophyll content in the leaves of seedlings. Treatment of wheat seeds with extract solutions enhanced chlorophyll a and b biosynthesis in seedlings by 10-15% compared to the salt-treated plants. Under salt stress conditions, in seedlings obtained from seeds treated with 0.1% and 0.01% solutions of the extract MDA levels were reduced by 10-12% compared to the untreated variant. These results demonstrate the phytoprotective effect of pine bark extract under salt stress.

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REDOX HOMEOSTASIS IN PLANT ADAPTATION TO STRESS CONDITIONS

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Climate change and increasing environmental stress factors significantly threaten agricultural productivity and global food security. Rising temperatures, irregular precipitation, drought, and the spread of phytopathogens destabilize agroecosystems and reduce crop yield potential. In this context, understanding plant adaptation mechanisms and developing sustainable approaches to enhance stress tolerance are of key importance.

The aim of this study was to elucidate the features of redox homeostasis formation in different wheat genotypes and soybean symbiotic systems under abiotic and biotic stress factors, and to develop scientifically grounded approaches for its regulation to enhance plant stress resistance and realize crop productivity potential. The research is based on the assumption that plant stress tolerance depends on the ability of a genotype to undergo adaptive metabolic adjustments and maintain the effective functioning of protective systems, which can be modulated through physiologically active compounds and optimization of plant–microbe symbiotic interactions.

The methods included a systems analysis of plant metabolism at physiological, biochemical, and molecular levels under laboratory, greenhouse, and field conditions. Spectrophotometric and refractometric methods were applied for quantitative assessment of metabolites and physiological parameters. Gas chromatography was used for profiling stress-related metabolites, while native disc electrophoresis enabled analysis of protein and enzyme spectra involved in adaptive responses. Statistical processing ensured the reliability of experimental data. Particular attention was paid to oxidative stress intensity, lipid peroxidation processes, and indicators of redox homeostasis in wheat genotypes and soybean–Bradyrhizobium japonicum symbiotic systems of different efficiency under stress conditions.

The results demonstrated that effective soybean symbiotic systems exhibit high stress tolerance due to coordinated interactions between macro- and microsymbionts and their ability to maintain active nitrogen fixation under water deficit. These systems are characterized by rapid activation of antioxidant defense, stable regulation of reactive oxygen species, and efficient maintenance of cellular redox balance. In contrast, inefficient symbiotic systems show metabolic imbalance, weak stress responsiveness, and reduced control over oxidative processes. Application of effective bacterial strains, as well as their modification with nanochelates of germanium and iron or salicylic acid, significantly improves antioxidant protection, enhances plant water status, and increases soybean productivity. Seed inoculation combined with fungicide treatment improves physiological regulation and reduces oxidative damage.

In winter wheat, drought-tolerant genotypes demonstrated a more efficient redox homeostasis system, ensuring the maintenance of cellular integrity and metabolic stability under water deficit. These genotypes showed lower oxidative stress intensity, reduced accumulation of reactive oxygen species, and higher membrane stability. Exogenous salicylic acid reduced lipid peroxidation, regulated antioxidant activity, and improved plant water balance, contributing to yield stabilization under drought conditions. Foliar nitrogen application activated stress-defense reactions but did not fully compensate for mineral nutrient deficiency.

Overall, redox homeostasis is a key regulatory mechanism of plant stress tolerance. Its stability determines the efficiency of metabolic adaptation and the ability of plants to maintain productivity under adverse conditions. The proposed agroecological approaches, based on efficient symbiotic systems, biological inoculants, and physiologically active compounds, enhance crop adaptive potential, stabilize yield formation, and ensure environmentally sustainable agricultural production. These findings provide a scientific basis for improving wheat and soybean cultivation under climate change and support the development of resilient agroecosystems.

LIPOSOMAL BIONANOCOMPOSITE MODULATES WHEAT ADAPTATION TO WHEAT STREAK MOSAIC VIRUS

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Crop production plays a strategic role in the sustainable development of the global economy. Cereal crops are not only staple foods but also important renewable resources for industrial and bioenergy applications. Among them, wheat (*Triticum aestivum* L.) is particularly significant due to its wide ecological adaptability, enabling cultivation across diverse agroecological zones and production systems. However, achieving consistently high yields remains challenging because of abiotic stress factors and increasing disruptions in plant–pathogen–environment interactions, which together cause substantial yield losses and threaten global food security.

Wheat streak mosaic virus (WSMV) is one of the most destructive viral pathogens of wheat worldwide, responsible for major productivity losses. It disrupts physiological and biochemical homeostasis and induces excessive production of reactive oxygen species (ROS), leading to oxidative stress, membrane lipid peroxidation, metabolic dysfunction, impaired growth, and reduced photosynthetic efficiency. Since chemical control of viral diseases is largely ineffective, increasing attention is focused on environmentally safe resistance inducers that enhance intrinsic plant defense mechanisms.

This study evaluated a liposomal bionanocomposite (BNC) based on a glycan–rhamnolipid complex as a seed treatment to enhance wheat resistance to WSMV. Wheat plants (*Triticum aestivum* L., cv. Zymoyarka) derived from treated seeds were grown under mock- and virus-inoculated conditions. Viral accumulation was quantified using DAS-ELISA. Physiological and biochemical responses were assessed by measuring hydrogen peroxide (H_2O_2), malondialdehyde (MDA) as an indicator of lipid peroxidation, and the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and phenylalanine ammonia-lyase (PAL). Ethylene emission, chlorophyll content, and gas exchange parameters were also analyzed.

The results showed that BNC treatment significantly reduced WSMV accumulation in infected plants compared to controls, indicating partial suppression of viral replication or spread. This was accompanied by reduced oxidative stress, with lower MDA levels and a more regulated H_2O_2 profile, reflecting improved ROS homeostasis. BNC treatment enhanced antioxidant defense capacity, increasing SOD and PAL activities, while maintaining more stable CAT activity. These changes indicate a coordinated strengthening of the antioxidant system and improved detoxification of ROS. In addition, BNC modulated physiological and hormonal responses. Reduced ethylene overproduction suggested a more balanced stress response and delayed senescence. Partial restoration of chlorophyll content, together with improved gas exchange and photosynthetic efficiency, indicated better preservation of chloroplast function under viral stress.

Overall, the findings demonstrate that BNC enhances wheat tolerance to WSMV by activating antioxidant defenses, regulating ROS balance, modulating stress signaling, and stabilizing photosynthetic processes. The liposomal bionanocomposite acts as a multifunctional resistance inducer that promotes pre-adaptive metabolic adjustments and improves physiological resilience without observable environmental drawbacks. Therefore, BNC represents a promising strategy for sustainable wheat protection under viral infection and environmental stress conditions.

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PRODUCTION OF TRANSGENIC *NICOTIANA* PLANTS WITH RUBY REPORTER FOR STRESS RESISTANCE RESEARCH

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RUBY is a novel noninvasive reporter system in plant genetic engineering. RUBY is consisting of three key genes: CYP76AD1, DODA, and glucosyl transferase, which synthesize betalain within four steps of catalysis. Tyrosine is used as the substrate for betalain biosynthesis (Sadowska-Bartosz, & Bartosz, 2021). Betalains are red colored natural pigments in plants of the order Caryophyllales where they replace anthocyanins. As usual plants synthesise one type of pigments either anthocyanins or betalains according to its taxonomic origin. In betalain containing plants these compounds are most often present in the flower petals, but may be observed in fruits, leaves, stems, and roots. Such properties make them a convenient tool for plant genetic engineering as reporter or marker that allows visual selection of transgenic tissues in plants. It has preferences before the reporter genes GUS, LUC, as it does not require sacrificing of plant tissues and expensive staining, and before GFP, for it doesn't need special equipment and darkness. Betalains can be easily measured on spectrophotometers to evaluate their quantity. CYP76AD1, DODA, and glucosyl transferase are responsible for the biosynthesis of betalains in multiple betalain-free species. RUBY has been successfully used for transformation of *Orysa sativa*, *Arabidopsis thaliana*, *Gossipium hyrsutum*, *Nicotiana tabacum*, *Petunia hybrida*, *Oryza sativa*, *Solanum lycopersicum*, *S. tuberosum*, *S. melongena*, *Plukenetia volubilis*, *Nicotiana benthamiana*, *Spirodella*, *Medicago truncatula*, *Zea mais*, and *Glicine mas*. Betalains are known to be strong antioxidants, that can biofortify edible agricultural species. Betalain production in engineered plants elevates their resistance to gray mold and confers resilience under oxidative conditions when stably integrated (Wang, & Sun, 2025).

RUBY reporter systems can be used for a variety of plant breeding studies, including the development of in vitro genetic transformation conditions, in-planta transformation, antibiotic-free transformation systems, gene expression studies, promoter characterization, environmental xenobiotic monitoring, long-distance protein transport, transgene stability assessment, and identification of T-DNA-free editing events. We proposed to create plants expressing RUBY reporter genes for studying the effects of viruses on the expression of heterologous proteins and metabolic engineering processes.

N. benthamiana and *N. tabacum* known as model plants in virology and genetic transformation, were used for transformation with RUBY. Although, the genetic transformation was conducted according to standard *Agrobacterium*-protocol (Horsch, et al, 1985), there were some peculiarities in transgenic plant selection. Regenerating clones differed in the amount of betalain pigment. Transgenic lines with the most intensive colour appeared later, than those with less intensive coloration. Probably metabolic overload and tyrosine deficiency were the reasons of such results that slowed the growth of the most pigmented transgenic lines. Thus, transgenic *N. benthamiana* and *N. tabacum* lines expressing different levels of betalain were obtained for our future virological experiments.

Genetic construct courtesy of Dr. Götz Hensel, Centre for plant genome engineering, and Dr. Olga Yaroshko, Institute of cell biology and genetic engineering

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BEYOND GENE-CENTERED MODELS: A SPATIAL SYSTEMS BIOLOGY VIEW OF PLANT DEVELOPMENT AND STRESS RESPONSES.

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Plants are multicellular organisms in which each cell type follows a distinct developmental trajectory characterized by specific geometry, local phytohormone balance, chromatin organization, and gene-expression profile. Under normal conditions, plant development depends on tightly coordinated interactions among heterogeneous cell types. Under stress conditions, however, individual tissues and cell populations exhibit distinct responses, including altered hormonal balance, accumulation of reactive oxygen species (ROS), chromatin remodeling, and changes in growth dynamics. Adaptive responses, therefore, require re-establishment of coordinated interactions and functional balance across the organ. Locally generated perturbations may propagate through tissues, producing systemic outcomes, analogous to multiscale interaction effects in other complex systems.

Importantly, the primary regulatory events responsible for a visible adaptive phenotype may occur in tissues distinct from those in which the phenotype is ultimately expressed. Consequently, understanding plant development and stress adaptation requires consideration of spatial organization, tissue geometry, mechanics, and multicellular interactions in addition to molecular regulation.

Here, we adopt a non–gene-centric perspective as a complement to established molecular regulatory frameworks. We propose that mechanosensing and cell–cell interactions contribute substantially to two classical root phenomena: root hair morphogenesis and vascular differentiation.

We suggest that root hair formation and stress-induced root hair remodeling do not arise solely from local gene expression within trichoblast, but instead emerge from differential cellular growth trajectories, cell-cycle dynamics, and geometric constraints imposed by neighboring tissues. In this framework, gene expression operates within preexisting mechanical and spatial contexts generated by coordinated multicellular growth.

Similarly, during vascular development, rapid radial expansion of metaxylem cells within a confined vascular cylinder may generate mechanical constraints affecting adjacent phloem tissues. These constraints correlate with nuclear remodeling, chromatin condensation, and phloem differentiation. Together, these examples suggest that growth heterogeneity generates mechanical and spatial contexts that contribute to coordinated cell-fate specification and adaptive responses in plant roots.

ESTABLISHING COLLECTION OF PETITE MUTANTS OF YEAST *SACCHAROMYCES CEREVISIAE* AND *S. PARADOXUS* FOR MODELING PLANT-FUNGI INTERACTIONS

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Yield losses due to damage to agricultural plants by pathogenic fungi are a problem of modern agriculture, which makes the study of the biology of fungal infection process an important task of modern science. The ability of the yeast *S. cerevisiae* and some closely related species to survive with significant damage to mitochondrial DNA (petit mutation) provides us with the opportunity to study the effect of mitochondrial DNA inhibition on infectious traits of fungi. Among them such are the formation of pseudohyphal growth, the ability to form complex colonies and biofilms, cell flocculation and their invasion into the nutrient medium, with the subsequent use of this information in the study of plant-fungal interactions. Using the collection of petit mutants of natural and laboratory strains of *S. cerevisiae* and the closely related species *S. paradoxus* allows us to model the state of fungal cells that have been treated with fungicides that inhibit mitochondrial respiration of pathogenic fungi.

To obtain rho^0 mutants, natural, laboratory and industrial strains of *S. cerevisiae* and strains of the closely related yeast species *S. paradoxus* were used, including those isolated from plant samples from the Chernobyl Exclusion Zone. They were isolated by double treatment of cells of the parent strain with ethidium bromide. Selection of petit mutants was carried out by loss of ability to grow on YPG medium containing a non-fermented carbon source glycerol. The loss of the mitochondrial genome was determined by the disappearance of mitochondrial nucleoids when analyzing DAPI-stained yeast cells using luminescent microscopy (Lumam-4 microscope).

The impact of mitochondrial DNA loss on the formation of traits characteristic to infectious stages of fungal growth was assessed. It was found that petite mutants lose the ability to form biofilms (mats)/ The formation of complex colonies and invasion into the nutrient medium was inhibited. The results obtained allowed us to identify the SK1strain of yeast *S. cerevisiae* as promising for modeling the dimorphic transition characteristic to infectious stages of some pathogenic fungi as well as the impact of mitochondrial DNA damage and the resulting respiratory disorder in mitochondria on this process.

Laboratory strains and their petits were used to study the influence of mitochondrial DNA status on the formation of adaptive regrowth due to appearance of adapted cell subpopulations under stress conditions and the restoration of active proliferation of cells that were in the stationary growth phase, which leads to the appearance of outgrowths on the surface of colonies. After prolonged cultivation on YPD, YP0,1D and YPD+G media, adaptive regrowth on petit mutant colonies was hardly recorded, unlike on parent strains colonies. On the contrary, on YPM medium we observed the formation of a small number of “warts” of adaptive regrowth on petits colonies. Our data indicate that the loss of mitochondrial DNA leads to a significant decrease in the adaptive response of yeast cells to changing living conditions by forming adaptive regrowth. Nevertheless, this ability remains at a low level in the population and can manifest itself under certain cultivation conditions.

Thus, we maintain and expand the collection of petit mutants of yeasts *S.cerevisiae* and *S.paradoxus*, which includes natural, laboratory and commercial strains. The collection will be utilized to model the impact of mitochondrial inhibition in phytopathogenic fungi on their interaction with plant tissues.

LAYERED DOUBLE HYDROXIDES AS NANOCARRIERS OF TRYPTOPHAN FOR PROMOTING PLANT GROWTH AND ENHANCING NUTRITIONAL STRESS TOLERANCE.

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Global crop production causes significant environmental impacts, including greenhouse gas emissions, biodiversity loss, intensive water consumption, and soil degradation[1]. As food demand continues to rise, interest in sustainable and efficient bioinputs has increased. Bio-based products such as amino acids, phytohormones, and plant growth stimulants are promising alternatives to synthetic agrochemicals, but their large-scale use is limited by low stability, rapid soil degradation, and poor bioavailability. In this context, combining modern technologies with agroecological practices could improve environmental sustainability and strengthen crop resilience to biotic and abiotic stresses. Tryptophan (Trp) is an essential amino acid and auxin precursor involved in plant growth, root development, and stress responses[2]. However, its agricultural application is limited because it rapidly degrades due to ultraviolet radiation, microbial activity, and chemical instability in soils. The encapsulation of Trp within Layered Double Hydroxides (LDH) nanostructures represents an innovative strategy to protect the molecule. The objective of this study was to develop and characterize LDH-based nanocarriers loaded with Trp and to evaluate their effects on plant growth promotion and nutritional stress tolerance. A Mg–Al LDH was synthesized via coprecipitation, followed by calcination and reconstruction in the presence of Trp to obtain the LDH–Trp nanohybrid through a memory-effect-driven rehydration process. The resulting material was characterized using X-ray diffraction, Fourier transform infrared spectroscopy, and zeta potential measurements[3]. The biological functionality of the LDH–Trp system was evaluated through a series of plant assays, including, nutrient deficiency experiments in lettuce and greenhouse trials in tomato plants (cv. Micro-Tom). Additionally, the effects of exogenous LDH–Trp application on the hormonal profile of tomato plants grown under greenhouse conditions were investigated. Under nutrient-deficient conditions, LDH–Trp treatment sustained lettuce growth by promoting changes in aerial biomass production and root system architecture. In greenhouse experiments, tomato plants treated with LDH–Trp showed higher aerial fresh and dry weights (14% and 17% respectively), as well as an increased the number and weight of fruits (20% and 6% respectively), compared with the controls treatments. The foliar application LDH–Trp produced differential effects on the hormonal content of tomato plants. Altogether, these results demonstrate that LDH-based nanocarriers are a promising strategy to improve Trp stability and delivery, enhancing plant growth, productivity, and stress tolerance, while contributing to the development of more sustainable agricultural practices.

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EXOGENOUS METHYL JASMONATE REGULATES PHOTOSYSTEM II EFFICIENCY AND METABOLIC REPROGRAMMING UNDER TERMINAL HEAT STRESS IN *BRASSICA JUNCEA* L.

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Terminal heat stress is one of the major constraints limiting productivity and physiological stability in oilseed crops including Indian mustard (*Brassica juncea* L.). The present study investigated the role of methyl jasmonate (MeJA) in alleviating heat stress-induced damage through modulation of photosynthetic pigments, chlorophyll fluorescence parameters, and metabolic biomarkers in contrasting mustard genotypes under field conditions. The experiment was conducted using two mustard genotypes differing in heat tolerance, namely Pusa Bahar (heat susceptible) and BPR-543-2 (heat tolerant), under timely and late sown environments during two consecutive growing seasons. Plants were treated with different concentrations of MeJA through foliar application at critical growth stages.

Heat stress significantly reduced chlorophyll a, chlorophyll b, and total chlorophyll content, indicating pigment degradation and impairment of the photosynthetic apparatus. However, exogenous application of MeJA substantially improved pigment retention under stress conditions. Chlorophyll fluorescence analysis revealed pronounced declines in maximum quantum efficiency of PSII (Fv/Fm), electron transport rate (ETR), and effective quantum yield of PSII [Y(II)] under late sown heat stress conditions, while non-photochemical quenching (NPQ) increased as a protective response mechanism. MeJA-treated plants exhibited enhanced Fv/Fm, ETR, and Y(II), along with regulated NPQ, suggesting improved photochemical efficiency and thermal energy dissipation. Comparative metabolite profiling further demonstrated distinct metabolic reprogramming under MeJA treatment. Enhanced accumulation of osmoprotective and antioxidant-associated metabolites, including amino acids, organic acids, phenolics, and stress-responsive secondary metabolites, was observed particularly in the tolerant genotype. Several metabolites exhibited strong association with improved photosynthetic performance under stress, indicating their potential utility as biomarkers for heat tolerance.

The findings demonstrate that MeJA-mediated physiological and metabolic adjustments contribute significantly to heat stress acclimation in mustard and may serve as an effective strategy for improving crop resilience under rising temperature scenarios.

IN SILICO SEARCH FOR POTENTIAL AMYLOIDOGENIC AND PRION-LIKE PLANT PROTEINS INDUCED BY X-RAY IRRADIATION

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Background and objectives. Amyloidogenic prion-like proteins were identified in plants as recently as 2016 [1]. In contrast to pathogenic animal prions [2], plant prion-like proteins are not implicated in disease processes and instead fulfill a range of beneficial physiological functions, including seed preservation and storage, stabilization of cellular homeostasis under stress, and antioxidant production. These properties make them promising candidates for use in agriculture, medicine and the development of novel biomaterials and biotechnological systems.

Previously, we hypothesised that radiation-induced senescence might result in proteomic reorganisation in plants by enhancing the accumulation of amyloidogenic and prion-like proteins in plant tissues [3]. To test this hypothesis, we studied plants grown from X-ray-treated pea seeds and found that several proteins were significantly more prevalent in irradiated plants than in the control group. This study aims to verify whether seed irradiation promotes amyloidogenesis and prionogenesis in the resulting plants by means of *in silico* analysis of this protein sample.

Materials and methods. A bioinformatic analysis was performed using the PLAAC (Prion-Like Amino Acid Composition) algorithm on a sample of 29 proteins that accumulated in excess in pea plants exposed to X-rays, compared to the control group.

Results and conclusions. The application of the PLAAC algorithm to 29 selected proteins identified four candidates that have the potential to be amyloidogenic or prionogenic: extensin 2 domain-containing protein, multiple organellar RNA editing factor (MORF), Argonaute RISC catalytic component 1 (AGO1) and Ole e 1-like protein. These proteins are functionally associated with cell wall biogenesis, RNA editing, RNA interference and vesicular trafficking, respectively. Notably, two proteins involved in RNA metabolism displayed the strongest prion-like propensity.

We also confirmed the expected relationship between prion-like behaviour and the intrinsic structural disorder of proteins. Ordered proteins, characterised by a positive folding index (FI), did not exhibit prionogenic potential. In contrast, disordered proteins with a negative FI were more likely to exhibit prion-like properties.

Overall, the *in silico* analysis suggests that proteins preferentially accumulated in plants derived from X-ray-irradiated seeds tend to exhibit elevated amyloidogenic and prion-like propensities. These properties may be a part of adaptive, stress-protective mechanism activated in response to irradiation exposure.

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COMPARATIVE ANALYSIS OF PHYTOACCUMULATION ABILITIES OF SEVERAL CROP PLANTS UNDER COMBINED HEAVY METAL CONTAMINATION

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Heavy metal contamination of agricultural soils has become a critical environmental issue, particularly in Ukraine, where military activities have caused significant soil pollution. In particular, huge amounts of heavy metals remain in the soil at the sites of military equipment combustion. Phytoremediation represents a cost-effective and environmentally friendly approach to soil decontamination, yet comparative data on crop species performance under combined metal stress remain scarce.

To conduct a pilot screening of eleven crop species common to Ukrainian agriculture for baseline metal tolerance and growth capacity under single and combined Pb^{2+}, Zn^{2+}, and Fe^{3+} contamination, as a first step toward identifying candidates worthy of further investigation for phytoremediation applications. As a supplementary objective, in silico optimization of the SpHMA2 metal transporter gene was carried out to adapt its sequence for subsequent GoldenGate cloning.

Eleven plant species were cultivated on Murashige-Skoog solid medium supplemented with individual and combined concentrations of Pb^{2+}, Zn^{2+}, and Fe^{3+}, starting at 500, 170 and 150 mg/L, respectively. These metal concentrations were selected because they represent the highest levels recorded at actual sites of military equipment combustion. Seed germination rate, vegetative growth, and linear dimensions were assessed. Heavy metal accumulation in roots and shoots was determined by spectrophotometric and atomic absorption spectrophotometric methods. Translocation factors (TF) were calculated to identify potential hyperaccumulators. Optimization of *SpHMA2* was performed using GeneOptimizer and Benchling software.

Combined metal contamination exhibited stronger phytotoxic effects than single-metal treatments. *Brassica napus* demonstrated the highest tolerance, maintaining germination efficiency above 88% across most Pb^{2+} and Zn^{2+}treatments, with TF values for Zn^{2+} reaching 17.1 at 42.5 mg/L. *Sorghum sudanense* in light-seeded form has also shown promising results, with TF for Pb^{2+} reaching 10.3. These findings align with published data: Abd-Elhady (2012) confirmed Sudan grass as an effective accumulator of Zn^{2+}, Cu^{2+}, and Pb^{2+}, while Angelova et al. (2017) and Szulc et al. (2014) demonstrated that *B. napus* maintains acceptable seed and oil quality even when grown on heavily contaminated soils, supporting its dual use for phytoremediation and biodiesel production. White clover and alfalfa exhibited moderate phytostabilization potential with TF < 1 for Pb^{2+}. Wheat, amaranth, and maize showed minimal or zero germination under combined contamination.

Brassica napus and *Sorghum sudanense* demonstrate the highest phytoremediation potential among the studied species and could be recommended for further trials on military-contaminated soils. The optimized *SpHMA2* sequence represents a foundation for generating transgenic *B. napus* lines with enhanced metal extraction capacity.

USNIC ACID AS A POTENTIAL BIOCONTROL AGENT AGAINST TOMATO MOSAIC VIRUS: EFFECT ON VIRAL DISTRIBUTION IN TOMATO PLANTS

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Tobamoviruses are important plant pathogens causing significant economic losses in agricultural production. Tomato mosaic virus (ToMV) is among the most widespread viruses infecting plants of the Solanaceae family. Due to the high stability of the virus, its easy mechanical transmission, and the lack of effective antiviral treatments, environmentally friendly strategies for controlling viral infections are increasingly required.

The aim of this study was to analyze the distribution of ToMV in different organs of tomato plants (Solanum lycopersicum L.) and to evaluate the inhibitory effect of usnic acid, a secondary metabolite of lichens, on the systemic spread of the virus. Tomato plants were experimentally infected with the ToMV SL-1 isolate and subsequently treated with two concentrations of usnic acid (2 μM and 20 μM). Analyses were performed on leaves, stems, fruits, and roots of infected plants.

The presence of the viral coat protein (CP) was detected by Western blot analysis and quantified using GelAnalyzer software. In addition, photosynthetic pigment content and molecular characterization of plant material were evaluated using PCR analysis.

The results confirmed the systemic spread of ToMV in all analyzed plant organs. The highest accumulation of CP protein was detected in leaf tissues, while the virus was also present in stems, fruits, and roots. Application of usnic acid reduced CP protein intensity in all analyzed organs compared with untreated infected plants. A stronger inhibitory effect was observed at the concentration of 20 μM, where no CP protein signal was detected during the second sampling. The results also indicated reduced systemic spread of the virus during advanced infection. Infected plants also showed changes in photosynthetic pigment content, while usnic acid partially alleviated the negative physiological effects associated with viral infection.

These findings suggest that usnic acid may represent a promising environmentally friendly biocontrol agent against tobamoviruses. Furthermore, the study provides new insights into the distribution of ToMV in individual tomato plant organs and highlights the importance of molecular and proteomic approaches in the study of plant viral infections.

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A WITHIN-CHAMBER CUTTING METHOD FOR REAL-TIME QUANTIFICATION OF LEAF WOUNDING VOLATILES AND GAS EXCHANGE

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Volatile organic compounds (VOCs) released by plant leaves play important role in plant–atmosphere interactions, stress signalling, and species-specific metabolic responses. Among these, wound-induced VOCs (wVOCs) are rapidly emitted in response to mechanical damage, herbivore attacks, or environmental disturbances such as strong winds or falling debris. These emissions include green leaf volatiles, methanol and terpenes. The kinetics of these releases are highly sensitive to the timing, severity, and method of tissue disruption. However, accurate quantification of VOC emissions following wounding remains technically challenging attributable to the time lag between injury and measurement, mechanical artifacts introduced during excision, and variations in environmental exposure.

The aim of the work was to present a standardized protocol for within-chamber leaf cutting, compatible with both attached and detached leaves, optimized for real-time monitoring of gas exchange and wVOCs. A surgical-grade cutter was integrated into the Walz GFS-3000 gas-exchange system to enable clean, controlled cuts within a sealed chamber under stable conditions of CO_2, light, humidity, and temperature. VOC emissions were continuously monitored using a proton transfer reaction time-of-flight mass-spectrometer (PTR-TOF-MS) positioned near the chamber outlet to minimize delays and signal distortion. This combined method allows precise tracking of emission onset, peak timing, maximum slope during emission rise, and total release. The protocol was demonstrated by using *Quercus rubra*, *Acer platanoides*, and *Gossypium hirsutum*.

As a result, this method revealed distinct species-specific differences in the magnitude and kinetics of wound-induced volatile bursts. Furthermore, it demonstrated the capability to capture the earliest phase of the response with high temporal fidelity. This protocol advances VOC research by overcoming major limitations of previous wound-emission studies, particularly delayed sampling and signal smearing. It is also broadly applicable to plant physiology, ecological biochemistry, and rapid stress-signalling research.

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ANALYSIS OF MICROCLONALLY PROPAGATED POPLAR AND WILLOW PLANTS EXPOSED TO AN EXCESSIVE LEVEL OF UV-B RADIATION USING ATR-FTIR MARKERS

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Ultraviolet B radiation is one of the key factors determining the course of metabolic processes in plants and their ability to maintain functional stability under stress conditions. This study analyzed the characteristics of the biochemical response of microclonally propagated plants of *Populus pyramidalis* × *P. laurifolia* 'Novoberlinska-3' and *Salix* sp. 'Zhytomyrska-1' following short-term UV-B irradiation at a dose of 17 kJ/m². Changes in primary and secondary metabolism, as well as in protein conformation, were assessed using mid-infrared spectroscopy.

The obtained data showed that both species responded to ultraviolet radiation, but the nature of these responses differs significantly. In poplar, an increase in the content of nucleic acids, lipid components, starch, and aliphatic fatty acid esters was observed at the initial stage. These changes were accompanied by an increase in the esterification index, indicating the activation of processes associated with lipid oxidation and the formation of reactive oxygen species. FTIR analysis demonstrated an increase in the ratio of amide I to amide II, which may indicate the emergence of degradation-resistant protein structures formed in response to stress. Concurrently, an increase in the proportion of phenylpropanoids was observed, reflecting the activation of the synthesis of protective phenolic compounds. At a later stage, a decrease in the content of structural polysaccharides and phenolic components was noted in the poplar, indicating a transition from an acute reaction to a phase of metabolic equilibration.

Willow, unlike poplar, responded much more rapidly and intensely. As early as the second day after irradiation, the content of lipids, carbohydrates, starch, as well as cellulose and hemicellulose in its leaves increased sharply. A decrease in the Amide I/Amide II ratio and a reduction in the proportion of proteins relative to nucleic acids indicated the suppression of protein synthesis and a reorientation of metabolism toward rapidly meeting energy needs. A significant increase in the proportion of phenylpropanoids confirmed the activation of secondary metabolism aimed at forming antioxidant defense. This response pattern is characteristic of plants that compensate for stress through rapid resource mobilization rather than structural stabilization.

A comparison of the two species revealed that the poplar employs a slower, structurally oriented adaptation strategy, whereas the willow exhibits a rapid, metabolically active response. Both species showed signs of stress tolerance, but the mechanisms underlying this tolerance differed significantly. The combination of spectroscopic and biochemical approaches allowed for the identification of species-specific adaptation markers, which may be useful for the further selection of promising *Salicaceae* clones for bioenergy, phytoremediation, and other areas of biotechnology.

TRANSGENERATIONAL MODULATION OF PHENOLIC SECONDARY METABOLITES IN CHAMOMILE FOLLOWING PARENTAL X-RAY IRRADIATION

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Introduction. Phenolic compounds, including flavonoids, serve as key secondary metabolites involved in plant antioxidant defense and stress adaptation. Previous studies have demonstrated that ionizing radiation, such as X-ray and UV-C, can modulate secondary metabolism in *Matricaria chamomilla* L. (Zhuk et al., 2021; Sokolova et al., 2024). However, the persistence and transgenerational stability of these radiation-induced effects remain insufficiently understood. The present study investigates dose-dependent and transgenerational changes in phenolic and flavonoid accumulation in two chamomile cultivars following parental (F0) X-ray irradiation.

Materials and Methods. Seeds of the F0 generation of *Matricaria chamomilla* L. cultivars "Perlyna Lisostepu" and "Goral" were exposed to X-ray irradiation at doses of 0, 5, 10, 15, and 20 Gy using an RUM-17 unit (200 kV, 10 mA, Cu filter 0.5 mm), following the methodology described in previous works (Zhuk et al., 2021; Sokolova et al., 2024). F0 plants were grown under field conditions in Lubny, Ukraine. F1 progeny were cultivated under controlled vegetation conditions. Total flavonoid content was determined by the aluminum chloride colorimetric method and expressed as mg rutin equivalents (RE) g^{-1} dry weight (DW). Total phenolic content was such as X-ray and UV-C, can modulate secondary metabolism in *Matricaria chamomilla* L. (Zhuk et al., 2021; Sokolova et al., 2024). However, the persistence and transgenerational stability of these radiation-induced effects remain insufficiently understood. The present study investigates dose-dependent and transgenerational changes in phenolic and flavonoid accumulation in two chamomile cultivars following parental (F0) X-ray irradiation.

Results. Control plants from different parental populations showed similar flavonoid levels, while total phenolic content exhibited higher variability. In line with earlier findings on non-monotonic responses to presowing irradiation (Zhuk et al., 2021; Sokolova et al., 2024), both flavonoids and phenolics in F1 plants demonstrated nonlinear, oscillatory dose–response relationships. In F1 plants of "Perlyna Lisostepu" (Botanical Garden, 2023 harvest), flavonoid content increased at 5 Gy (≈100 to 115 mg RE g^{-1} DW), decreased at 10 Gy (≈80 mg RE g^{-1} DW), and recovered at 15–20 Gy (≈115 mg RE g^{-1} DW). Similar oscillatory patterns were observed in "Goral" and other variants, though with genotype-dependent differences in amplitude and thresholds. Phenolic compounds displayed weaker but consistently non-linear responses. Notably, dose-specific maxima observed in F0 plants were not directly conserved in F1 progeny. In several cases, stimulatory effects in F0 were reversed in F1, indicating transgenerational reprogramming of secondary metabolism, consistent with epigenetic mechanisms previously reported for X-ray and UV-C exposure (Sokolova et al., 2024).

Conclusion. Parental X-ray irradiation induces stable but non-linear transgenerational effects on phenolic secondary metabolism in chamomile. The observed oscillatory dose–response patterns suggest complex feedback interactions between radiation-induced stress, antioxidant defense systems, and epigenetic reprogramming mechanisms across generations (Sokolova et al., 2024; Sokolova et al., 2024b). These findings expand our understanding of plant stress memory and long-term protective reactions triggered by ionizing radiation.

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CRISPRING BARLEY FOR SUSTAINABLE BEER PRODUCTION

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Beer has long been a popular refreshment, originally because clean water was scarce. Today, although water is no longer a limiting factor, crops such as barley face significant new challenges. Climate change and the spread of pathogens demand crop varieties with complex, locally adapted traits that ensure stable yields. Traditional breeding techniques—such as radiation mutagenesis and crossbreeding—have produced many successful varieties but are lengthy and labour-intensive. Barley, a diploid crop that thrives in harsh environments, is particularly amenable to genetic transformation and serves as a model for other cereals, such as wheat. Because tolerance to biotic and abiotic stresses depends on intricate intracellular processes, understanding the genetic networks of crops like barley is critical to future breeding strategies.

This talk will explore the use of functional genomics—specifically, gene inactivation via CRISPR/Cas—to identify gene functions relevant to stress responses. Targeted manipulation of genes involved in retrograde signalling and hormone regulation has yielded promising findings with practical applications. Several case studies will be presented, highlighting genes that influence light perception, pathogen resistance, and drought tolerance.

EPIGENETICS AND MOBILE ELEMENTS: HIDDEN TREASURES FOR CROP BREEDING?

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Plant genomes are much more dynamic than generally perceived. This is exemplified by the frequent occurrence of variants, for instance in clonally propagated plants such as apple and grapes. Regularly, one can observe variations in pigmentations in their fruits and leaves. These traits are easily detected due to their visual appearance.

This, however, implies that the vast majority of changes remain hidden and undetected, because they do not result in visible phenotypic changes. It could very well be that other important trait changes, such as disease resistance, remain undetected. Furthermore, genetic changes can be even more challenging to detect phenotypically in plants with polyploid genomes due to the higher number of alleles.

While mutations can come in many forms, important drivers of genetic and epigenetic changes are transposable elements (TEs). TEs are potent mutagens because they can cause a broad palette of changes to the genome: they move, copy and knock-out genes, they change gene expression patterns and can create new gene regulatory pathways. Because they are targeted by the host DNA methylation machinery, they can also create epigenetic diversity. Finally, epimutations (heritable changes in DNA methylation patterns) can also occur spontaneously.

In this presentation I will discuss how we have used transposable elements and induced epigenetic changes to create phenotypic diversity in model and crop plants (Arabidopsis, rice and wheat). I will show how experimentally induced TE mobilization can lead to the acquisition of drought tolerance and key traits in crops. Finally, I will provide an outlook on the use of induced TE mobilization and epigenetic changes for crop breeding.

BIOFORTIFICATION OF CROPS

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Global food production must undergo a major upheaval to feed a burgeoning human population despite multiple disruptors, ranging from environmental challenges that result from climate change to emerging geopolitical conflicts. A policy shift that focuses on the Bioeconomy could address these challenges for many nations. The following Presentation describes the potential of plant cellular agriculture technologies, including molecular farming and plant cell-based products, to meet those needs.

CUCURBIT ROOT TRANSFORMATION SYSTEM: A TOOL FOR STUDYING FUNCTIONAL GENES

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Cucurbit crops are economically important horticultural plants and excellent models for studying long-distance signaling and shoot–root crosstalk due to their accessible vascular saps and graft compatibility. However, functional genomic studies in many cucurbit species have been hindered by the lack of efficient and rapid genetic transformation systems. To overcome this limitation, an *Agrobacterium rhizogenes*-mediated root transformation system was developed for several cucurbit crops, including pumpkin, cucumber, melon, bottle gourd, and luffa gourd. This system enables the generation of healthy composite plants with fully transformed roots within six weeks, providing a practical platform for gene overexpression, CRISPR/Cas9-mediated knockout, subcellular localization, physiological assays, and stress-response studies.

The utility of this transformation platform was demonstrated through the functional analysis of key stress-responsive genes in cucurbits. In cucumber/pumpkin-grafted plants, CRISPR/Cas9-mediated editing of the pumpkin sodium transporter gene *CmoHKT1;1* disturbed Na^+ transport and increased salt sensitivity, confirming its role in limiting long-distance Na^+ movement from rootstock to scion. In addition, overexpression of the pumpkin tonoplast Na^+/H^+ antiporter gene *CmoNHX4* in cucumber roots improved salinity tolerance by regulating Na^+ and K^+ homeostasis. Furthermore, the same root transformation strategy was successfully applied to investigate the role of *CmoPIP1-4*, a plasma membrane intrinsic protein, in pumpkin drought tolerance. CRISPR-mediated knockout of *CmoPIP1-4* increased drought sensitivity by reducing hydrogen sulfide and abscisic acid levels, impairing antioxidant activity, altering stomatal behavior, increasing water loss, and damaging root architecture. Overall, this cucurbit root transformation system provides a rapid, reliable, and versatile tool for functional gene characterization. It is especially valuable for studying root-specific gene functions, abiotic stress tolerance mechanisms, and shoot–root communication in cucurbit crops.

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VARIABILITY OF NUTRITIONAL AND FUNCTIONAL PROPERTIES OF CEREALS IN RELATION TO YEAR AND TILLAGE SYSTEM

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Nutritional and functional properties of cereals result from complex interactions between genetic, environmental, and agronomic factors. This study evaluated the effects of conventional and conservation (no-till, minimum tillage, and mulching) farming systems on major nutritional components in three cereal species (spring barley, winter wheat, and maize) during a two-year field experiment (2023–2024).

The experiment was conducted at the Research Station in Borovce (NPPC Piešťany). Standard analytical methods were used to determine nutritional components: proteins (Dumas method), starch (Ewers polarimetric method), lipids (microwave-assisted extraction), total dietary fiber (enzymatic-gravimetric method) and *β*-D-glucans (McCleary method). All results were statistically evaluated.

The content of nutritional components varied considerably among cereal species, with protein content ranging from 6.85 to 13.45%, starch from 58.35 to 79.46%, and lipids from 1.89 to 4.14%, depending on crop species and year. Maize was characterized by the highest starch (79.92%) and lipid contents (4.14%), but lower protein content, whereas barley showed higher levels of dietary fibre (15.30%) and *β*-D-glucans (3.99%). Wheat exhibited intermediate values for most of the studied parameters.

Three-factor ANOVA identified crop species (F1) and year (F2) as the main factors affecting nutritional composition ($p < 0.05$). Variance component analysis indicated that crop species explained 73.7–99.3% of the variability, with the highest contribution observed for *β*-D-glucan (99.3%) and starch (93.6%) content. The effect of year was lower but significant, particularly for protein (15.7%) and fibre (11.6%) content. In contrast, the effect of the tillage system (F3) was limited, explaining only a small portion of the variability.

The most pronounced interaction occurred between crop species and year (F1×F2), explaining up to 12.3% of variability in lipid content and 11.5% in fibre content, indicating species-specific responses to environmental conditions. The interaction between year and tillage system (F2×F3) was relatively low, accounting for a small proportion of variability (0.0–1.1% for most parameters, with a slight increase to 7.5% for fibre).

Correlation analysis revealed strong relationships among parameters. A strong positive correlation was found between starch and lipids ($r = 0.874$), indicating their parallel accumulation. In contrast, protein content was negatively correlated with lipids ($r = -0.797$), fibre ($r = -0.665$) and *β*-D-glucans ($r = -0.606$). These relationships indicate differential accumulation of storage and structural components in the grain, primarily depending on crop species and year.

Overall, the results demonstrate that variability in cereal nutritional composition is primarily determined by crop species and environmental conditions, while the influence of tillage systems is less pronounced but may contribute to specific variations in grain composition depending on environmental conditions. A more precise evaluation of their effects will require analysis of data from additional years of the ongoing field experiment.

This work was supported by the research project “Potential for Risk Reduction in Sustainable Farming Systems” and project FPPV-60-2026.

THE ROLE OF PHYTOXINS PRODUCED BY PATHOGENS IN THE DEVELOPMENT OF DISEASE-TOLERANT TOMATO PLANTS

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A promising method for breeding disease-resistant plants is based on the use of protein and non-protein pathotoxins—which are typically synthesized by pathogens—as selective agents [1-3]. Using a method of co-culturing plants with infected tissues and pathogen zoosporangia, *in vitro* systems were developed for selecting cell lines and plants resistant to the tomato late blight pathogen. As a rule, most toxins have a single target in host cells, and the resistance trait is under simple genetic control. The feasibility of *in vitro* selection schemes used to select variants resistant to other metabolites, namely antibiotics and pathotoxins, has been demonstrated. The validity of cell-based selection schemes for resistance is determined by the mechanisms of toxin action. Some pathogens first infect cells and then release toxins. Others first release toxins that destroy cells and then use the products of their breakdown for nutrition. It is entirely natural that in the first case, there will be no correlation between *in vitro* and *in vivo* resistance; therefore, conducting selection at the cellular level will be appropriate only in the second scenario. It should also be borne in mind that resistance to toxins does not always correlate with resistance to disease-causing agents.

The experimental study found that the structures of the pathogenic fungus *Phytophthora infestans* (Mont.) de Bary with β-1,3 and β-1,6-glucosidic bonds in concentrations $5 – 15 \times 10^{-6}$ mg/l can be successfully used as selective agents in the cellular selection of tomato plants for resistance to this pathogen. It was also demonstrated that a change in the ratio of sterol groups—an important biochemical indicator—including during pathogenesis, alters the level of resistance to late blight in tomato plants obtained through cell-based selection for resistance to polyene antibiotics and sterol biosynthesis inhibitors.

As a result of the studies, by altering the expression of specific genes through silencing induced by modifying sterol metabolism, it was possible to isolate the TF-4 line, which have resistance to the tomato pathogen *Phytophthora infestans* (Mont.) de Bary.

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EFFECT OF KNOCKOUT THE CML30 GENE IN POTATO PLANTS ON RESISTANCE TO *R.SOLANACEARUM*

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The development of plant resistance to pathogens depends on many factors, among these are endogenous plant signalling components which inhibit or activate the immune response of the host. Calmodulin-like proteins (CMLs) are specialised Ca^{2+} sensors unique to plants, characterised by a plant-specific EF-Hand domain. In eukaryotic cells, calcium ions (Ca^{2+}) act as one of the most common second messengers. Calcium (Ca^{2+}) is a vital and dynamic component that acts as one of the key messengers in plant cell signalling processes. Fluctuations in cellular Ca^{2+} levels, caused by external factors or Ca^{2+} influx, can induce physiological changes that facilitate adaptive responses during growth, development, and under abiotic and biotic stress conditions. However, in some cases, CML genes may play a negative role in the formation of the immune response in plants and may be used as potential targets for knockout in molecular breeding. Our group studies the resistance and susceptibility mechanisms of potato plants with regards to *Ralstonia solanacearum* infections causing bacterial wilt and rot (Jose et al. 2023). By CRISPR-knockout, we already proved the importance of polyphenol oxidase genes in the resistance (Jose et al. 2026).

Here we present the results of genomic editing of the CML30 gene in *Solanum tuberosum*. A bioinformatics analysis was carried out to identify a potential negative regulator among potato CML genes. In order to find CRISPR-target within the chosen gene, CML30, a section of the sequence from four potato varieties (“Désirée”, “Balatoni Rózsa”, “Botond”, “DG-82-330”) was analysed. Genetic transformation and CRISPR/Cas9 genome editing of Désirée potato plants was carried out using an *Agrobacterium tumefaciens*-mediated method, employing pKSE401 plasmid harbouring a CML30-specific target. Molecular analysis was carried out using T7I endonuclease, which enabled the selection of lines for further study, whilst sequencing confirmed the genetic editing of the target gene regions (with the target focused on the first exon).

To date, knockout of the CML30 gene has been confirmed in eight lines. Further assessments of the plants revealed noticeable differences in stem morphology (increased length due to elongation of the internodes) in two lines compared with the control and other mutant lines in this category. In addition, a noticeable change in the colour of the tuber skin was observed, with the tubers taking on a more yellowish hue. A more detailed study revealed a reduction in anthocyanin levels in the tuber skin and leaves of these lines; changes were also detected in the anthocyanin spectrum of these two lines, specifically the absence of pelargonidin. An experiment was conducted to infect plants with the pathogen *Ralstonia solanacearum* under *in vitro* conditions. The ability of the bacterium to penetrate the plant and the severity of wilting or plant death were assessed. The mutants were found to be more resistant than the control plants. Experiments are currently planned to study mutant plants with a knockout of the CML30 gene when grown in soil.

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VISUALIZATION OF EARLY REGENERATION STAGES AND FORMATION OF CHIMERIC TISSUES IN TRANSGENIC ZUCCHINI USING THE RUBY REPORTER

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The RUBY reporter system, based on betalain biosynthesis, enables direct visualization of transformed cells without the need for fluorescence microscopy or exogenous substrates. The accumulation of a bright red pigment in living tissues provides a simple and non-destructive approach for monitoring transformation and regeneration processes. Previous studies have demonstrated the successful application of RUBY in various plant species [1]; however, its use in *Cucurbitaceae* species remains poorly investigated.

The aim of this study was to evaluate the potential of the RUBY reporter for real-time visualization of transformed cells during *Agrobacterium*-mediated organogenesis in zucchini (*Cucurbita pepo* L.) cv. Zebra, as well as to assess its applicability for detecting chimerism in regenerants.

Agrobacterium tumefaciens strain GV3101 carrying the 35S-RUBY construct was used for transformation. Cotyledon explants were cultured on MS medium supplemented with 1 mg/L 6-benzylaminopurine and 3% sucrose. A total of 50 explants were analyzed in three biological replicates. RUBY expression was assessed based on betalain accumulation, while transgene integration was confirmed by PCR analysis. The first signs of RUBY expression were observed 2–3 days after co-cultivation as intense red pigmentation of the explants. Such early signal detection suggests that RUBY efficiently visualizes primary transformation events and initial T-DNA expression prior to the formation of morphogenetic structures. Similar early expression patterns have previously been reported in regenerating tissues and callus cultures of other plant species [1]. During subsequent cultivation, both pigmented and non-pigmented shoots were regenerated. As organogenesis progressed, the pigmentation pattern changed. In some regenerants, initially uniform coloration gradually became restricted predominantly to vascular tissues of the leaves, followed by the appearance of pronounced mosaic patterns in both leaves and roots. No regenerants exhibiting stable uniform pigmentation across all tissues were obtained. The observed spatial heterogeneity of RUBY expression suggests the chimeric nature of the regenerated shoots. Most likely, shoot formation originated from multiple cell clones differing in transformation status, with only a subset carrying the integrated transgene. Such phenomena are characteristic of organogenesis in recalcitrant species, where shoot regeneration is initiated from multicellular origins. The observed mosaic pigmentation patterns may indicate the formation of periclinal or sectorial chimeras during shoot organogenesis [2]. Particular interest was associated with the preferential localization of pigment in vascular tissues. According to previous studies, such distribution may result from both tissue-specific activity of the CaMV 35S promoter and metabolic constraints affecting betalain accumulation in different cell types [3].

In conclusion, this study demonstrates the potential of the RUBY reporter for real-time visualization of transformed cell fate during zucchini organogenesis. To the best of our knowledge, this is the first report describing the application of RUBY for monitoring the spatial and temporal dynamics of transformation and chimerism formation in *Cucurbita pepo*.

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IMPROVING THE EFFICIENCY OF AGROBACTERIAL TRANSFORMATION OF *N. TABACUM* USING ABIOTIC STRESSORS

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Introduction. It is known that T-DNA integrates into the plant genome via various molecular mechanisms, including double-strand break repair. Our work is based on the idea of artificially inducing double-stranded DNA breaks through the action of stressful factors. This increases the number of sites available for T-DNA integration and activates reparative cell systems involved in integrating T-DNA or other DNA vectors into the host genome. Our **aim** is to develop an effective, reliable and simple method of genetically transforming plants using stress factors.

Materials and methods. In our experiments, we used explants of *N. tabacum* cv. Petit Havana, transformed using a standard *Agrobacterium tumefaciens* (strain GV3101) protocol with the vector constructs pCB063 and pCB064, which encode the synthesis of the ESAT-6 protein antigen of *Mycobacterium tuberculosis* (fusion protein ESAT-6-Ag85b), as well as the *nptII* gene. The presence of the heterologous gene insertion was confirmed by PCR analysis of regenerant leaves, which were sampled after 10 passages. The following stress factors were used to modulate the efficiency of transformation: 1. Heat shock (incubation of explants in a thermostat at +40 °C and +45 °C for 60 min prior to transformation); 2. Salt stress (explants were incubated for 30 min at +27 °C in 0.15 M and 0.25 M NaCl solutions prior to transformation, followed by washing with distilled water); 3. Oxidative stress: incubation of explants for 30 min at +27 °C in a 0.3% H_2O_2 solution, followed by washing with distilled water; 4. Acute ionizing radiation (doses of 7 Gy and 10 Gy at a dose rate of 53.4 Gy/h). The experiments were repeated three times.

Results and discussion. The experiment revealed only minor differences in the transformation efficiency of tobacco explants when using the pCB063 and pCB064 constructs. Therefore, we averaged the results. Taking into account the survival of regenerants, the transformation efficiency in the control group averaged 72%. Following acute irradiation at a dose of 7 Gy, the transformation efficiency increased to 96%; however, when the dose was increased to 10 Gy, it decreased to 46%. Incubating the explants in a 0.3% H_2O_2 solution significantly increased the transformation efficiency from 72% to 92%. As with ionizing radiation, a dose-dependent effect was observed when tobacco leaf explants were incubated with NaCl: at a lower concentration of 0.15 M, the transformation efficiency increased to 81%, whereas at a higher concentration of 0.25 M, it decreased to 63%. Heat shock at temperatures of +40 °C and +45 °C for 1 h reduced the efficiency of transformation to 56% and 40%, respectively. Our data show that stress factors can increase or decrease the efficiency of Agrobacterium-mediated transformation. Generally, those abiotic factors and their doses that activate the expression of proteins of the inducible double-strand DNA break repair systems exert a stimulatory effect (e.g. ionizing radiation at a dose of 7 Gy and treatment with 0.3% H_2O_2). Conversely, factors and doses that repress protein synthesis in host cells (e.g. heat shock, exposure to high concentrations of NaCl and threshold doses of ionizing radiation) reduce the efficiency of Agrobacterium-mediated transformation.

Conclusions. A relatively small change in the dose of acute ionizing radiation can lead to radically different outcomes in terms of transformation efficiency; therefore, the use of ionizing radiation as a stimulant for the integration of vector constructs into the plant genome appears to be methodologically unreliable and difficult to reproduce. From this perspective, treatment of explants with 0.3% H_2O_2 proved to be the most suitable abiotic stressor among those tested.

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EVALUATION OF *RUBY* GENE TRANSIENT EXPRESSION IN ORGANS OF *SOLANACEAE* SPECIES

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The aim of this work was to compare effectiveness of *Agrobacterium*-mediated transformation and evaluation of *Ruby* gene transient expression in tissues of *Physalis peruviana, P.ixocarpa* (variety Likhtaryk), *P. pubescence* (variety Zharynka), *Petunia* line M1K, *Petunia hybrida* line 5P, *Nicotiana tabacum* cv. Wisconsin, *Nicotiana benthamiana*.

Methods. *Agrobacterium tumefaciens* strain GV3101 carrying the 35S::Ruby genetic vector was grown in Luria-Bertani (LB) medium with antibiotics (50 mg/L rifampicin, 25 mg/L gentamicin, 50 mg/L spectinomycin) for 24 h with shaking. Agrobacterial suspension was centrifuged (5000 rpm, 12 min) and resuspended in ½ MS medium supplemented with 15 g/L sucrose (½ MS_{15}). Acetosyringone was added to a final concentration of 200 μM in 50 mL of *Agrobacterium* suspension.

Cotyledon explants from 7–10-day-old *in vitro* seedlings of *Physalis* species, and leaf explants from 21-day-old plants of *Petunia*, *N. tabacum*, and N. *benthamiana*, were co-cultivated with the *Agrobacterium* suspension for 30 min. Explants were then transferred to MS medium with 30 g/L sucrose (MS_{30}) without antibiotics for 24 h, followed by transfer to MS_{30} with 650 mg/L cefotaxime (to inhibit *Agrobacterium*) and 25 mg/L hygromycin (for plant selection). Petri dishes with control explants and with explants that were transformed were cultivated at 22–25°C, 3000–4500 lx, and a 14-h photoperiod. Transient *Ruby* expression was assessed visually; positive results were indicated by a color change from green to bright pink.

Results. Transient expression (pink color) was observed from the 3rd day; the maximum expression was observed on the 5th day after cocultivation of explants with *Agrobacterium*. The efficiency of transformation was determined by calculating the percentage of explants with areas stained pink from the total number of explants used in each variant of the experiment. The percentage of plants in the tissues of which transient expression was observed was for *N. tabacum* - 100%, *N. benthamiana* - 56.4%, *P. hybrida* line M1K - 79%, 5P - 26.3%, *P. peruviana* - 31.7%, *P. ixocarpa* (variety Likhtaryk) - 8.6% and *P. pubescens* (variety Zharynka) - 21.16%.

Conclusions. Therefore, the obtained results indicate that, the 35S:RUBY vector is suitable for use in experiments aimed at obtaining transient expression of marker transgenes and genes of interest in transformed plants.

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ENGINEERING INDUSTRIAL AND NITRITIONAL ISOPRENOIDS IN SOLANACEAE PLATFORMS

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Chemical refining is a highly efficient process that has driven industrialisation and globalisation. Carotenoid production using chemical synthesis has traditionally been the method of choice. However, dwindling fuel reserves and climatic fluctuation, are now imposing key societal and economic challenges to health and welfare provision, agriculture, manufacturing, and energy. This has resulted in the development of sustainable production platforms for carotenoids. At scale plants are one of the best biobased modes of production.

In this presentation the development of Solanaceae crops, particularly tomato for the production of the carotenoid's lycopene, beta-carotene and xanthophylls will be described using both metabolic engineering and introgression approaches from natural variation. Ketocarotenoids will be of particular focus showing industrial feasibility in aquaculture and as poultry feeds will be demonstrated [1]. Fundamentally, the multi-omic characterisation of these varieties has illustrated the metabolic re-programming required to obtain high level production [2]. In addition, the sequestration mechanisms available to store carotenoids elucidated through multiplex CRISPR approaches to elucidate the modification of plastids, plastoglobuli formation and esterification will be described [3].

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TARGETED ENGINEERING OF PLANT-MADE VOLATILES FOR SUSTAINABLE CROP PROTECTION

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Insect sex pheromones are among the most precise tools in sustainable pest management: species-specific volatile signals that can monitor and disrupt pest populations without chemical residues. Yet their widespread use is limited by the high cost of chemical synthesis. An attractive alternative is to engineer plants, natural masters of volatile production, to biosynthesize these compounds directly.

Our group has pursued this vision across several projects, demonstrating that *tobacco plants* can be engineered to produce and emit functional moth sex pheromone components at biologically active levels, and that synthetic gene circuits can tune pheromone output while managing the metabolic burden on the host plant.

Building on this, we recently identified TNDS, a novel irregular isopentenyl diphosphate synthase from *Lavandula* that synthesizes the precursor to *trans*-α-necrodyl acetate, a key pheromone component of *Delottococcus aberiae*, an invasive mealybug pest threatening European citrus crops. Tobacco plants expressing TNDS produced and emitted the active compound at levels sufficient to attract male insects under controlled conditions. Structural comparisons with related enzymes are beginning to reveal the molecular rules governing product specificity, paving the way for rational enzyme design and broader deployment of plant-based pheromone bioproduction.

THE EFFECT OF SELECTED ELICITORS ON TRITERPENOID PRODUCTION IN *CALENDULA OFFICINALIS* PLANTS AND HAIRY ROOT CULTURE

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In plant biotechnology, elicitation is considered a highly effective, controlled, and practically feasible strategy used for increasing the production and accumulation of valuable phytochemicals. It involves the induction or enhancement of biosynthesis of plant metabolites in entire plants, or – more often – in plant *in vitro* cultures, through the application of biotic or abiotic agents known as elicitors. The aim of the study was the comparison of the effects exerted by jasmonic acid and chitosan on triterpenoid production in two experimental models, *Calendula officinalis* hairy root cultures and greenhouse-cultivated plants (pot plants). *C. officinalis* (marigold) is a medicinal plant belonging to Asteraceae family, appreciated as a rich source of bioactive compounds suitable for pharmacological and cosmetic applications. This plant contains various steroids and triterpenoids, including oleanolic acid saponins exerting antibacterial and antiparasitic activity. In the experiments, *C. officinalis* pot plants and hairy roots were exposed to 100 μM jasmonic acid or 50 mg/L chitosan treatment during two weeks. Research involved the gas chromatography-mass spectrometry (GC-MS) qualitative and quantitative analyses of the composition and content of steroids and triterpenoids occurring in free and conjugated forms (esters and glycosides of sterols, as well as saponins, i.e., triterpenoid glycosides; the conjugated forms were analyzed after hydrolysis).

Jasmonic acid was selected as a plant stress hormone mediating plant defense response, widely used to enhance the production of specialized metabolites in plant *in vitro* cultures. The obtained results confirmed that jasmonic acid can be regarded as the strong and efficient triterpenoid biosynthesis elicitor - in the pot plants, the accumulation of saponins increased over two-fold, whereas in hairy roots, saponin accumulation in the biomass increased up to 86-fold, and saponin release into the medium increased up to 533-fold.

Chitosan is a biostimulant and biofertilizer commonly applied in organic agriculture to stimulate plant growth, abiotic stress tolerance and pathogen resistance. Surprisingly, in the present study the effect of chitosan was not as explicitly positive as expected. *C. officinalis* plants and hairy root cultures exposed to chitosan treatment displayed reduced biomass and altered steroid and triterpenoid metabolism, e.g., the biosynthesis of free forms of sterols (particularly the most abundant stigmasterol) was inhibited, the rate of sterol esterification increased, the content of triterpenoid (oleanolic and ursolic) acids was slightly enhanced (by approx. 80%), but the biosynthesis of triterpenoid saponins was negatively affected (by 20%).

Generally, the effects observed in hairy roots were more instantaneous and profound than in pot plants. In hairy root cultures, the influence of an applied factor is constant during the experiment, and the excess synthesized metabolites can more easily accumulate in the tissue or be secreted into the surrounding medium. Moreover, in the case of entire plants, various plant parts (i.e., shoots, roots) reacted differently to the same stressor. Thus, hairy root cultures may be a valuable model to track metabolic changes; however, direct extrapolation of study results to plants under field conditions can be misleading.

SMALL BUT DIVERSE: HIDDEN GENETIC COMPLEXITY IN DUCKWEED (WOLFFIA)

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Duckweeds are emerging crop plants with enormous, as yet untapped potential. They represent an ancient group of morphologically reduced aquatic monocotyledonous plants, divided into five genera. Recent studies have revealed phylogeny and genomic diversity within the genus Lemna and identified the potential for future domestication by crossing of different duckweed ecotypes (Stepanenko et al, 2025; Lee et al., 2026).

Over the last decade, the biological features of duckweeds have fuelled significant commercial interest in duckweeds, and also established them as a novel model for plant development, genomics, and stress biology. This requires an in-depth understanding of the genetic and physiological diversity of duckweeds. While the genus Lemna is well characterised (Morello et al, 2026), the genera Wolffiella and Wolffia remain unclear in terms of infrageneric phylogeny and taxonomic status of many accessions due to similar and partially overlapping morphological features.

In our study, we employed a novel and comprehensive approach, integrating flow cytometry, in situ hybridisation, and chloroplast and nuclear DNA markers. The combination of these methods was instrumental in the preliminary evaluation of the status of the duckweed genus Wolffia.

Genotyping Wolffia clones using direct sequencing of chloroplast spacers, 5S rDNA NTS and TBP sequence polymorphisms together with genome size and ploidy level revealed distinct species clusters and potential species complexes, including hybrids and their parental species. Genome size measurements revealed a high variability as well as tri- and tetraploids among the Wolffia accessions. Hybridity has been confirmed by chromosome counting and genomic in situ hybridisation (GISH) with nuclear DNA from potential diploid parental species as probes.

These results demonstrate the immense hidden potential of the genus Wolffia. Further research is required to understand its phylogenetic status and species differentiation, comprehensively, with implications for molecular taxonomy as well as for various applications in agriculture and biotechnology.

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INFLUENCE OF CRYOPRESERVATION AND MELATONIN PRIMING ON THE SEED GERMINATION AND DYNAMICS OF THE BULGARIAN ENDEMIC *PAPAVER DEGENII*

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Cryopreservation, the procedure of storing tissues at ultra-low temperatures (–196°C), offers numerous advantages over conventional seed banking methods for the long-term storage of endangered and rare plant species. On the other hand, priming is becoming an increasingly popular approach for improving seed germination. The application of both techniques, individually and in combination, on the Bulgarian endemic and vulnerable species *Papaver degenii* aims to investigate the possibilities for their application in the long-term conservation of the species. The objective of this study was to examine their influence on seed germination percentage and germination dynamics.

The seeds of *P. degenii* were collected from native plants of the species grown in Pirin Mountain, Bulgaria. Seeds were surface-sterilized and divided into three groups: (1) Seeds primed with melatonin (MT) at concentrations of 0.1, 0.5, 1 μM, and 1 mM for 24 hours and sown on B5 medium; (2) Seeds primed with MT (0.1, 0.5, 1 μM, and 1 mM) and immersed in liquid nitrogen (LN_2) for 7 days, thawed in a 40 °C water bath for 2 minutes and sown on B5 medium; (3) Seeds osmoprotected in a loading solution (B5 supplemented with 2 M sucrose) for 30 minutes, treated with PVS2 or PVS3 for 20 minutes and immersed in LN_2 for 7 days. For recovery, seeds were thawed in a 40 °C water bath for 2 minutes, unloaded in B5 medium containing 1.2 M sucrose for 30 minutes and sown on B5 medium. After sowing, all seed cultures were maintained at 12 ± 2 °C in the dark for 7 days and subsequently transferred to 23 ± 2 °C under a 16/8 h light/dark photoperiod. Non-primed (NP) and non-treated but cryopreserved (NTC) seeds were used as controls. Germination percentage (GP), Mean Germination Time (MGT), and Synchrony of germination were determined for all variants.

Primed seeds maintained a high GP across all tested MT concentrations, 95.73% on average, compared to 97.22% for NP. Increasing the concentration from 0.1 μM to 1 mM increased the MGT from 12 to 15 days. However, this delay in germination also resulted in more synchronized germination at the 1 mM concentration. Cryopreservation of primed seeds led to a decrease in GP and a decline in germination dynamics at the lowest and highest MT concentrations applied. Concentrations of 0.5 μM MT and 1 μM MT helped maintain germination and dynamics close to those of the NTC variant. Seed vitrification with PVS2 and PVS3 shortened the MGT and maintained high germination rates (94.4% and 97.6%, respectively), with the PVS2 variant also enhancing germination synchrony.

A dose-dependent effect of melatonin was established both in individual priming and in the cryopreservation of primed seeds. Vitrification of seeds in PVS2 and PVS3 showed the most promising results across all studied parameters for seed cryopreservation.

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IN VITRO BANKS AS A CONSTITUENT OF THE SYSTEM OF PLANT BIODIVERSITY CONSERVATION AND USE IN BIOTECHNOLOGICAL RESEARCH

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Plant biodiversity is preserved both *in situ* (in natural habitats) and *ex situ* (in different types of collections). *In situ* approach is conservation of species in their natural habitats which provide optimal growth conditions and possibilities of evolutionary changes. *Ex situ* approaches are considered as necessary components of the joint global system of biodiversity conservation. They provide a wide range of possibilities: from seed banks and living plant collections to application of methods of modern plant biotechnology (*in vitro* culture and cryoconservation).

Conservation in seed banks is relatively simple and economically efficient but cannot be used for a range of plant species, ex. if seeds are of limited longevity and do not withstand long-term storage, if seed dormancy is deep and difficult-to-regulate etc. In cases where seed storage is impossible or ineffective, the other methods should be used. Plant material can be stored *in vitro* either via regular subcultivations on culture media in aseptic conditions, or with using so-called "slow-growth" approach which is another option for subcultivations. Encapsulation of *in vitro*-derived explants is a field of research that is rapidly developing in recent years.

The report summarises more than 30-years' experience of maintaining and studying of plant large-scale *ex situ* collection of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine. Started in 1993 the object includes the seed bank, *in vitro* bank with its aseptic plants and cell lines, collection of extracts and corresponding data bases. Seed bank contains more than 7 thousands seed samples of 188 higher plants families. Seed samples are used as conservation units and for induction *in vitro* cultures (aseptic plants, callus and embryogenic cultures). *In vitro* collection contains wild-growing plants of different taxonomic groups including endangered species (Red Book of Ukraine, endemics) and species of practical interest, ex. those of potential pharmacological significance. They are used in different directions of research in the field of biotechnology (genetic transformation, hairy-root production, search for valuable secondary metabolites, green synthesis of nanoparticles etc).

IN VITRO BANK OF ICBGE: HOW WE (COULD) USE IT

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We try to use the in vitro bank of ICBGE in very different directions. Some of them are described in this talk. They include revealing new metabolites in callus cultures, investigations into the content of known biologically active substances, as well as ways to increase the biosynthetic potential of plants. They include the use of both "traditional" elicitors and so-called epigenetic ones. More recent directions of study using the in vitro bank are war-driven and include, for example, screening of plant species for their ability to accumulate heavy metals under combined pollution and their tolerance to polycyclic aromatic hydrocarbons.

For example, two new compounds have been found in the plants from our collection, with our direct participation; the chemical part of the work was performed by our collaborator, Prof. Sławomir Wybraniec (Cracow University of Technology). Both substances are betalains; one of them, malonyl-amaranthin and its isomers, was found in Celosia cristata. It is not surprising that such new compounds are found specifically in cell cultures and aseptic in vitro plants, as the metabolite profile in such cultures shifts very often toward a significantly larger number of polar compounds - suggesting that they are more highly glycosylated. In the case of betalains, this is exactly what can lead to the appearance of new substances that are not typical for intact plants.

Among the biologically active metabolites, the antidepressant substances of Hypericum - hypericins and hyperforins - were actively studied by us. Additionally, we use these substances to study the effect of elicitors on them, specifically methyl jasmonate. We offer a different way of elicitor treatment - the application of drops of the solution near the plant without direct contact with it. Such treatment is easy to implement both in greenhouses as well as in the field - one should only open a vessel with MeJA solution or apply its drops near the plants (the required volume depends on the number of treated plants and the density of their growth). It is a very simple and fast method of MeJA application. The results show that gaseous MeJA, applied by drops, strongly influences the hypericin content in H. perforatum shoots in a dose-dependent manner. It is also interesting that the most effective concentrations of MeJA are roughly comparable to the ones which exist and act on plants in nature.

Continuing the theme of searching for ways to unlock the biosynthetic potential of plants, we became interested in the idea of so-called epigenetic elicitors. One of such substances which we used was valproic acid, a histone deacetylase inhibitor. We observed a number of effects on seed germination, plant growth, content of recombinant protein, as well as low-molecular-weight compounds. Each of these effects, if taken individually, could be considered incidental or minor. However, taken together, they create a consistent and comprehensive picture of metabolic enhancement. This pattern aligns perfectly with the proposed mechanism of valproic acid as a histone deacetylase inhibitor, which is expected to lead to an overall upregulation of gene expression within the cell.

The primary objective of our war-driven screening is to identify plant species that can be effectively utilized for the remediation of small-scale areas contaminated with heavy metals and specific organic pollutants. Under the current circumstances, we are specifically focusing on locally contaminated zones, such as shell craters, sites of destroyed military equipment, residential gardens affected by fires, and areas surrounding burnt fueling stations.

PLANTS AS SOURCES OF PROTEASES WITH FIBRINOGENOLYTIC ACTIVITY

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Fibrinogen is an important plasma protein required for blood coagulation. Upon vascular injury, a series of reactions occurs that leads to the conversion of fibrinogen into fibrin. Fibrin molecules are capable of polymerization, resulting in the formation of a clot framework that prevents blood loss. However, during inflammatory responses, strokes, and myocardial infarctions, the concentration of fibrinogen in the blood increases significantly, thereby elevating the risk of thrombosis. The use of specific proteases capable of cleaving functionally active sites from the fibrinogen molecule represents a promising approach for the rapid and controlled reduction of fibrinogen levels in thrombotic complications. The study of such enzymes is also relevant for fundamental research. With the help of specific proteases, unique fragments of the fibrinogen molecule can be obtained, enabling investigation of the structure and functions of individual protein regions.

That is why the aim of this study was to search for proteases of plant origin with fibrinogenolytic activity for their potential application in medicine and basic research.

Plant extracts were provided by the Department of Genetic Engineering of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine. Aseptic plants used to prepare extract are maintained within the Germplasm bank of the Ukrainian and the world flora. They were cultured *in vitro* on Murashige and Skoog medium at 22 ± 2°C at 16-h photoperiod; the subculturing interval was 1.5 months. To prepare extracts, biomass was mixed with buffer (50 mM Tris-HCl, pH 8.2) and homogenized using a laboratory ball mill for 3 minutes. The extract was then centrifuged (14,000 rpm, 5 minutes), and the supernatant was collected. The presence of proteases with fibrinogenolytic activity was determined using zymography. Fibrinogen hydrolysis was carried out in 0.05 M Tris-HCl buffer (pH 7.4) containing 0.13 M NaCl at 37°C. The final substrate concentration in the reaction mixture was 2 mg/mL. Extracts were diluted 50-fold in the final mixture and incubated for 10–80 minutes. The specificity of protease action on the fibrinogen molecule was determined using polyacrylamide gel electrophoresis and Western blot analysis.

A total of 250 plant-derived extracts were characterized. Among the studied samples, eight were selected for their pronounced ability to degrade fibrinogen. These sources included leaves and roots of *Nicotiana tabacum* L., *Delosperma cooperi* (Hook. f.) L.Bolus, *Allium aflatunense* B.Fedtsch., *Hypericum tetrapterum* Fr., *Arnica montana* L., *Bletilla striata* (Thunb.) Rchb. f., and *Dendrobium linguella* Rchb. f. The most active fibrinogenolytic proteases were found in the extract of *D. linguella*, with molecular masses of approximately 55 and 65 kDa. It was found that proteases from this extract most specifically cleave the Aα-chain of fibrinogen, acting at its C-terminus.

The screening of plant extracts revealed promising sources of proteases with fibrinogenolytic activity. Particular attention should be given to *D. linguella* as the most active producer of fibrinogenases.

IDENTIFICATION OF AAE15/16 GENES IN THE PANGENOME OF CAMELINA FOR METABOLIC ENGINEERING

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Camelina sativa is a promising oilseed crop for the biotechnological improvement of seed fatty acid composition, particularly toward the accumulation of medium-chain fatty acids, which are valuable for the production of biofuel and biojet fuel components (Iskandarov et al., 2017). Among the potentially important targets for such improvement are the *AAE15/16* genes, which are associated with the activation of short- and medium-chain fatty acids and their reincorporation into the fatty acid biosynthesis cycle. In *Arabidopsis thaliana*, disruption of these genes has been shown to promote increased accumulation of 10:0 and other jet-fuel-type fatty acids (Tjellström et al., 2013).

In this study, we aimed to enhance the value of camelina seed oil for bio-jet fuel by introduction of seed-specific transgenes for a specialized a FatB thioesterase and a lysophosphatidic acid acyltransferase (LPAT) and diacylgylcerol acyltransferase (DGAT) from *Cuphea* species to produce oils enriched in medium-chain fatty acids (C10, C12, C14). Levels of medium chain fatty acids were further increased in camelina seed oil by RNAi-mediated silencing of genes for acyl-acyl carrier protein synthases that limit accumulation of these fatty acids. For the precise silencing of *AAE15/16* genes, a genome-wide search and comparative characterization of *AAE15* and *AAE16* genes in 12 *C. sativa* genome assemblies available in Phytozome has been performed. A stable set of genes was detected in all analyzed genome assemblies: three copies of *AAE15* and three copies of *AAE16* in each genotype. In total, 72 *AAE15*/*16* genes were identified across 12 genome assemblies of different *C. sativa* cultivars and accessions. This stable copy number is consistent with the allohexaploid nature of the *C. sativa* genome and indicates conservation of this set of homeologous genes after polyploidization. Analysis of structural organization also points to the overall conservation of these genes in the genomes of the studied genotypes.

Overall, our studies demonstrated the feasibility of engineering camelina, including a Ukraine-adapted variety, for production of oils with up to 30% of medium-chain fatty acids that are more readily convertible to bio-jet fuel than conventional camelina oil that is rich in C16-C22 fatty acids. The obtained results made it possible to establish a reference set of *AAE15/16* genes for *C. sativa*, providing a basis for further analysis of their expression, variability of intronic regions, development of molecular markers, and assessment of the role of these genes in shaping the fatty acid composition of seeds, as well as for the use of *AAE15/16* as targets for metabolic engineering. The conservation of *AAE15/16* genes across different *Camelina* genotypes is an important prerequisite for further targeted improvement of this crop as a source of oil with a valuable fatty acid profile suitable for the production of aviation biofuel.

This work was carried out within the framework of the research project of the Department for Targeted Training of Taras Shevchenko National University of Kyiv at the National Academy of Sciences of Ukraine "Metabolic engineering of the fatty acid composition of Camelina oil — an improved source for aviation biofuel production" (2026–2027) (State Registration No. 0126U003053).

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STUDY OF THE ANTI-RADIATION PROPERTIES OF SOME PHYTOEXTRACTS FROM AZERBAIJAN FLORA

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The Republic of Azerbaijan is rich in beneficial medicinal plants. Therefore, developing phytoextracts with protective properties based on them is highly promising. The aim of this work is to evaluate the radioprotective properties of the extract from the flowers of calendula officinalis (Calendula officinalis L.) and phytoextracts consisting of calendula officinalis (Calendula officinalis), dandelion officinalis (Taráxacum officinále) and yarrow (Achillea millefolium) obtained from medicinal flora of Azerbaijan.

The medicinal plants included in the collection were collected in the summer-autumn period. To study the radiobiological activity, extracts of calendula and a plant extract containing calendula, dandelion and yarrow were obtained. Dried and crushed plant masses were extracted with chloroform, followed by evaporation of the solvent.

To determine the presence of the radiobiological activity of the extracts obtained, a test was conducted for the 30-day survival of irradiated animals. In experiments, mongrel mice weighing 20– 25 g were used. Acute toxicity was determined when the test extracts were administered in aqueous solution once by the intraperitoneal route in full accordance with the generally accepted pharmacology method

The radiobiological experiment was carried out on mice that received a calendula extract and phytoextracts at a concentration of 300 mg / kg 30 minutes prior to irradiation at a dose of 9,0 Gy and fixed the lifetime of irradiated animals for a month. As a comparison of the radioprotective action, a radioprotector-standard cystamine and a control group of animals were used, which was replaced with distilled water instead of the radioprotector.

It was found out that the extract of calendula possesses radioprotective activity (55%), somewhat inferior to the activity of the protector-standard of cystamine (80%), and phytoextracts decreasing the lethal effect of ionizing radiation, has radioprotective activity (62%) and increases survival and life expectancy lethally irradiated mice more than the extract of calendula (55%) and less cystamine (80%).

The obtained results indicate that with the introduction of the extract of calendula, a significant increase in the life span of animals (almost 2-fold) occurs, although the survival after 30 days is somewhat inferior to that of a radioprotectorstandard. In the case of phytoextract, the lifespan of irradiated animals also increases, but the survival percentage after the end of the experiment – after 30 days – is greater than the extract of calendula. The data obtained by us testify to the prospects of using the extract of calendula separately and in a complex of extracts of dandelion and yarrow in the development of medicinal compositions intended for preventive purposes and in order to prevent local radiation injuries.

GENOME-WIDE IDENTIFICATION AND ANALYSIS OF THE CYTOKININ OXIDASE/DEHYDROGENASE (CKX) GENE FAMILY IN FINGER MILLET (*ELEUSINE CORACANA*)

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Cytokinin oxidase/dehydrogenase (CKX) enzymes play a key role in the regulation of cytokinin homeostasis in plants by catalyzing the irreversible degradation of excess cytokinins. Since cytokinins control meristem activity, shoot architecture, inflorescence development, stress responses, biomass accumulation, and grain productivity, CKX genes represent important targets for crop improvement and metabolic regulation. Downregulation or silencing of CKX genes has been shown to increase cytokinin levels in different plant tissues and may contribute to improved productivity-related traits. In finger millet (*Eleusine coracana*), a stress-tolerant and nutritionally valuable cereal crop, this effect was previously observed in the SE7 somaclonal line, in which attenuated expression of *EcCKX1* and *EcCKX2* was associated with altered cytokinin homeostasis, increased meristem activity, higher inflorescence number, and enhanced grain yield.

The aim of this study was to perform a comprehensive genome-wide identification and comparative analysis of the CKX gene family in finger millet and to connect these genomic data with the previously characterized cytokinin-related phenotype of the SE7 line. Functional CKX genes were identified in the *E. coracana* genome using known CKX sequences from model monocot and dicot species, including previously characterized *EcCKX1* and *EcCKX2*, followed by comparative analysis of coding sequences, protein domains, exon-intron organization, promoter regions, phylogenetic relationships, and syntenic conservation. Particular attention was given to the presence of conserved FAD- and cytokinin-binding domains, which are essential for CKX enzymatic function, as well as to the organization of cytokinin-responsive cis-regulatory elements in upstream gene regions.

The genome-wide analysis also clarified the relationship between the previously identified *EcCKX1* and *EcCKX2* genes and the broader CKX family of finger millet. These genes belong to different CKX groups but encode highly conserved homeologous copies and are among the most relevant candidates for explaining the SE7 phenotype. Earlier physiological and transcriptomic studies showed that SE7 plants accumulate higher levels of physiologically active isopentenyl adenine during early inflorescence development, while transcript levels of cytokinin-degrading enzymes are reduced in young leaves, seedlings, and initiating inflorescences. This attenuation of cytokinin degradation is associated with prolonged meristematic activity, increased numbers of inflorescences and seeds per plant, greater bushiness, enhanced biomass accumulation, and a shorter vegetation cycle.

Overall, the obtained results provide the first comprehensive characterization of the CKX gene family in finger millet and establish a genomic framework for understanding cytokinin-dependent regulation of productivity traits in this crop. The integration of genome-wide CKX identification with the SE7 somaclonal line data confirms that reduced CKX expression can be linked to beneficial changes in plant architecture and yield formation. These findings provide a basis for further functional studies of individual *EcCKX* genes, development of molecular markers, targeted gene expression analysis, and potential biotechnological or breeding approaches aimed at improving finger millet productivity, biomass accumulation, and adaptation to marginal agricultural environments.

IMPACT OF GREEN SYNTHESIS PROCESS CONDITIONS ON THE SILVER NANOPARTICLES CHARACTERISTICS

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The use of biotechnological approaches to obtain valuable secondary metabolites from plants and their subsequent application is a relevant issue today. Hairy root cultures can serve as factories for the production of biologically active secondary metabolites, such as phenolic acids, flavonoids, and stilbenes. The advantage of this approach is the synthesis of plant-specific compounds in quantities that can exceed those in the parent plants by a significant margin. The reducing activity of the obtained metabolites is of particular interest, as it can be used for the "green" synthesis of metal nanoparticles. The process of nanoparticle synthesis can be heavily influenced by the conditions of colloid solution preparation. Main parameters include reaction time, temperature, precursor concentration, reducing agent type, and the presence of stabilizing agents or surfactants. That is why the aim of this study was to investigate the influence of one of the main parameters of the synthesis process, namely its duration, on the characteristics of colloidal solutions of silver nanoparticles.

Three samples (70%) of plant extracts were used for the "green" synthesis of silver nanoparticles, namely ethanolic extracts of untransformed roots and two lines of "hairy" chicory roots carrying heterologous human interferon-α2b genes. Nanoparticles were synthesized by mixing 5 ml of an aqueous solution of 1 mM $AgNO_3$ (Sigma) with 400 µl of root extracts, as previously it was shown that a higher concentration (400 µl per 5 ml of $AgNO_3$ instead of 200 µl per 5 ml) brings better results – more efficient formation of nanoparticles. The parameter of interest was the duration of the synthesis; thus, after mixing the solutions, the mixture was heated in a water bath at 80°C for 1 or 2 hours. A characteristic change in the color of the colloidal solutions from colorless to brown was observed. Spectrophotometry of the samples in the UV-visible region of the spectrum confirmed the presence of characteristic peaks at 420–470 nm. In all samples, the peaks of the colloidal solutions obtained with longer heating were significantly higher. As well, it was shown that the peaks of the spectra of colloidal solutions of nanoparticles obtained using extracts from "hairy" roots were much higher than those of the control roots. Among the "hairy" root samples, the second line resulted in a more concentrated colloid solution, which may be linked to the differences in the extract composition used for the study.

The results of the UV-vis spectroscopy suggest that for the effective preparation of colloidal solutions of silver nanoparticles by "green" synthesis from extracts of chicory roots, it is advisable to use a longer heating time (up to two hours) of the mixture of extracts and $AgNO_3$ solution.

The work was carried out within the framework of the scientific and research work of young scientists of the National Academy of Sciences of Ukraine, "Features of the formation of silver nanoparticles by 'green' synthesis using chicory extracts".

ANTIOXIDANT AND ANTIMUTAGENIC EFFECTS OF AQUEOUS AND ALCOHOL *EXTRACTS OF ASTRAGALUS DASYANTHUS*

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This study was aimed to evaluate the mutagenic/antimutagenic and antioxidant effects of extracts of *Astragalus dasyanthus* grown *in vitro* on human lymphocytes using the comet assay.

Aqueous, ethanol and methanol extracts were prepared from dried *Astragalus dasyanthus* plants grown *in vitro*. The content of polyfructans in the obtained aqueous extracts was determined by the McRARY and Slattery method, based on the Selivanov reaction. The total flavonoid content in alcohol extracts was quantified spectrophotometrically at a wavelength of 419 nm. Human lymphocytes were isolated from the peripheral blood of healthy donors by rate-zonal centrifugation and then incubated in RPMI 1640 medium with extracts for 2 hours at 37 °C. As a control, cells were incubated in the same medium without extracts. To induce DNA damage, the Fenton reaction was performed: the lymphocyte suspension was incubated with RPMI 1640 medium, 0.75 mM $FeSO_4$ and 0.75 mM H_2O_2 for 2 hours at 37 °C. Additionally, an aliquot of lymphocytes was subjected to the Fenton reaction in the presence of methanol extracts under the same conditions. To perform comet assay, cells were immobilized on microscope slides in a layer of 1% low-melting agarose and lysed in a high-salt buffer with detergent, after which electrophoresis was performed in the TBE buffer (pH 7.5) for 10 min at 4 °C and the electric field strength of 1 V/cm. After electrophoresis, 100–200 randomly selected cells were analyzed using CometScore software. To evaluate the mutagenic/antimutagenic and antioxidant effects of *Astragalus* extracts, the relative DNA amount in the comet tail was measured.

We established that the content of water-soluble sugars in aqueous extracts was 47.72±2.08 μg/μl, while the flavonoids content in methanol and ethanol extracts was 33.57±2.53 μg/μl and 22.15±1.26 μg/μl, respectively. These results indicate effective accumulation of these substances in plants grown *in vitro* and confirm the validity of the selected extraction protocols.

Incubation of cells with aqueous extracts at a concentration of 6 μg/μl led to a statistically significant increase in the relative number of single- and double-stranded DNA breaks in cells, whereas lower concentrations did not increase the level of DNA damage compared with the control. This may indicate that aqueous extracts of *Astragalus dasyanthus* exhibit a weak but pronounced genotoxic effect. Ethanol and methanol extracts at a concentration of 3 μg/μl demonstrated antimutagenic and/or reparogenic effects, reducing the level of DNA damage in lymphocytes almost threefold (to 2.17±0.39% and 1.86±0.1% for ethanol and methanol extracts, respectively) compared with untreated lymphocytes (5.7±0.47%).

The Fenton reaction was used to evaluate the antioxidant activity of the methanol extract. It was found that incubation of cells with H_2O_2 and Fe^{2+} at concentrations of 0.75 mM resulted in an almost twofold increase in the level of DNA breaks compared with the control. At the same time, incubation of cells in the presence of methanol extract (3 μg/μl) significantly reduced the level of induced damage to 3.74±0.31%, indicating pronounced antioxidant activity of this extract.

INDUCING *IN VITRO* REGENERATION FOR OVERCOMING RECALCITRANCE IN WOODY PLANTS

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Recalcitrance is the biological resistance/inability of plant cells or tissues to regenerate *in vitro* is often related to genetic factors (McCown 2000). Woody plants with a strongly episodic type of growth have low proliferative activity, so it is very difficult to achieve uniform, continuous growth of microshoots *in vitro* (McCown 2000). With age, the regenerative ability decreases, so it is difficult to reproduce old plants (Chmielarz et al. 2023). According to Nelson et al. (2025), to overcome recalcitrance, it is necessary to activate regeneration genes. For this purpose, it is necessary to develop individual procedures for stabilizing the growth of *in vitro* plant tissues. In previous studies, microshoots were obtained from 120-year-old English oak (*Quercus robur* L.), 50-year-old Norway maple (*Acer platanoides* L.) and 30-year-old large-leaved linden (*Tilia platyphyllos* Scop.), which have a low regeneration ability. The aim of this study is to induce regeneration of *Q. robur, A. platanoides* and *T. platyphyllos in vitro* explants for micropropagation.

For the studies, 1.0–1.5 cm nodal stem segments from aseptic *Q. robur, A. platanoides, T. platyphyllos* plants were used. The studies were carried out in July. A two-stage stabilization procedure was used. At the first stage, the explants were cultivated on the basic hormone-free Woody Plant Medium (WPM) (McCown & Lloyd 1981) in Petri dishes (8–10 pcs.) in a horizontal position at a temperature of +4±1°C in the dark for 3 days. At the second stage, they were cultivated in a vertical position on WPM with 6 consecutive subcultivations for 18 days. A medium supplemented with 2.0 g/l activated charcoal and 2.0 mg/l povidone was used (3 days); thereafter the explants were subcultured on medium supplemented with 0.25−0.50 mg/l kinetin (3 days); the medium was changed every 3 days for 18 days. All nutrient media were supplemented with 100 mg/l inositol, 30 g/l sucrose, and 7.0−7.3 g/l microbiological agar, the pH adjusted to the level of 5.7–5.9. The plant material was cultivated in a laboratory room at a temperature of 24±1 °C and illumination of 2.0−3.0 klx with a 16-hour photoperiod and a relative humidity of 70–75%.

According to the specified cultivation procedure of nodal stem segments a survival rate was 90–100 % on day 6. The percentage of proliferated *Q. robur* explants was 90 %, *A. platanoides* – 80 %, *T. platyphyllos* – 70 % on day 15. The regeneration rate of *Q. robur* explants was 80 %, *A. platanoides* – 60 %, *T. platyphyllos* – 50 % on day 30. The regeneration rate of explants was 10–20 % without the stabilization procedure (control) on the 30th day of cultivation. Microshoots had typical anatomy, morphology and pigmentation, no vitrification were detected. 60 % of *Q. robur* and 70 % of *A. platanoides* microshoots produced callus tissue of dense consistency and different pigmentation (light green/green/cream) on the 45th day of cultivation. Callus grew at the base of the microshoot. *T. platyphyllos* microshoots did not produce callus. Therefore, the two-stage stabilization procedure can overcome recalcitrance and provide actively growing microshoots of *Q. robur, A. platanoides, T. platyphyllos* for further studies.

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A MODEL SYSTEM FOR STUDYING RHIZOSPHERE EFFECTS IN PLANTS

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Higher plants are involved in complex interactions with other organisms in biocenoses—viruses, bacteria, fungi, plants, animals, and soil algae. Algae protect the soil from erosion and enrich it with nitrogen and various substances, including biologically active ones. Algal colonies increase the soil's water-holding capacity, and cells that naturally die off during ontogenesis become fertilizer, supplying the plant root system with beneficial organic substances. Algae, in turn, can obtain organic substances and specific secondary metabolites from plants for use in their life processes. This is confirmed by the biocenotic species-specificity of the coexistence of certain taxonomic groups of higher and lower plants and microorganisms. To support photosynthesis, algae require a daylight intensity of 10^{-3} to 10^{-6}, depending on the taxonomic classification of the species. Light of this intensity can penetrate the soil to a depth of a few millimeters. The abundance of algae in the immediate vicinity of the root hairs of higher plants is always higher than in soil particles that do not come into contact with the roots. Thus, higher plants can potentially provide algae with light and thereby support their active growth.

The objective of study was to identify a strain of soil algae symbiotic with *Phaseolus vulgaris* L. for use in subsequent model experiments. During the work, the effect of co-culturing two species of soil algae – the obligate photoautotroph *Chlamydomonas moevusii* and the mixotroph *Gleospheridium* sp. – with bean plants was investigated. The algae were grown in a liquid nutrient medium (3 MBBN) at a concentration of 10^5 cells/ml and added to the soil placed in glass jars covered with light-blocking foil. Then, a 10 cm soil layer was added to the jars. Germinated bean seeds were placed in the top layer of the soil to a depth of about 2 cm. The study revealed differences in bean growth parameters in both treatments compared to the control after 3 vegetation weeks. A stimulating effect of the algae *C.moevusii* on the growth parameters of the aboveground parts of beans and an inhibitory effect of the *Gleospheridium* sp. were identified. A difference in the accumulation of beans biomass between two experimental variants was determined after 5 vegetation weeks. The inhibitory effect of *Gleospheridium* sp. algae on beans growth led to a ≈70% reduction in biomass gain and a ≈1.5-fold increase in the average biomass of plants when co-cultured with *C.moevusii* algae compared to the control. Bacteria and fungi obtained data show coexist in culture with *C.moevusii*. Three strains of filamentous fungi and 11 strains of bacterial cultures were isolated. The fungi were identified as *Geotrichum candidum*, *Mycelia sterilia* (white), and *Penicillium* sp.

Thus, a model system for further studies of the role of rhizosphere light in the coexistence of soil algae and higher plants was obtained.

ENGINEERING OF NEW IRREGULAR TERPENE BIOSYNTHETIC PATHWAYS IN *NICOTIANA BENTHAMIANA*

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Natural plant terpene biosynthesis delivers a vast variety of structures, but its diversity can still be increased by introducing new enzyme activities. Formation of terpenes starts by joining activated C5 isoprene precursors catalysed by isoprene diphosphate synthases (IDS). All living organisms contain regular IDS building linear chains of different length by head to tail coupling. These basic structures are rearranged and modified by subsequent enzymes, most notably terpene synthases and cytochromes P450. In rare cases, branched or cyclized skeletons are formed directly by head to middle coupling performed by irregular IDS. Irregular monoterpene molecules have a variety of valuable functions, acting as pheromones and insecticides, and demonstrating cytotoxic and antibiotic properties. By integrating irregular IDS into metabolic networks of heterologous hosts, we can potentially initiate new biosynthetic pathways and expand the repertoire of irregular monoterpene structures.

Transient expression of three IDS that form branched and cyclic monoterpenes in *Nicotiana benthamiana* resulted in formation of six major new components, 6-*O*-malonyl-*β*-D-glucopyranoside and 6-*O*-malonyl-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside derivatives of chrysanthemol, lavandulol and cyclolavandulol. Alleviating the bottlenecks in the biosynthesis of dimethylallyl diphosphate (DMAPP), a substrate for irregular IDS enzymes, in plastids and cytoplasm allowed for a substantial increase in the accumulation of new metabolites. Consequently, we achieved the level of cyclolavandulyl glucosides up to around 4 mmol/g FW. This yield represents the highest amount of irregular monoterpenes produced in plant systems.

To enlarge the spectrum of irregular monoterpenes further, we included in the engineered pathways cytochromes P450 from human liver (hCYPs). These enzymes are responsible for detoxification of xenobiotics and therefore display broad substrate specificities. Two hCYPs converted branched and cyclopropane monoterpenes into their hydroxylated derivatives. Although hCYPs do not require obligatory co-expression of cognate redox partners to display their activity *in planta*, addition of human P450 reductase improved the accumulation hydroxylated products.

We established a sustainable platform for the efficient plant-based production of valuable irregular monoterpenes in non-volatile glycosylated form. Using a network of heterologous genes derived from different groups of organisms allowed us to initiate new biosynthetic pathways and expand the range of irregular monoterpene structures available.

IMPROVEMENT OF *IN VITRO* MICROPROPAGATION OF *SIDA CORDIFOLIA* L. PROMISING FOR BIOTECHNOLOGICAL PRODUCTION OF SECONDARY METABOLITES

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In vitro biotechnological approaches represent effective strategies for enhancing the production of plant secondary metabolites and optimizing their biosynthesis under controlled conditions (Selwal et al., 2023). Efficient micropropagation systems provide a basis for such studies by ensuring the availability of uniform and sufficient plant biomass. *Sida cordifolia* L. is a medicinal plant known for its diverse pharmacological activities, including anti-inflammatory and antimicrobial effects (Kumar et al., 2024). Phytochemical studies have identified a wide range of bioactive secondary metabolites, particularly alkaloids such as ephedrine and pseudoephedrine, as well as flavonoids. However, *in vitro* propagation of *S. cordifolia* is often constrained by limited shoot and root elongation, which restricts the efficient production of uniform plant material for biotechnological applications.

This study aimed to improve shoot and root elongation of *S. cordifolia* from two-node explants by simple modifications of Murashige and Skoog (MS) medium. A comparison of full- and half-strength MS media revealed that half-strength MS medium (1/2MS) significantly enhanced shoot and root elongation (3.9-fold and 4.2-fold, respectively) compared to full-strength MS. Further treatments included supplementation of 1/2MS medium with KH_2PO_4, plant growth regulators (TDZ, BAP, 2iP, GA_3), or pretreatment of explants with Norit buffer.

KH_2PO_4 significantly improved shoot and root elongation compared to MS medium, with the highest effect observed at 170 mg/L. Pretreatment with Norit buffer produced a comparable stimulatory effect on both shoot and root growth. In contrast, the addition of cytokinins and GA_3 inhibited elongation compared to hormone-free medium. Overall, the results indicate that hormone-free half-strength MS medium supplemented with 170 mg/L of KH_2PO_4 was effective for the elongation of shoots and roots from nodal segments. This simple modification of basal MS medium enables efficient regeneration of plant material and provides a suitable platform for *in vitro* plant production, followed by secondary metabolite production. In addition, this biotechnological approach can contribute to solving another problem of this species – the need to protect the biodiversity of this medicinally used plant.

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PLANT CELL, TISSUE, AND ORGAN CULTURES: DEVELOPMENTS AND APPLICATIONS WITHIN OUR RESEARCH ACTIVITIES

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Plants are a source of food for direct consumption and of raw materials for producing food for humans. They are also a reservoir of chemical substances with a wide range of applications. These substances are used not only in the food and feed industry but also in industrial sectors such as chemicals, pharmaceuticals, cosmetics, woodworking, energy, and others, as well as in medicine and agriculture. The growing demand for plants and their chemicals means more of them need to be grown in fields. However, environmental, climatic, social, demographic, civilisational, and other factors present barriers to meeting these demands. The greater demand for plants and their chemicals requires more of them to be cultivated in fields. For some plant species, therefore, alternatives are sought "out of the field", in *in vitro* systems. In sterile conditions, it is not necessary to grow whole plants (for example, by micropropagation), but only their organs, tissues, or cells. In addition to a wide range of other applications, such *in vitro* cultivation systems are particularly well-suited to producing cell biomass, plant stem cells, and metabolites, especially secondary metabolites. These areas of research are current trends in plant biotechnology with significant application outcomes.

The research programme of our "*Plant in vitro cultures group*" is focused on the cultivation of plant organs, tissues, and cells under *in vitro* conditions. It encompasses micropropagation, callus cultures, cell suspension systems, and root cultures, as well as the establishment of plant stem cells and the production of valuable biomass and bioactive secondary metabolites. The main objective is to develop reliable, efficient, and scalable protocols that consider different types of explants, nutrient media compositions, phytohormone balances, and cultivation conditions.

Current research addresses a wide range of medicinal, aromatic, ornamental, and industrial plant species, particularly those containing pharmacologically and biocidal active compounds. The group works with various *in vitro* plant cultivation systems, such as callus, cell suspension, and root cultures, in a wide range of medicinal, aromatic, ornamental, and industrial plant species (*Datura stramonium* L., *Calendula officinalis* L., *Chelidonium majus* L., *Lavandula* spp., *Sida* spp., *Tagetes* spp., *Sempervivum tectorum* L., and *Cannabis sativa* L.). Special emphasis is placed on the isolation and propagation of plant stem cells or stem cell-like cells, representing an innovative strategy with the potential for stable, uniform, and highly efficient production systems. These cultures are also being assessed for their biotechnological potential to generate natural compounds for the pharmaceutical, cosmetic, nutraceutical, and agricultural sectors. The results of this research group have been published for several plant species – *S. hermaphrodita*, *C. officinalis*, and *D. stramonium* (Kaňuková et al., 2022, 2024, 2025).

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BIOTECHNOLOGICAL PRODUCTIVITY OF THE UV-15 STRAIN OF *UNGERNIA VICTORIS* TISSUE CULTURE

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Ungernia victoris Vved. ex Artjushenko is a rare medicinal plant belonging to the Amaryllidaceae family that is endemic to the mountains of Tajikistan and Uzbekistan. The pharmacological value of the species is due to the accumulation of isoquinoline alkaloids galantamine, lycorine and their derivatives; as well as coumarins, polysaccharide complexes and other biologically active compounds (BAC). Preparations based on *U. victoris* extracts have a normalizing effect on the central and peripheral nervous systems [1].

Since 1995, we have established and maintained a tissue culture of *U. victoris*, the extracts of which exhibit a wide range of pharmacological activities: antimutagenic, radioprotective, general stimulating, regenerating and antitumor [2]. Through long-term cell selection, the strain UV-15 was derived from the initial UV-2 strain.

The aim of the work was to investigate the biotechnological characteristics of the UV-15 strain of *U. victoris* tissue culture, and to assess the content of BAC in cellular biomass.

Callus was cultured on a specially developed solid hormone free medium 5C [3] in 380mL glass jars filled with 50mL of medium. The subculture duration was 45–50 days for tissue culture propagation and 55–65 days for harvesting biomass. To determine the yield of wet and dry biomass in a subculture cycle, wet and dry tissue were weighed, respectively, when transplanted onto fresh medium and every 10 days for 90 days.

The content of water-soluble mono- and polysaccharides in extracts from dry cell biomass was examined using the method according to DSTU5059:2008. The content of polyphenols was determined using Folin-Ciocalteu method in mg rutin equivalent. Chromatographic screening of extracts was performed using an Agilent 1260 Infinity II HPLC-MS.

The UV-15 strain tissue culture was heterogeneous, consisted of slightly friable aggregates ranging in size from 0.1 to 0.8cm and was yellow to light yellow in color. The growth type was unorganized, the growth index was 6-7. The maximum yield on the 60^{th} day of subculture was 384.4g/L of medium for wet biomass and 20.8g/L for dry biomass. The content of dry biomass in the wet in the stationary phase of growth was 5.4–7.8%.

The polyphenol content in the dry biomass extract was 0.031%w/w. The monosaccharide content in the wet biomass was 0.11%w/w, while the hydrolysable polysaccharide content was 0.47%w/w. The content of water-soluble polysaccharides in terms of dry mass was 4%. Chromato-mass spectrometry revealed the most intense peaks at m/z 288 (ion $[M+H]^+$galantamine, lycorine or their isomers), m/z 183 (ion $[M+H]^+$harmane or isomers), and m/z 166 (ion $[M+H]^+$hordenine or isomers).

Thus, the UV-15 strain of *U. victoris* tissue culture is highly productive. The cell biomass accumulates polyphenols, mono- and polysaccharides, as well as compounds that are likely to be derivatives and/or isomers of galantamine or other alkaloids, according to ESI mass spectra. These compounds require further detailed study.

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FEATURES OF BIOMASS GROWTH AND NAPHTHOQUINONES ACCUMULATION IN THE CELL LINE 3Ep OF *ECHIUM PLANTAGINEUM* TISSUE CULTURE

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Echium plantagineum, a medicinal plant belonging to the Boraginaceae family, contains phenolic compounds, terpenoids, and naphthoquinones, including shikonin and its derivatives. The presence of these biologically active compounds (BAC) gives the plant wound-healing, antibacterial, fungicidal, and anti-inflammatory activities [1]. Given the lack of industrial sources of shikonin in Ukraine, *E. plantagineum* tissue culture could serve as an innovative source of BAC with wound-healing properties.

The aim of the study was to investigate features of biomass growth in the cell line 3Ep of *E. plantagineum* tissue culture and the content of naphthoquinone compounds in the cell biomass.

The starting material was the cell line 3Ep of root origin [2], which had been cultivated for 5 years on the Linsmaier and Skoog medium modified for enhanced naphthoquinone synthesis. Cell biomass was cultivated in 210mL glass jars filled with 40mL of the medium in the dark, at a temperature from 24.5°C to 27.0°C and relative humidity of 70–80%. The subculture duration was 21 days. To determine the wet biomass yield, the callus was weighed during subculturing onto fresh medium and every three days thereafter for a period of 34 days. To determine the dry biomass content, the tissue was dried for 24 hours at 55°C and 56% ambient humidity. The dry matter content in the wet biomass was determined after drying as relative to weight of the original wet biomass of the tissue culture. The content of naphthoquinone compounds was studied in ethyl acetate extracts from dry biomass using an Agilent 1260 Infinity II high-performance liquid chromatograph with mass spectrometric detection.

The tissue culture of the cell line 3Ep consists of dedifferentiated cells that exhibit no signs of morphogenesis, has a brownish-red color and fine-grained conglomerates. The growth index was 3–4. The growth curve of the culture was S-shaped, reaching the stationary phase on the 14th day of subculture. The maximum wet biomass yield was observed on the 21st day of subculture, reaching 148.5g/L of medium, while the dry biomass yield was 9.2g/L. The dry matter content in the wet biomass during the stationary phase ranged from 6.2 to 6.7%.

Shikonin and its derivatives: acetylshikonin, isovalerylshikonin, and hydroxyvalerylshikonin were identified in the *E. plantagineum* tissue culture extracts. The total content of naphthoquinone compounds was estimated at 2.11–2.43% of the dry weight, which is several times higher than in the roots of natural plants, which accumulate up to 0.45% of naphthoquinone compounds according to literature [3].

It was found that during subculture, the line 3Ep of *E. plantagineum* tissue culture accumulates 148.5g/L of wet biomass and 9.2g/L of dry biomass, which contains 2-3% shikonin and its derivatives, which significantly exceeds the indicators of wild-grown plants and confirms the potential of its use in the pharmaceutical and biotechnological industries.

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IMPACT OF VALPROATE ON GROWTH AND BIOCHEMICAL CHARACTERISTICS IN OILSEED RADISH AND RAPESEED

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Plants are an important source of valuable compounds for humans, including both primary and secondary metabolites, which are widely used across various industries. Therefore, increasing their productivity and more effectively exploiting their biosynthetic potential remains a relevant challenge. A promising approach involves using substances capable of influencing the cellular epigenome to stimulate the biosynthesis of secondary metabolites and regulate plant metabolism.

Over the past two decades, a substantial amount of data on epigenetic regulation in plants has emerged, along with studies summarizing the accumulated knowledge, however, many questions remain unresolved. Despite existing data on the effects of other epigenetic modifiers on plants, the comprehensive impact of valproic acid specifically on vegetative seedling growth and the biochemical profile of oilseed radish and rapeseed remains unexplored. This study is the first to test the hypothesis that valproate treatment may stimulate the synthesis of secondary metabolites in *Raphanus sativus* var. *oleifera* and *Brassica napus*.

The aim of the study was to investigate the effect of valproate on the growth characteristics of two species of the *Brassicaceae* family and to compare the accumulation of phenolic compounds in the obtained plant cultures.

Seeds of rapeseed and oilseed radish were introduced into aseptic culture by surface sterilization (70% ethanol, followed by a 50% solution of the commercial bleach agent "Bilyzna" (2% sodium hypochlorite) and then rinsed three times with sterile distilled water) with subsequent sowing onto ½ MS (Murashige and Skoog) nutrient medium. Various concentrations of the valproic acid (0.005–10 μmol/L) were added directly to the medium prior to inoculation. Cultivation was carried out at room temperature under a 16-hour photoperiod. Seed germination was recorded starting from the third day after planting on the medium. A seed was considered germinated when it had formed two cotyledon leaves at the time of assessment. On days 21–22, morphometric parameters (root and hypocotyl length) were measured using a millimeter ruler. The total phenolic content was determined by the spectrophotometric Folin–Ciocalteu method. Absorbance was measured at 785 nm. Phenolic content was calculated from a calibration curve and expressed as mg of ferulic acid per gram of dry weight. All experiments were performed in triplicate, the significance of differences was assessed using Student's t-test ($p \leq 0.05$).

Valproate affected seed germination in a species-specific manner, with a stimulatory effect observed across a broader concentration range in rapeseed (0.005, 1, 5, and 10 μmol/L) than in oilseed radish (0.005 and 0.1 μmol/L). For both species, hypocotyl length was greatest in the control plants, while root growth was selectively stimulated at certain concentrations — in rapeseed at 0.01, 5, and 10 μmol/L, and in oilseed radish at 0.01, 0.05, 0.5, and 5 μmol/L. Phenolic compound content was consistently higher in shoots than in roots, and valproate promoted secondary metabolite accumulation in shoots at lower concentrations for oilseed radish (0.005 and 0.01 μmol/L) and at intermediate concentrations for rapeseed (0.05, 0.1, 0.5, and 5 μmol/L).

Thus, the obtained data demonstrate the potential of valproate as a regulator of metabolic processes in plants, which opens up prospects for the use of this epigenetic modifier as a tool for enhancing plant productivity.

EFFECTS OF GENOTYPE AND CULTURE MEDIA ON FORMATION OF PRODUCTIVE SPELT REGENERANTS IN IMMATURE EMBRYO CULTURE

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Spelt wheat (*Triticum spelta* L.) is a hexaploid species most closely related to common wheat [1]. Due to high grain quality, resistance to pathogens and adaptation to low-input cultivation conditions, spelt is an attractive target for organic farming, as well as for biotechnology, for example, to increase the genetic diversity in wheat breeding programs. Somaclonal variation arising during *in vitro* culture can contribute to increasing biodiversity within a single genotype without introducing foreign genes. Genotype, explant type, and culture conditions are the main factors influencing somaclonal variation [2]. The aim of this study was to develop effective protocols for obtaining productive spelt regenerants in immature embryo culture and to investigate the effects of genotype and culture media on the induction of somaclonal variation.

Methods of *in vitro* culture and greenhouse cultivation of plant regenerants were applied. Statistical analysis of the results was performed according to standard methods, using formulas for qualitative variability. The significance of differences among experimental variants was assessed by comparing the confidence intervals of the values at the level of significance $p < 0.05$.

Immature seeds of five genotypes (Ukrainian cultivar Zorya Ukrainy, German cultivars Zuricher Rotkorn and Filderweiss, Swiss cultivar Oberkulmer Rothkorn, and Ukrainian breeding line *T. spelta* 4 (UK 4C/15)) were collected 10-20 days after pollination. Immature embryos were cultured using eight combinations of nutrient media (two for callus induction and four for plant regeneration). Approximately 1,300 spelt regenerants were transferred to soil, and about half successfully adapted to *ex vitro* conditions. Regenerants of cultivars Zuricher Rotkorn and Filderweiss demonstrated significantly higher adaptation frequencies compared to the other genotypes. Seeds of the R_1 generation were obtained for all studied genotypes. In total, 85 regenerants produced seeds, representing more than 6% of all plants transferred to soil. The highest number of seed-producing plants was observed among regenerants of the Filderweiss cultivar. The overall germination of R_1 seeds was approximately 60%. Somaclones capable of producing R_2 seeds were identified as productive somaclones. In total, 51 productive somaclons have been obtained. The German cultivars Filderweiss and Zuricher Rotkorn, as well as the Ukrainian breeding line UK 4C/15, were found to be the most suitable genotypes for obtaining productive somaclones.

Productive plant regenerants were obtained using all eight combinations of nutrient media for culturing of immature embryos. The composition of the culture media influenced the frequency of productive regenerant and somaclone formation, as well as the long-term viability of R_1 seeds. The use of regeneration media with high content of 6-benzylaminopurine (1-3 mg/L), regardless of the callus induction medium applied, resulted in a higher number of productive somaclones with significant somaclonal variation in grain size and plant phenotypic traits in regenerant progeny.

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DNA ANALYSIS OF SPELT GENOTYPES BY THE IRAP MARKERS

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A large part of the plant genome is represented by mobile genetic elements. Their share in the wheat genome is approximately 68 % of all DNA [1]. High copy number and polymorphism of integration sites in long terminal repeats (LTR) makes retrotransposons a perfect target for the development of molecular markers [2]. Inter-retrotransposon amplified polymorphism (IRAP) could be used widely to assess genetic diversity and genome stability control. To date, IRAP has not been conducted to analyze the spelt genome.

The aim of this work was to determine the conditions of IRAP analysis for the molecular genetic characterization of spelt genotypes. In our study we used seeds of five varieties (Ukrainian cultivar Zorya Ukrainy, German cultivars Zuricher Rotkorn and Filderweiss, Swiss cultivar Oberkulmer Rothkorn and Ukrainian breeding line *T. spelta* 4 (UK 4C/15, reproduction 2022), generously provided by the Institute of Plant Physiology and Genetics of the NAS of Ukraine. Total plant DNA was extracted and amplified with primers to the LTR retrotransposons using IRAP-PCR.

The following 8 primers Da, Sa, Ni, Wis, Wi, Wh, Su, and St homologous to LTR of retrotransposons Daniella, Sabrina, Nikita, Wis2, Wilma, Wham, Sukkula, and Stowaway consequently as well as 7 combinations of pair of IRAP primers were used to analyze the DNA of spelt genotypes. The effectiveness of using each primer for spelt molecular genetic analysis was assessed by the results of electrophoretic separation of plant amplified DNA. After amplicon analysis, each primer and pair of IRAP primers were divided into 4 groups according to the efficiency of their use [2]. The list of groups goes as follows: non-working (Sab/Wh, Su/Ni), because when they were used for the analysis, PCR did not occur; ineffective primers (Wh, Su/St), the use of which led to increased noise in sample lane, complicating the distinction of individual amplicons; low-efficient (Su, Wis), using which only one clear amplicon was identified; and highly efficient (Da, Sa, Ni, St, Da/Ni, Da/Su, Da/Sa, Su/Sa, Sa/St), primers, that resulted in three or more clearly identifiable amplicons in PCR product spectra.

After the effective primers for analysis were determined, the genetic homogeneity of spelt plants at the certain loci was checked. For all highly effective primers, almost identical spectra of amplification products with minor differences were observed. This indicates the absence or minimal level of polymorphism at the studied regions of the genome among genotypes.

Thus, the effectiveness of primers for IRAP analysis of the spelt genome was assessed. It was found that the Da primer and the Sa/Su primer combination are the most informative of all, as they provide the formation of a large number of clear spectra of amplified fragments. It was established that when using IRAP primers in the study of the spelt genotypes at different loci, both practically identical spectra of amplification products and those that reveal polymorphism among genotypes are observed.

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THEORETICAL AND EXPERIMENTAL RATIONALE OF THE CREATION OF MEDICINAL PLATES WITH WOUND-HEALING EFFECTS BASED ON MEDICINAL PLANTS – ADAPTOGENS

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Currently, it is important to find optimal medicinal forms of wound healing action based on natural remedies due to the high resistance of the human body to synthetic active ingredients. The rich chemical composition of sweet rue (*Ruta graveolens*), which contains alkaloids (0,2–1,4%), essential oils (0,7%), furocoumarins and coumarins, flavonoids, tannins and resins, fatty acids, vitamins, macro- and microelements, allows us to consider this plant as a component of a dosage form with analgesic, anti-inflammatory, antibacterial effects. The most vividly presented flavonoid rutin in *Ruta graveolens* is a bioflavonoid that strengthens the walls of blood vessels, improves blood circulation, and has antioxidant, anti-inflammatory, and anti-edema properties, as well as protects cells from damage, improves the functioning of the cardiovascular system and immunity, improves the absorption of vitamin C, promotes tissue healing. *Ruta graveolens,* restoring capillary blood circulation in tissues, promotes the resorption of scar seals and the disappearance of pain. The research on homeopathic matrix tincture (HMT) *Ruta graveolens* showed that rutin significantly exceeds the content of other phenolic compounds, therefore HMT can be used to provide capillary strengthening, wound healing, and pain-relieving effects. The tinctures of burdock, mallow, and celandine are chosen as active ingredients due to the content of biologically active substances that have antimicrobial, wound-healing, and anti-inflammatory effects. A burdock (*Arctium lappa L.*), which is an adaptogen that exhibits anti-inflammatory, antispasmodic, multivitamin, and antibacterial activity, can be considered as a component of therapeutic films for the treatment of wounds and trophic ulcers.

The studies have confirmed that burdock leaves in tincture form perform an explicit antimicrobial activity, particularly against *Staphylococcus aureus* and *Bacillus subtilis*. The therapeutic activity of burdock is due to organic acids, essential oils (anti-inflammatory activity), bitterness (stimulates blood circulation, affects the state of skin regeneration), phenolic compounds (antibacterial, antioxidant effects). A forest mallow herb (*Malva sylvestris L.*), due to its flavonoid content, has an antioxidant and anti-inflammatory mechanism of action. Extracts and tinctures of wild mallow herb have an antimicrobial effect, significantly affecting both gram-positive and gram-negative bacteria, perform antioxidant and immunostimulating activity, which makes their use advisable for the treatment of purulent wounds. A well-known alkaloid-containing plant is celandine (*Chelidonium majus L.*), among the biologically active components in its chemical composition, the main ones are alkaloids, which have anti-inflammatory, antispasmodic, antiseptic and analgesic effects. A well-known adaptogen *Ginkgo biloba* contains a number of powerful antioxidants that can help to protect the body's cells from damage caused by free radicals. We have studied the use of a medical film based on a 3% Na carboxymethylcellulose and a HMT of *Ruta graveolens* with wound-healing, reparative, and pain-relieving effects, and the antioxidant activity of tinctures of burdock leaves, mallow and sweet rue, and Ginkgo biloba leaves. The use of medical films based on a 3% Na carboxymethylcellulose and HMT *Ruta graveolens* showed positive dynamics in the healing of trophic ulcers and can be recommended for further study for the use as a wound-healing and pain-relieving herbal remedy. The films were used in the complex treatment of trophic ulcers after a stroke. The films were applied at night under a bandage. There was a decrease in pain and relief of sensation in the affected area. The results of studies on tinctures of burdock leaves, wild mallow and sweet rue, and ginkgo biloba leaves showed their high antioxidant activity.

RECOMBINATION FREQUENCY OF FLOWER COLOR, LEAF SHAPE AND NUMBER OF PODS PER NODE IN INTERSPECIFIC CROSSES OF CHICKPEA

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In genetics, recombination, the process that produces new gametes with allele combinations not present in the parents, has been the primary source of variation and evolution (Handerson 2012). This study was the first to define recombination frequencies for leaf shape, flower color, and the number of pods per node in chickpea (*Cicer arietinum* L.). Leaf shape and the number of pods per node directly affect yield or play a crucial role in mitigating the harmful effects of climate change. The study aimed to determine the recombination frequency of leaf shape and the number of pods per node in interspecific crosses between cultivated chickpea and a mutant of the chickpea progenitor, *C. reticulatum* Ladiz. AWC 612M, a mutant of *C. reticulatum* and resistant to leafmine (*Liriomyza cicerina* Rond.), has multipinnate leaves, pink flowers, small dark brown seeds with pigment on plants, and is single-podded per node (Toker et al. 20199). It was crossed with a recombinant inbred line (RIL) of cultivated chickpea with unifoliate leaves, white flowers, large cream seeds without pigment, and double-podded per node (Eker et al. 2022).

After interspecific crossing, *xenia* was seen in the seed shape and color. Not only were heterosis and heterobeltiosis determined for agro-morphological traits, but F_1 plants also had fern-like imparipinnate leaves. Flower and seed colors were found to be linked to plant pigmentation. In the F_2 population, recombination frequencies were determined for the following combinations: (*i*) pinkish-purple flowers, single-podded and fern-like imparipinnate leaves (25%); (*ii*) pinkish-purple flowers, single-podded and unifoliate leaves (16.7%); (*iii*) white flowers, single-podded and fern-like imparipinnate leaves (12.5%); (*iv*) white flowers, single-podded and unifoliate leaves (9%); (*v*) white flowers, single-podded and multipinnate leaves (2.5%); (*vi*) pinkish-purple flowers, double-podded and fern-like imparipinnate leaves (10.8%); (*vii*) pinkish-purple flowers, double-podded and unifoliate leaves (4%); (*viii*) pinkish-puple flowers, double-podded and multipinnate leaves (1.7%); (*ix*) white flowers, double-podded and fern-like imparipinnate leaves (2.5%); and (*x*) white flowers, double-podded and multipinnate leaves (0.8%), while frequencies of the non-recombinants were detected for pinkish-purple flowers, single-podded and multipinnate (11.7) and white flowers, double-podded and unifoliate leaves (2.5%). Recombination frequency was significantly higher (85.8%) than that of non-recombinants (14.2%). To create wide variation, interspecific crosses with phenotypically divergent parents were suggested, since higher recombination frequency yields greater variation in the F_2 population.

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VIGOR IN OFFSPRINGS FROM INTERGENERIC GRAFTING BETWEEN BEAN AND COWPEA

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Grafting, as one of the old analytical inventions, is carried out as a means of vegetative propagation of plants for the magnificent, enormous advantages, including resistance to pests *via* the rootstock or scion, increased yield, and earliness *via* the scion. Productive reciprocal intergeneric grafting between common bean (*Phaseolus vulgaris* L.) and cowpea [*Vigna unguiculata* (L.) Walp.] has not been achieved, despite colossal yield increases between interspecific grafting of *Phaseolus* species. The aims of this study were (*i*) to achieve productive reciprocal grafting between common bean and cowpea landraces, and (*ii*) to prove grafting vigor in offspring derived from intergeneric grafting between bean and cowpea. A landrace of common bean was reciprocally grafted with a cowpea landrace grown by smallholder farmers in Antalya, Turkiye.

The rootstock and scion must essentially be closely related for a grafting operation to be successful. Both bean and cowpea varieties had an indeterminate growth habit, that is, they act as vines or climbers by climbing supports or trailing along the ground. The mature seed coat of the bean landrace was white in color, whereas the pod had striped pigmentation before maturing. The mature seed coat of the cowpea landrace was cream in color with a black-eyed circle due to the presence of a distinctive black spot on its hilum, while the pod had 13 kidney-shaped seeds without pigmentation. The green leaves were similar to both the scions and the rootstock, indicating that the leaf shapes and colors were affected by both the rootstock and the scion. When the bean was used as rootstock, the scion cowpea produced pinkish pods at maturity, but, interestingly, the scion had red seeds at harvest, like the bean. The *P* gene, which controls pigmentation in beans, may be governed by the rootstock. On the other hand, the scion bean produced fully dried cream seeds at maturity and larger, longer pods than those of non-grafted cowpea when cowpea was used as rootstock. The grafted plants had larger seeds and pods than those of non-grafted beans and cowpeas. The offspring from the grafted plants exhibited not only a surprisingly determinate growth habit but also heterotic effects, such as heterobeltiosis in the first generation after grafting.

Grafting vigor was recorded not only in stomata at the cellular level but also in agro-morphological traits, including seed and pod yields. Intergeneric grafting was performed by hand in a greenhouse under above-optimum growth conditions for two seasons. Plants were subjected to extreme temperatures, including chilling (up to 1°C in the greenhouse) and heat stress (over 40°C in the greenhouse), over two seasons. The offspring of grafted plants were found to be superior compared to non-grafted plants. The offspring, called “magic beans,” derived from reciprocal intergeneric grafting between bean and cowpea, will mitigate the negative impacts of climate change on global food security and illuminate the lesser-known side of genetics and plant evolution, as the magic beans have enormously gained genetic advances and grafting vigor comparable to those of hybrid breeding.

GYTOGENETIC ANALYSIS OF *RHODIOLA KIRILOWII* PLANTS *IN VITRO*

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Rhodiola kirilowii (Regel) Maxim is a medicinal plant of the Crassulaceae family, which is used in herbal medicine, mainly in Tibetan, as an adaptogen with immunomodulatory, detoxifying and anti-edematous properties. Modern pharmacological studies have identified a number of biologically active compounds, including salidroside and rosavins, which indicate its similarity to *R. rosea* [Grech-Baran et al., 2015]. Genome studying of the species can provide insight into the biosynthetic pathways of compounds, as well as identify new genes that may contribute to plant adaptation to high-altitude growth conditions. There is limited research on *R. kirilowii* genome, therefore we conducted a cytogenetic analysis of *in vitro* plants of the species.

We have created a collection of *R. kirilowii* plants, which are grown under sterile conditions on the MS nutrient medium [Murashige, Skoog, 1962] with half the content of macro- and microsalts at the light (3000 lux), 20–22°C temperature, 55–65% humidity and a light day of 16 hours.

Cytological studies of this species are a complex process due to widespread polyploidy and small chromosome size. Cytogenetic analysis of *in vitro R. kirilowii* plants showed that the number of chromosomes in metaphase plates ranged from 10 to 102, with a diploid number of 2n=22. The size of the chromosomes of the species was 1.5-2 μm. Almost all the studied roots were mixoploid. Among the analyzed cells, 11% were diploid. We found two haploid cells (n=11) numbering 1.69% of the total analyzed metaphases. The proportion of polyploid cells was high: the modal class was formed by hexaploid and pentaploid cells, which were 15.25% and 14.41%, respectively. Among all analyzed cells, 32.2% were aneuploid. An increased number of nuclei (2-3) was found in the cells, which confirmed polyploidy for this species.

It is known that *R. kirilowii* can be both diploid (2n=22) and tetraploid (4n=40+1), in the male karyotype of which, in addition to 40 homeologous chromosomes, the presence of one sex chromosome was confirmed [Zhang et al., 2025]. Such a phenomenon can be detected only for dioecious plants, the species of *Rhodiola* genus. We also composed a collection of indoor plants cultivated in the thermal room of the Department of Cell Population Genetics. Some of the *R. kirilowii* plants bloomed after four months of cultivation. Due to flowering we determined the flower formula and showed the presence of both stamens and pistils, which indicates that the species can be monoecious. Such a phenomenon may occur in polyploid forms, under stress, in culture and as a result of hybridization.

Summarising, the cytogenetic analysis evidenced the predominance of hexa- and pentaploid cells in the roots of *R. kirilowii* indicating its polyploid origin. The species' ability to aneu- and mixoploidy was also revealed. These phenomena may be a consequence of anthropogenic load and growth in mountainous conditions, as well as evidence of the increased adaptive potential of plants with a changed number of chromosomes to survive in adverse environmental conditions. These mechanisms are the main driving forces in the reticular evolution of plants.

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EFFECT OF ELICITATION ON THE CONTENT OF TRITERPENOID SAPONINS IN SUSPENSION CULTURE OF SOAPWORT *SAPONARIA OFFICINALIS* L.

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Soapwort (*Saponaria officinalis* L.) is widely utilized in various industrial sectors due to its triterpenoid saponins, which possess diverse biological properties. This study investigated the impact of two biotic elicitors, jasmonic acid and chitosan, on triterpenoid accumulation in soapwort suspension cultures. The most significant enhancement was achieved with 50 μM jasmonic acid, which yielded a 2.89- to 6.05-fold increase in triterpenoid levels (depending on the culture's origin) without adversely affecting growth or morphology. Chitosan at a concentration of 25 mg/l showed a much weaker effect, elevating triterpenoid content only 1.05- to 1.68-fold.

MACHINE LEARNING-BASED ANALYSIS OF CALLUS INDUCTION AND BIOMASS IN *LAVANDULA* × *INTERMEDIA*

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Aim of the study: Production of secondary metabolites in Lavandula species is influenced by cultivation conditions, while in vitro systems provide controlled environments for their consistent production. However, callus induction and biomass accumulation depend on interacting effects of explant origin, plant growth `regulator composition, and cultivation duration, which are difficult to resolve using conventional approaches. This study aimed to determine how these factors influence callus induction and biomass production in Lavandula × intermedia and to evaluate their predictive contribution using machine learning models.

Methods: Fifty-seven plant growth regulator combinations were tested using root- and stem-derived explants over a 15-week in vitro cultivation period. Callus induction frequency and fresh biomass were recorded at three-week intervals. The dataset was analysed using Beta regression, generalized additive models (GAM), Random Forest (RF), Support Vector Regression (SVR), and Extreme Gradient Boosting (XGBoost). Multi-objective optimization was performed using the Non-Dominated Sorting Genetic Algorithm II (NSGA-II).

Results: Callus induction occurred on 49 media, with frequencies ranging from 10.52 – 100.00% for root explants and 6.25 – 100.00% for stem explants, with most induction observed within the first three weeks. Biomass accumulation followed a biphasic trajectory, with low values up to week 6 (0.04 – 6.30 g), followed by a marked increase, reaching 3.31 –35.44 g by week 15 depending on culture medium and explant type. The combination of 0.5 mg/L 2,4-D and 0.5 mg/L kinetin produced the highest biomass. Principal component analysis indicated that cultivation time explained 75.50% of total variance. To quantify these relationships, machine learning models were applied to predict callus induction and biomass. XGBoost showed the highest predictive performance ($R^2 = 0.9373$, RMSE = 1.7610 for biomass; $R^2 = 0.8305$, RMSE = 12.0026 for induction). Feature importance analysis indicated that cultivation duration was the dominant factor influencing biomass, whereas explant type primarily affected callus induction.

Conclusions: Overall, callus induction and biomass accumulation in *L.* × *intermedia* are governed by nonlinear interactions among experimental factors, with distinct determinants for each response. These patterns were consistently captured by machine learning models. XGBoost showed the highest predictive accuracy and, together with NSGA-II, identified high-performing culture conditions within the tested experimental space.

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THE ACUTE X-RAY IRRADIATION EFFECTS IN *SALVIA OFFICINALIS L.* AND *HYPERICUM PERFORATUM L. IN VITRO* PLANTS

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In continuation of studies of the effect of ionizing radiation on the processes of secondary metabolism in medicinal plants, to investigate the effect of IR on the accumulation of secondary metabolites in medicinal *in vitro* plants culture was proposed. So the aim of the work was to identify radiation doses capable of stimulating the biosynthesis of pharmaceutically valuable substances.

Plants were grown *in vitro* on hormone-free Murashige-Skoog medium supplemented with 30 g/l sucrose. Irradiation was carried out on a RUM-17 X-ray unit at doses of 5 Gy, 10 Gy, 15 Gy and 30 Gy for *Salvia officinalis L.* and 1 Gy, 5 Gy, 10 Gy, 15 Gy for *Hypericum perforatum L.* at a dose rate of 1.42 cGy/s. Samples for determination of the content of secondary metabolites – phenols and flavonoids were taken one, three and six weeks after irradiation. Plants were freeze-dried and extracted with 70% ethanol.

The changes in the content of pharmaceutically important secondary metabolism substances in plant extracts of *Salvia officinalis L.* and *Hypericum perforatum L.*, grown *in vitro* and exposed to IR was investigated. The stimulating effect of irradiation on the content of phenols and flavonoids in 3-week-old *Hypericum perforatum L. in vitro* plants and hypericins in 6-week-old plants was shown for all radiation doses. Also for 1-week-old *Salvia officinalis L. in vitro* plants an increase in the yield of phenolic compounds, flavonoids and rosmarinic acid in ethanol extracts was observed for all irradiation doses compared to the control. The greatest stimulation of the release of phenolic compounds into ethanol fr *Salvia officinalis L. in vitro* plants extracts was observed irradiated at a dose of 10 Gy.

A significant increase in the content of flavonoids in extracts from irradiated *Salvia officinalis L. in vitro* plants was observed one and three weeks after irradiation. After 6 weeks, no significant difference was observed. The greatest increase in the yield of flavonoids in extracts from irradiated plants compared to controls was observed for 5 Gy both after a week and after three weeks. In addition, after 3 weeks, a significant increase was also observed for the irradiation dose of 15 Gy. After 6 weeks after irradiation, a slight increase was observed for the variants irradiated at doses of 15 Gy and 30 Gy. A significantly higher release of rosmarinic acid into ethanol extracts for the irradiated variants is observed a week after irradiation. Moreover, the use of irradiation at a dose of 10 Gy exceeds the control level by 4 times. For three and six weeks after irradiation, the effect was not preserved.

Irradiation of *Hypericum perforatum L. in vitro* plants culture at doses of 1 Gy, 5 Gy, 10 Gy, 15 Gy caused an increase in the content of flavonoids in the leaves of 3-week-old plants by 20% - an irradiation dose of 1 Gy, and by 30% - in the leaves of 6-week-old plants, doses of 5 Gy and 10 Gy. An increase in the content of phenolic compounds was noted for 3-week-old plants - for all irradiation doses: by 13-22%. For 6-week-old plants, changes in the content of phenolic compounds for irradiated plants differed little from non-irradiated ones. Three months after irradiation, changes in the content of both phenols and flavonoids were practically not noted for all irradiation doses. Hypericin production was stimulated to some extent three weeks after irradiation at doses of 1, 5 and 15 Gy, while no such effect was observed in plants that received a dose of 10 Gy. After 6 weeks after irradiation, a significant stimulating effect was demonstrated for all doses used - the total content of hypericins increased by approximately 1,5 times regardless of the dose used. Three months after irradiation, the effect of lower doses (1 and 5 Gy) was practically not detected, while higher doses (10 and 15 Gy) significantly suppressed hypericin biosynthesis.

Thus, confirmation of the possibility of using ionizing radiation to modify the accumulation of pharmaceutically valuable substances in *Hypericum perforatum L. in vitro* plants culture was obtained.