

Serbian Plant Physiology Society

Institute for Biological Research "Siniša Stanković"

– National Institute of the Republic of Serbia, University of Belgrade

Faculty of Sciences, University of Novi Sad

University of Belgrade, Faculty of Biology

Institute for Multidisciplinary Research

– National Institute of the Republic of Serbia, University of Belgrade

Serbian Biological Society "Stevan Jakovljević" Kragujevac

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Freiberg, Germany



PROGRAMME

THURSDAY 5TH NOVEMBER

from 10:00 Registration
12:00-12:10 Opening Ceremony

SECTION 1 • PLANT GROWTH AND DEVELOPMENT

Chairs: TIJANA CVETIĆ ANTIĆ & MARZENA WARCHOŁ

12:10-12:40 **Keynote:** KALLIOPE K. PAPADOPOULOU
Decoding endophyte-mediated plant resilience to abiotic stress
12:40-13:10 **Plenary lecture:** GUIDO GROSSMANN
Shaping a cell to invade - dissecting root hair emergence, growth and (surprising) functions
13:10-13:30 **Invited talk:** DANIJELA PAUNOVIĆ
*The Arabinogalactan proteins of centaury (*Centaureum erythraea* Rafn.)*

13:30-15:00 LUNCH BREAK

15:00-15:20 **Invited talk:** VÁCLAV MOTYKA
Interplay of auxin transport, phytohormones, and plant growth-promoting rhizobacteria in maize responses to aluminium toxicity
15:20-15:35 **Selected talk:** DAVID HONYS
Pollen Thermotolerance: A Translational Perspective
15:35-15:50 **Selected talk:** BOGDAN NIKOLIĆ
Brassinosteroids and their influence on seed germination

SECTION 2 • METABOLISM AND BIOCHEMISTRY

Chairs: DANIJELA MIŠIĆ & VUK MAKSIMOVIĆ

16:00-16:30 **Plenary lecture:** ALAIN TISSIER
How light and temperature affect glandular trichome development and metabolism in tomato
16:30-17:00 **Plenary lecture:** JAN ZUBER
Unveiling the molecular complexity of plant biopolymers with ultrahigh-resolution FT-ICR-MS
17:00-17:30 COFFEE BREAK
17:30-17:50 **Invited talk:** DRAGANA JAKOVLJEVIĆ
*Basil (*Ocimum L.*) tissue culture: a tool for the production and characterization of secondary metabolites*
17:50-18:10 **Invited talk:** JAIME MORANTE
*Exploring the bioactive profile of elicited *Morus alba* cell cultures*
18:10-18:25 **Selected talk:** JELENA OŽEGOVIĆ
*Chemical composition and antioxidant potential of *Raphidiopsis raciborskii* strains*
18:25-18:40 **Selected talk:** MILOŠ TODOROVIĆ
*Transcriptomic and metabolomics insights into the flower color polymorphism of two *Centaureum* species*
18:40-20:00 Poster session – sections 1 and 2
20:00-22:00 WELCOMING COCKTAIL AT THE DBE

FRIDAY 6TH NOVEMBER

SECTION 3 · PLANT STRESS PHYSIOLOGY

Chairs: JELENA DRAGIŠIĆ MAKSIMOVIĆ & JAN ZUBER

- 09:00-09:30 **Plenary lecture:** BILJANA KUKAVICA
Seed priming as a strategy to improve maize resilience to hypoxia
- 09:30-09:50 **Invited lecture:** ANA NIKOLIĆ
Decoding chilling resilience: molecular, biochemical, and phenotypic adaptations during early maize development in standard and quality protein maize
- 09:50-10:10 **Invited talk:** MILAN ŽIŽIĆ
Illuminating microalgae with synchrotron light
- 10:10-10:30 **Invited talk:** DRAGANA NIKOLIĆ
Secret messages in small packages – extracellular vesicles in inter-kingdom war and peace
- 10:30-11:00 **COFFEE BREAK**
- 11:00-11:15 **Selected talk:** TATIANA ILINA
*Desiccation-driven structural transitions of LEA4 proteins from *Ramonda serbica**
- 11:15-11:30 **Selected talk:** JAN FILA
Deciphering the role of bZIP transcription factor family E under heat stress conditions
- 11:30-11:45 **Selected talk:** KRISTINA MAJSEC
*Moderately elevated temperatures make potato more susceptible to *Pseudomonas syringae* infection*
- 11:45-12:45 **Poster session- section 3**
- 12:45-13:30 **MDPI workshop for students - AI in Publishing**
- 12:45/13:30-15:00 **LUNCH BREAK**

SECTION 4 · ECOLOGY, GENETICS AND EVOLUTION OF PLANTS

Chairs: TIJANA BANJANAC & MILAN BORIŠEV

- 15:00-15:30 **Plenary lecture:** MARKO SABOVLJEVIĆ
Tiny plants, big impact: recent needs and modern solutions in bryophyte conservation
- 15:30-15:50 **Invited talk:** EDYTA SKRZYPEK
Progress in oat DH technology and functional characteristics of oat × maize hybrids
- 15:50-16:10 **Invited talk:** DRAGANA TRKULJA
Integrating genetic resources, phenomics, genomics, and transcriptomics to enhance wheat drought resilience
- 16:10-16:45 **COFFEE BREAK**
- 16:45-17:00 **Selected talk:** VELIMIR MLADENOV
From Field to Genome: UAV Phenotyping and SSR Markers Reveal Drought-Tolerance Signatures Across Maize Hybrid Environments
- 17:00-17:15 **Selected talk:** UROŠ ALEKSIĆ
Intercropping modifies species-specific responses to multiple metal stress in flotation tailings
- 17:15-17:35 **Sponsor talk** Analysis d.o.o.
- 17:35-19:00 **Poster session – sections 4 and 5**
- 21:00-00:30 **GALA DINNER AT THE RESTAURANT "SOKAČE"**

SATURDAY 7TH NOVEMBER

10:00-11:00 SPPS Assembly

SECTION 5 · PLANT BIOTECHNOLOGY AND APPLICATIONS

Chairs: JELENA SAMARDŽIĆ & BILJANA KUKAVICA

11:00-11:30 **Plenary lecture:** ANGELOS K. KANELIS

*Genotype-driven regulation of phenylpropanoid biosynthesis in *Cistus creticus*: a combined metabolomic, transcriptomic, and functional characterization approach*

11:30-11:50 **Invited talk:** IVANA MOMČILOVIĆ

Aeroponics: from the space station, through high-yield potato farming, to a tool for studying the process of tuberization

11:50-12:10 **Invited talk:** MIRJANA PEŠIĆ

From plants to bioactive compounds: Metabolomics, food applications and bioaccessibility

12:10-12:30 **COFFEE BREAK**

12:30-12:50 **Invited talk:** MARINA SOKOVIĆ

Essential Oils Revisited: Three Decades of Research from Traditional Remedies to Next-Generation Biobased Therapeutics

12:50-13:05 **Selected talk:** DRAGANA MILADINOVIĆ

Optimizing CRISPR/Cas9 Genome Editing Protocols in Sunflower: Overcoming Recalcitrance and Microbial Bottlenecks in Hypocotyl-Derived Explants

13:05-13:15 **Closing Ceremony**

13:15-15:00 **LUNCH BREAK**

15:00-18:30 **EXCURSION SREMSKI KARLOVCI**



PLANT GROWTH AND DEVELOPMENT

Decoding Endophyte-Mediated Plant Resilience to Abiotic Stress

Papadopoulou, Kalliope K.¹

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Climate-driven abiotic stress is increasingly challenging crop productivity. The plant microbiome offers a sustainable route to resilience. Accumulating evidence from multi-scale studies illustrated how beneficial root-residing fungal endophytes can effectively reprogram the plant stress response: at the transcriptome level, stressed colonized plants acquire a molecular profile that closely resembles that of unstressed plants. This shift involves extensive rewiring of hormone signalling, recruitment of stress-responsive transcription factors, and modulation of canonical defence pathways. Integration of transcriptomics and untargeted metabolomics revealed a shift toward metabolites that contribute to water conservation, antioxidant capacity, and redox homeostasis, providing a mechanistic link between endophyte colonization and improved stress performance. Importantly, the interaction is not initiated only after physical contact. Evidence of early cross-talk indicates that endophytes can influence their hosts before and upon colonization that help shape root development and potentially facilitate subsequent establishment. The benefits also extend beyond individual microbial partnerships. Combining fungal endophytes with mycorrhizal fungi produced additive improvements in plant growth and water status under stress, while stress conditions themselves enhanced endophyte colonization. Finally, whole-genome sequencing provided insight into the potential of fungal traits underlying these effects, supporting the transition from mechanistic discovery toward the responsible deployment of defined microbial communities in agriculture.

KEYWORDS: fungal endophytes; drought; defined microbial communities; kingdom cross-talk

ACKNOWLEDGEMENT: This work is partially funded by the PRIMA programme (project SALSA), supported by the European Union.

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Shaping a Cell to Invade – Dissecting Root Hair Emergence, Growth and (Surprising) Functions

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Root hairs are highly polarized tip-growing cells that invade the surrounding soil, contributing to root anchorage, nutrient mobilization and uptake, and interactions with soil microbes. Their stereotypic finger-like shape is defined by the formation of a polar domain at the plasma membrane, established through dynamic protein–protein and protein–lipid interactions. How this domain is established, how its diameter is defined, and how the associated growth machinery integrates environmental cues are key questions addressed in our research. Using root hair development in *Arabidopsis thaliana* as a model, we identified mutants with altered root hair shape. Live-cell super-resolution imaging and analysis of single-molecule dynamics allowed us to map protein mobility over time. Based on these data, we propose a model in which the dynamic membrane association of ROP GTPases and their activating ROP guanine nucleotide exchange factors (ROP-GEFs) defines the diameter and maintains the integrity of the polar membrane domain. Proximity labeling of ROP2 and GEF3 further identified components of the molecular networks coordinating polarity, growth regulation and cell integrity, providing potential entry points for environmental modulation. Following root hair emergence and microbial colonization in microfluidic devices revealed a previously unknown mechanism by which root hairs attract microbes and enhance colonization by beneficial rhizobacteria. In turn, these bacteria modulate root hair growth and shape, pointing to crosstalk between microbial signaling and the mechanisms controlling polarity and growth.

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The Arabinogalactan Proteins of Centaury (*Centaurea erythraea* Rafn.)

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Arabinogalactan proteins (AGPs) are extensively glycosylated cell wall proteins belonging to the superfamily of O-glycosylated hydroxyproline-rich glycoproteins (HRGPs). They are characterized by high primary structure diversity, are encoded by a large gene family and are associated with a wide array of physiological roles in plant growth and development, which makes them the most attractive group among HRGPs. Common centaury (*Centaurea erythraea* Rafn.) is characterized by an exceptional developmental plasticity and strong morphogenetic potential and is a suitable model organism for developmental biology studies. AGPs have been identified as significant factors during somatic embryogenesis and organogenesis of common centaury and as such, can be important for the regeneration process in both plants grown *in vitro* and plants from nature. AGPs are intrinsically disordered proteins, which hinders their homology-based identification. Use of bioinformatic tools, such as the *ragp* R package has enabled the identification of a large number of HRGP and AGP sequences in the *C. erythraea* transcriptome. A model for predicting proline hydroxylation probability based on local protein sequence has been incorporated into the *ragp* R package, together with a number of diverse tools that allow detailed analysis of protein sequences. The information gathered provides a clearer picture of AGPs as links between cell wall mechanics and intracellular signal transduction during plant regeneration.

KEYWORDS: cell wall; arabinogalactan proteins; *Centaurea erythraea* Rafn.; *in vitro*; *ragp*

ACKNOWLEDGEMENT: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-33/2026-03/ 200007)

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Interplay of Auxin Transport, Phytohormones, and Plant Growth-Promoting Rhizobacteria in Maize Responses to Aluminium Toxicity

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In plants, concentrations and distributions of auxins and cytokinins (CKs) underpin many developmental processes. Plant Growth-Promoting Rhizobacteria (PGPR) can influence plant development by modulating phytohormone activity and thus represent a promising biological approach to improving plant growth and resilience under adverse soil conditions. To investigate this, the beneficial effects of *Bacillus toyonensis* strain Bt04 (Bt04) in mitigating aluminium (Al) toxicity in maize roots, with particular emphasis on its potential role in regulating plant hormonal responses, were examined. Bt04 produced several phytohormones, including auxins and CKs, and its application promoted maize growth and root development under Al stress. Bacterial inoculation also reduced Al accumulation in maize roots and decreased lipid peroxidation, indicating enhanced tolerance to Al-induced stress. Further experiments using the auxin transport inhibitors NPA and 1-NOA showed that auxin transport pathways contribute to the response of maize roots to both Bt04 inoculation and Al stress. Bt04 also influenced the levels of stress-related hormones, particularly abscisic acid and jasmonic acid, and modulated their responses to auxin transport inhibition.

Overall, these findings indicate that Bt04 can alleviate the adverse effects of Al toxicity through interconnected processes involving root development, stress protection, hormonal regulation, and phytohormone transport. The results highlight the potential of beneficial rhizobacteria as a biological strategy for improving crop resilience in acidic, Al-affected soils.

KEYWORDS: aluminium; auxin transport; *Bacillus toyonensis*; maize; plant growth-promoting rhizobacteria

ACKNOWLEDGEMENT: The presenting author's work was supported by the Czech Science Foundation (26-23352S)

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Pollen Thermotolerance: A Translational Perspective

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Pollen germination and pollen tube growth are among the most heat-sensitive stages of plant reproduction. Elevated temperatures trigger a broad reduction in protein synthesis while promoting the selective translation of proteins required for stress protection, protein homeostasis, protection against protein misfolding, and cellular survival. Our work shows that components of the translational machinery play important roles in maintaining pollen performance under heat stress. Mutants affecting specific translation initiation factors display altered pollen germination, tube growth, and reproductive success, with some genotypes unexpectedly showing improved tolerance to elevated temperatures. This enhanced thermotolerance is associated with increased expression of protective chaperones and stress-responsive proteins. In particular, translation factors appear to modulate the expression of molecular chaperones involved in maintaining protein folding, membrane integrity, and pollen tube stability during stress. Physical interactions between translation-related proteins and heat shock proteins further support the existence of a broader regulatory network linking translational control with pollen thermotolerance. Together, these findings highlight selective translation as a key mechanism safeguarding reproductive success under climate-induced temperature extremes.

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Brassinosteroids and their Influence on Seed Germination

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Regulation of seed germination rate is crucial for high seedling growth, resulting in weed suppression and efficient crop production, especially under suboptimal growing conditions. Previously it was thought that only GA and ABA interactions regulate germination, but there is also evidence that other phytohormones, especially BR, positively regulate seed germination in *Arabidopsis* and other plants. Exogenous application of BR and examination of BR-associated mutants significantly affect seed germination in various plants under the inhibitory influence of abscisic acid. Recent evidence suggests that the antagonistic effect of BR on seed ABA inhibition of germination is mediated in part by some small BRs-binding proteins. ABA-induced germination inhibition was overcome by overexpression of the BRs biosynthetic gene under the control of a seed-specific promoter in *Arabidopsis*. There are additional putative genes involved in BRs biosynthesis that affect seed dormancy and germination in *Arabidopsis*. It remains to be determined whether this physiological effect is correlated with the increased content of endogenous BR in transgenic seeds. It has recently been observed that some sterols have a positive function on seed germination. Since seed germination is *par excellence* an energetic phenomenon, the effect of exogenously added phytohormone BR on energetics and metabolism in the seeds of some plants has recently been examined, confirming the positive effect of phytohormone BR on energy changes and carbohydrate metabolism, but also on other important molecules from primary and secondary metabolism during seed germination. Therefore, modification of sterol and BR content in seeds can have important agronomic applications in both normal and stressful conditions under which seeds germinate.

KEYWORDS: Brassinosteroids; seed germination; phytohormone crossroads; energetic changes; metabolic changes

ACKNOWLEDGEMENT: The research was financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grants No. 451-03-33/2026-03/ 200214

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Potential of Coconut Water as a Natural Growth Regulator for Microtuber Formation in Potato (*Solanum tuberosum* L.)

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Microtuberization represents an important stage in potato tissue culture and seed tuber production. Due to the increasing interest in sustainable tissue culture systems, coconut water (CW) has been investigated as a potential natural alternative to synthetic plant growth regulators. The aim of this study was to evaluate the effect of CW on microtuber formation in potato cultivar Arnova and to compare its effectiveness with 6-benzylaminopurine (BAP). Single-node explants were cultured on solid Murashige and Skoog (MS) medium containing 8% sucrose and 0.7% agar, supplemented with either BAP (2 mg/L) or CW (50, 100, and 150 mL/L). Medium without additional supplements served as the control. Cultures were maintained in darkness for five weeks, after which the percentage of tuberization, average microtuber diameter, and average microtuber mass were evaluated. The addition of BAP significantly increased the percentage of tuberization (98.33%) compared with the control (37.50%). CW treatments also improved microtuber formation, with the highest tuberization percentage observed at 50 mL/L (54.17%). Compared with the control treatment, BAP induced the greatest increase in microtuber diameter and mass, reaching 1.41-fold and 2.94-fold higher values, respectively. CW treatments showed intermediate effects, resulting in 1.20–1.25-fold higher microtuber diameter and 2.00–2.09-fold higher microtuber mass compared with the control. The results indicate that CW exhibits biostimulatory activity and may represent a promising supplement for improving potato microtuberization. Although BAP remained more effective, CW showed potential as a natural alternative for the partial replacement of synthetic growth regulators in potato tissue culture.

KEYWORDS: potato; microtuberization; coconut water; biostimulant

ACKNOWLEDGEMENT: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract number 451-03-33/2026-03/200007. The presented results are in line with Sustainable Development Goal 12 (Responsible Consumption and Production) of the United Nations 2030 Agenda.

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Effect of Coconut Water as a Natural Biostimulant on Callus Induction in Potato (*Solanum tuberosum* L.)

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Potato tissue culture protocols predominantly rely on synthetic growth regulators for callus induction and plant regeneration. In recent years, coconut water (CW) has gained attention as a natural biostimulant due to its rich content of bioactive compounds. This study compared CW and the synthetic cytokinin 6-benzylaminopurine (BAP) for callus induction from nodal explants of potato cultivar Arnova and evaluated the regeneration potential of calli. Single-node explants were cultured on Murashige and Skoog (MS) medium supplemented with BAP (2 mg/L) or CW (50, 100, and 150 mL/L), while MS medium without additional supplements served as control. Supplemented media induced callus formation, with 100% of explants forming calli within 12 days, whereas no callus developed on control medium. The induced calli were green to yellowish-green and exhibited a compact nodular structure. Significant differences among treatments were observed for callus growth parameters (diameter, fresh weight, and dry weight), with the highest values recorded on MS medium supplemented with BAP. In CW treatments, measured growth parameter values increased in a concentration-dependent manner, reaching highest values at 150 mL/L. After five weeks, calli were transferred to MS medium containing different plant growth regulator compositions: BAP; BAP and gibberellic acid (GA₃); thidiazuron and naphthaleneacetic acid (NAA). Shoot regeneration was achieved only on the medium supplemented with BAP (2 mg/L) and GA₃ (0.25 mg/L), indicating specific hormonal response and confirming organogenic potential. Overall, CW induces morphogenic callus formation and shows potential as a natural biostimulant in potato tissue culture. Further optimization of regeneration protocol may improve efficiency.

KEYWORDS: *Solanum tuberosum* L.; callogenesis; natural growth regulator; shoot regeneration; *in vitro* culture

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Endogenous Phytohormone Profiles in Different Types of *Iris reichenbachii* Callus

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The subject of this study was dwarf bearded yellow iris (*Iris reichenbachii*. Heuffel), an endemic plant of the Balkan Peninsula. Zygotic embryo culture is a useful method for *ex situ* conservation of endemic plants because it preserves genetic diversity. Zygotic embryos of *I. reichenbachii* were cultured on solid MS medium with 3% sucrose, supplemented with 2,4-dichlorophenoxyacetic acid and kinetin (1.0 mg/L each). Based on color and consistency, three types of callus could be distinguished after four weeks of culture: i) white, friable callus, referred to as embryogenic callus (EC); ii) green, compact, organogenic callus (OC); and iii) yellow, nodular callus, referred to as non-embryogenic callus (NEC). The aim of this study was endogenous phytohormone profiling of the different types of callus of *I. reichenbachii*. Phytohormone analyses showed a significant differences in phytohormone status in EC, OC and NEC. The lowest total content of cytokinins was observed in NEC, while their content in EC (2.1-fold) and OC (3.8-fold) was higher than in NEC. Significantly higher total auxin content was recorded in NEC in comparison with EC and OC. The lowest total content of ABA was recorded in OC, while in NEC (2.3-fold) and EC (8.8-fold) the ABA content was higher in comparison with OC. In addition, significant differences in the content of jasmonic, salicylic and sinapic acids were recorded in EC, OC and NEC. Our results indicate that the different levels of endogenous hormones in the various types of iris callus underlie the distinct regeneration pathways in *in vitro* cultures.

KEYWORDS: embryogenic callus; non-embryogenic callus; organogenic callus; dwarf iris

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Dynamics of Carbohydrate Metabolism During Early *In Vitro* Kohlrabi Development in Response to Elevated Sugar and Cytokinins

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Plant hormones, particularly cytokinins, and their interactions with sucrose are considered important regulators of stem swelling in kohlrabi (*Brassica oleracea* L. var. *gongylodes*), yet the underlying mechanisms remain poorly understood. An optimized *in vitro* regeneration system was used to investigate carbohydrate metabolism during four early developmental stages (T1–T4) of young kohlrabi plants and to evaluate effects of elevated sucrose concentrations (6% and 9%) and cytokinins (trans-zeatin, transZ; thidiazuron, TDZ). Soluble sugar content and expression of genes involved in sucrose metabolism, transport, and starch synthesis were analyzed. TDZ induced the strongest increase in soluble sugars at T2 (plants with two emerged leaves). Elevated glucose and fructose levels persisted at T3 under this treatment, while at T4 (plants with fully developed leaves /differentiation of de novo shoots) the most pronounced responses were observed after 9% sucrose application. Gene expression patterns varied considerably with developmental stage and treatment, with the strongest changes generally occurring at T2 under TDZ and/or 9% sucrose. At T4, relationships between sugar accumulation and gene expression indicated treatment-specific metabolic responses. The 9% sucrose treatment significantly increased monosaccharide levels and enhanced SuSy1 and SWEET expression, implying stimulated sucrose degradation and sugar transport. In contrast, 6% sucrose reduced SPS1, STP4, and SS1 expression, suggesting a shift in resource allocation toward stress adaptation rather than sucrose transport and starch synthesis. Overall, young kohlrabi plants exhibited clear capacity to modulate carbohydrate metabolism in response to sucrose and cytokinin treatments revealing sophisticated mechanisms governing sugar partitioning in accordance with internal and external signals.

KEYWORDS: kohlrabi; sucrose; cytokinins; carbohydrate metabolism; *in vitro* culture

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Comparative Stem Anatomy of *In Vitro*-Grown, Acclimatized, and Wild Plants of *Micromeria myrtifolia* Boiss & Hohen

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The transition from *in vitro* culture to *ex vitro* conditions is accompanied by anatomical adjustments that are essential for the successful establishment of micropropagated plants. The aim of this study was to investigate stem anatomical changes associated with acclimatization by comparing *in vitro*-grown, *ex vitro*-acclimatized, and wild plants of *Micromeria myrtifolia* Boiss. & Hohen. (Lamiaceae). Several quantitative anatomical traits were analyzed in stem cross-sections, including stem diameter, pith diameter, cortex thickness, corner collenchyma thickness, and cuticle thickness. The analyzed stem anatomical traits differed significantly among *in vitro*-grown, *ex vitro*-acclimatized, and wild plants. Wild plants exhibited the highest values of stem diameter, pith diameter, corner collenchyma thickness, and cuticle thickness whereas *in vitro*-grown plantlets were characterized by the greatest cortex thickness. *Ex vitro*-acclimatized plants displayed intermediate stem anatomical characteristics between *in vitro*-grown and wild plants, suggesting progressive structural adaptation during acclimatization. These findings contribute to a better understanding of the acclimatization process and anatomical adaptations of *M. myrtifolia* during the transition from *in vitro* culture to *ex vitro* conditions. To the best of our knowledge, this is the first study to characterize stem anatomical responses associated with the acclimatization of micropropagated plants in the genus *Micromeria*, using *M. myrtifolia* as a model species, and provides insights into the structural adjustments accompanying the establishment of micropropagated plants.

KEYWORDS: *Micromeria myrtifolia*; stem anatomy; *in vitro* culture; acclimatization; wild plants

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Effects of Different Light Sources on the Morphological Traits of *Hyssopus officinalis* L.

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Light is one of the most important factors affecting plant growth and development. In addition to providing energy for photosynthesis, the type and intensity of light can significantly influence plant morphology. The aim of this study was to examine the effects of different light sources on the morphological traits of *Hyssopus officinalis* plants. Plants were grown in soil culture under uniform air temperature throughout the experiment. One group was grown in a greenhouse under daylight, while the other two groups were grown in plant growth boxes under LED Clone Bar (18 W, 6500 K, spectrum 380–800 nm) and LED Quantum Board (240 W, full spectrum). At four weeks of age, the following morphometric parameters were measured: shoot fresh weight, shoot length, number of internodes, average internode length, leaf length, and number of leaves. ANOVA revealed statistically significant differences in all measured morphometric traits among the three light treatments. The longest internodes were recorded in plants exposed to daylight, while the shortest were observed under LED Quantum Board. The remaining parameters showed the opposite trend, with the highest values under LED Quantum Board, except for leaf length, which was greatest under LED Clone Bar. The lowest values were recorded under daylight.

The choice of light source can significantly influence the growth and morphological characteristics of *H. officinalis*, highlighting its potential to improve cultivation practices and emphasizing the importance of appropriate light conditions for the cultivation of this medicinal and aromatic plant.

KEYWORDS: *Hyssopus officinalis* L.; soil culture; light sources; morphometric traits; plant growth

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EcoLotus Within AgroServ: Field Evaluation and High-Throughput Phenotyping of Common Lotus in Serbia and Poland

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The EU-funded Horizon Europe AgroServ project supports the transition towards sustainable and resilient agriculture through agroecology, multidisciplinary research, and access to scientific services and infrastructures. It enabled the realization of the Transnational access project: Common lotus – sustainable crop and valuable forage for cows on agroecological farms (EcoLotus). The two year experiment was conducted in 2025 and 2026 in Serbia, and in Poland, using five common lotus (*Lotus corniculatus* L.) cultivars differing in genetic and geographical origin. Field performance was evaluated under two cutting regimes (two and three cuts per season), two levels of nitrogen fertilizer, and three replicates. Yield and quality of green biomass were assessed, together with basic physical and chemical soil and plant properties. To provide detailed information on crop performance, extensive high-throughput phenotyping of experimental plots was performed in Serbia using unmanned aerial vehicles and vegetation indices. In addition, rapid, non-destructive handheld measurements were applied for early assessment of green biomass, photosynthetic activity, and chlorophyll content in both countries. These approaches enabled comparison of biomass production under contrasting agricultural input levels and agroecological conditions.

The wider EcoLotus project also addresses the hay quality, the chemical composition of lotus plants, including proanthocyanidins and carotenoids, as well as the effects of different lotus inclusion rates in cattle feed on milk production, milk quality, and methane emissions. By generating new field-based evidence on soil quality, land coverage, biomass yield, and forage quality, EcoLotus will contribute valuable scientific data for assessing the potential of common lotus in sustainable agricultural systems.

KEYWORDS: AgroServ; *Lotus corniculatus* L.; sustainable agriculture; high-throughput phenotyping; cultivation intensity.

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Cadmium Effects on Morphogenesis of *Drepanocladus aduncus* and *Drepanocladus fluitans* Under Controlled Conditions

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Bryophytes have long been adapted to metal-rich environments, with species varying in their sensitivity, tolerance, and resistance. *Drepanocladus aduncus* (Hedw.) Warnst. and *Drepanocladus fluitans* (Hedw.) Warnst. are wetland and aquatic mosses, both belonging to the family Amblystegiaceae. *D. aduncus* is a circumboreal, almost cosmopolitan species with broad ecological tolerance, occurring in a range of wetlands from salty to temporary dried, whereas *D. fluitans* is an acidophilic aquatic moss associated with acidic lakes, streams, swamps, and peat bogs. Both species have been reported to accumulate various metals from their environments, indicating their potential for metal accumulation and biomonitoring. Previous *in vitro* investigations have demonstrated that metal exposure can affect bryophyte survival, multiplication, and physiological responses. In this study, cadmium (Cd), a toxic non-essential heavy metal, was applied in the form of cadmium acetate dihydrate ($\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) at concentrations of 0, 10, 100, and 200 μM to assess the *in vitro* responses of the two moss species to heavy metal stress. Cadmium acetate dehydrate exposure inhibited multiplication in both *D. aduncus* and *D. fluitans*, with the inhibitory effect increased significantly with increasing cadmium concentration. Survival was 100% at 10 and 100 μM in both species. In contrast, exposure to 200 μM cadmium acetate dihydrate had lethal effects on *D. fluitans* explants, whereas 54.17% of *D. aduncus* explants survived. Despite their ability to accumulate metals and tolerate metal-enriched environments, both species were sensitive to elevated cadmium concentrations, with *D. fluitans* exhibiting a lower tolerance than *D. aduncus*, although it is known to accumulate and tolerate arsenic in high amounts, for example.

KEYWORDS: bryophytes; *in vitro*; mosses; heavy metals; wetland

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Physiological Traits of the Axenically Grown Rare and Endangered Moss *Paludella squarrosa* (Meesiaceae): Effects of Growth Medium Type

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Paludella squarrosa (Hedw.) Brid. is a circumpolar boreo-arctic wetland moss species inhabiting calcareous wet fens, rare groundwater-fed peatlands characterized by cold, mineral-rich water with high calcium and magnesium content. The species is highly sensitive to habitat disturbance and degradation. On the European continent, it is classified as Near Threatened (IUCN: NT), while it is regionally extinct (IUCN: RE) in Great Britain and the Netherlands, critically endangered (IUCN: CR) in Ireland and Germany, and endangered (IUCN: EN) in Italy, the Czech Republic, Poland, Slovakia, Romania, and Ukraine. For *ex situ* conservation purposes, the first reported axenic culture of the species was established from gametophytes using material collected in Belarus, and optimization of *in vitro* cultivation followed. The experiment was conducted under controlled long-day conditions (16 h light), at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 18 °C. Several growth media were tested (KNOP, MS/2, BCD, Lukavsky, and Benecke), and physiological responses were assessed. Plants grown on MS/2 medium produced the highest number of new shoots, the largest secondary protonemal patches, and the highest number of buds on secondary protonemata. Furthermore, they exhibited the highest chlorophyll content. The maximum quantum yield of photosystem II (QY_{max}) was also highest under MS/2, although no significant differences in photochemical (qP) or nonphotochemical (NPQ) quenching were observed among plants grown on different media. The lowest pigment content, together with relatively low multiplication and QY_{max} values, was recorded on KNOP minimal medium. Further studies are on-going to support the development of effective *ex situ* conservation protocols for this threatened species.

KEYWORDS: conservation physiology; photosynthesis; morphogenesis; *in vitro*; bryophytes

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Effect of Different Growth Media on the Chemical Composition of *In Vitro* Cultivated Liverwort *Trichocolea tomentella*

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Trichocolea tomentella is a liverwort rare species scatterly distributed throughout Central and Western Europe, where its populations are categorized as Near Threatened. In addition to its ecological importance, *T. tomentella* is of particular interest due to its diverse chemical profile. Therefore, optimizing the growth of this species under *in vitro* conditions and further investigating its biological potential are of high significance. This study investigated the effects of different growth media (BCD, Benecke, KNOP, Lukavsky, and MS/4) on selected groups of chemical compounds and the reducing potential of *T. tomentella* under equal, controlled temperature, humidity and light conditions. The results showed that the Lukavsky and MS/4 media promoted the accumulation of total phenolic compounds. However, plants cultivated on KNOP medium synthesized higher levels of individual groups of phenolic compounds, particularly phenolic acids and flavonoids. In addition to KNOP and Benecke medium exhibited a stimulatory effect on the accumulation of total terpenoids in the investigated liverwort. Furthermore, triterpenoid compounds accumulated predominantly in plants cultivated on KNOP medium. These differences indicate that the composition of the growth medium can significantly influence the accumulation and distribution of individual groups of secondary metabolites in *T. tomentella*. The highest reducing potential was observed in plants cultivated on KNOP medium, suggesting enhanced antioxidant activity. Based on the monitored parameters, KNOP medium had the most pronounced overall effect on the synthesis and accumulation of several groups of chemical compounds, distinguishing it from the other media investigated. These findings highlight the importance of optimizing culture conditions for *T. tomentella* and provide a basis for further studies of its phytochemical composition and biological potential, as well as use it as green factory.

KEYWORDS: liverwort; secondary metabolites; antioxidant activity

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Stage-dependent Auxin Metabolic Profiles and *AUX1* Expression in Wild-type and Carotene-Deficient Strains of *Phycomyces blakesleeanus*

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Although auxin metabolism and homeostasis have been extensively characterized in plants, increasing evidence indicates that auxin-related metabolic processes may also contribute to fungal growth and development. Our previous study showed that exogenous indole-3-acetic acid (IAA) influences sporangiophore growth and tropic responses in the zygomycete fungus *Phycomyces blakesleeanus* and identified putative auxin-influx carrier genes, suggesting an endogenous auxin-related regulatory system. Here, we investigated stage-dependent variations in auxin profiles and *AUX1* gene expression in the wild-type (Wt) strain NRRL1555 (-) and two carotene-deficient mutants, C2 *carA5geo3* (-) and C148 *carA5madC119geo3* (-). Endogenous IAA, its biosynthetic precursors and intermediates, the oxidative catabolite oxIAA, and the conjugates IAA-Asp and IAA-Glu were analysed in mycelium and sporangiophores at stages I and IV. Auxin profiling revealed pronounced developmental and strain-specific variation. Significant strain × stage interactions for TRA, IAA, IAA-Asp, and IAA-Glu indicated that their developmental trajectories differed among strains. Detection of indole-3-pyruvic acid (IPyA) at all stages, together with indole-3-acetamide (IAM) only at stage IV, is consistent with the involvement of the IPyA pathway in IAA biosynthesis. Marked developmental changes in IAA and its catabolites and conjugates indicated active regulation of IAA metabolism during sporangiophore development. In addition, *AUX1* gene expression showed a stage-dependent pattern, with no significant difference between mycelium and stage I, followed by strong upregulation at stage IV. Collectively, these findings reveal stage- and strain-dependent modulation of auxin metabolic profiles alongside developmentally regulated *AUX1* gene expression, supporting auxin-associated regulation of sporangiophore development in *P. blakesleeanus*.

KEYWORDS: auxin biosynthesis and homeostasis; fungus; indole-3-acetic acid; mycelium; sporangiophore development

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Biostimulant Effect of Dihydroquercetin on Seed Germination and Early Growth of Soybean

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Seed germination and early seedling development are critical determinants of successful crop establishment and may be improved by the application of natural biostimulants. This study evaluated the effects of dihydroquercetin (DHQ) on seed germination and early seedling development in three soybean (*Glycine max* L.) cultivars, Beluga, Rubin, and Rovina. Seeds were treated with DHQ at concentrations of 0.01%, 0.05%, and 0.1% and germinated under controlled laboratory conditions. Germination percentage, shoot and root length, total seedling length, biomass, and seedling vigor indices were determined. The activities of two key enzymes of the antioxidant defense system, catalase (CAT) and peroxidase (POD), were also analyzed during early seedling development.

DHQ treatment significantly enhanced germination and seedling growth compared with the untreated control, with the magnitude of the response depending on treatment concentration and cultivar. The 0.1% DHQ treatment generally produced the strongest positive effects, resulting in higher germination percentage, greater root and shoot elongation, increased seedling biomass, and improved vigor indices. DHQ treatment also modified antioxidant enzyme activity, with CAT activity generally decreasing during seedling development and POD activity increasing. Treated seedlings exhibited higher enzyme activities than the control. Overall, the results show that DHQ positively affects soybean germination and early seedling development and may enhance antioxidant defense during seedling establishment. These findings support its potential use as a natural biostimulant for soybean seed treatment in sustainable crop production systems.

KEYWORDS: *Glycine max*; dihydroquercetin; seed germination; vigor index; oxidative stress

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Cellulose Fibril Organization of Parenchyma Cell Walls in Twining Stem of *Dioscorea balcanica* Studied by FDLD Microscopy

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Dioscorea balcanica Košanin is a liana plant whose stem undergoes pronounced mechanical and structural adaptation during growth and development, making it a suitable model for investigating cell wall organization associated with stems twisting. Fluorescence-detected linear dichroism (FDLD) microscopy enables the assessment of the preferential orientation and structural order of cellulose fibrils within plant cell walls. FDLD microscopy was applied to examine the relative orientation and structural order of cellulose fibrils in parenchyma cell walls of straight and twisted stem internodes. The analysis revealed no significant differences in the degree of cellulose fibril ordering between the examined internodes, indicating that stem twisting is not accompanied by substantial changes in the overall structural order of cellulose within parenchyma cell walls. These findings suggest that the mechanical adaptation of the twining stem may involve changes in cell wall organization other than alterations in the relative ordering of cellulose fibrils.

KEYWORDS: *Dioscorea balcanica*; cellulose; Fluorescence-detected linear dichroism (FDLD) microscopy; fibril order

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Effect of Biostimulants on the Growth of Date Palm Seedlings Differing in Initial Height

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Biostimulants are becoming increasingly important in agriculture, especially in recent years. They are substances or microorganisms that may stimulate natural plant processes, improve nutrient uptake, tolerance to abiotic stress, and crop quality, regardless of nutrient content. The aim of this study was to evaluate the effects of algae- and *Bacillus*-based biostimulants on the growth of date palm (*Phoenix dactylifera* L.) seedlings. One seed from commercially available fresh date fruits was sown per pot filled with plant-growth substrate. Fifteen weeks later, seedlings were classified into three groups based on their initial height and subjected to treatments by biostimulants, with five plants from each group per treatment. The experiment comprised 5 treatments: drenching with a algae- *Bacillus*-based solutions of biostimulants prepared in two concentrations each (as recommended and a tenfold higher), and a water as a control. The treatments were applied once a month, three times in total. During the experiment, plant height was measured weekly, as was the appearance (growth) of new leaves. Data were analyzed using one-way ANOVA, followed by Fisher's LSD test. These results suggest that the effectiveness of biostimulant application may vary depending on the developmental stage of the seedlings, and that should be taken into account in further research.

KEYWORDS: biostimulants; *Phoenix dactylifera*; *Ascomphyllum nodosum*; *Bacillus* spp.; plant growth

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The Effect of Black Soldier Fly Frass on The Growth of Kurtovska Kapia Pepper: A Preliminary Study

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Black soldier fly (*Hermetia illucens* L.) frass functions as a multifunctional fertilizer because, in addition to nutrients, it contains chitin and other bioactive compounds. Kurtovska kapia is a highly prized sweet red pepper variety throughout the Balkans. The aim of this preliminary study was to assess the effect of black soldier fly frass on the growth and physiological parameters of Kurtovska kapia pepper. The frass used in this study was obtained from larvae reared for 17 days on a mixture of vegetable waste and brewing industry by-products. Following larval rearing, the frass was separated by sieving, dried at 70 °C for 1 h, and subsequently passed through a 2 mm sieve to remove coarse fractions. Plants were grown under greenhouse conditions and treated with this frass 60 days after sowing. There were four treatment groups: a control group, two groups treated with frass powder at different concentrations, and a group irrigated with an aqueous frass solution. Plants treated with frass powder showed a lower maximum quantum yield of PSII (QY) and a higher H₂O₂ content in leaves compared with the other groups. In contrast, plants irrigated with the aqueous frass solution maintained QY values comparable to the control and reached the highest chlorophyll content (3.21 mg/g FW compared with 2.83 mg/g in the control and 1.77-1.99 mg/g in the powder treatments). These results indicate that the aqueous frass solution preserves the functional integrity of the photosynthetic apparatus in Kurtovska kapia, providing a baseline for further studies on molecular responses.

KEYWORDS: *Capsicum annuum*; plant biostimulation; insect manure; photosynthesis; *Hermetia illucens*

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Adaptations to Mechanical Strain/twining in Liana Plants – Sclerenchyma Cell Wall Structure in *Dioscorea balcanica* Košanin

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In response to gravitropic environmental stimuli, woody angiosperms develop tension wood (TW) on the tension side of the stem and opposite wood (OW) on the opposite side. In response to mechanical stimuli (unidirectional bending), they develop tensile flexure wood (TFW) and compressive flexure wood (CFW) on the tension and opposite (compression) sides of the stem, respectively. Both TW and TFW fibers, although showing some differences in cell wall structure/composition, are specialized sclerenchyma cells, gelatinous fibers (G-fibers), characterized by a specialized innermost cell wall layer, gelatinous layer (G-layer). G-fibers are also responsible for twining of liana stems; they appear only in the region of contact with the support and are absent or rare in regions of the stem that do not twine. Continuous high squeezing forces of liana stems during twining, bending, and fixing around the support (thigmomorphogenesis) represents mechanical stimuli/stress. We suggest *Dioscorea balcanica* Košanin, a monocotyledonous, perennial liana, an endangered species, and a Balkan Peninsula Tertiary relict, as a model plant for TFW and CFW formation/structure investigations. The cell wall structure/composition of the entire ring of sclerenchyma cells was studied on stem cross sections of *D. balcanica* twisted internodes using light microscopy, scanning electron microscopy (SEM), and Raman microspectroscopy. Our results indicate that adaptations to mechanical strain/twining in lianas rely largely on changes in compound middle lamellae (CML) composition, rather than the G-layer, and are more pronounced in CFW than in TFW fibers: variations in lignin, cellulose, hemicellulose, and pectin structure, and cellulose microfibril orientation.

KEYWORDS: cell wall; compressive flexure wood; gelatinous fibers; liana plants; tensile flexure wood

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ZnO Nanoparticle-Mediated Modulation of Maize (*Zea mays* L.) Growth

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Nanomaterial-mediated modulation of plant growth is an emerging area of plant physiology with potential applications in sustainable agriculture. This study investigated the effects of ZnO nanoparticles obtained by green sol-gel synthesis using *C. reticulata* extract and Zn-nitrate precursor, with thermal treatment at 400 °C for 4 h and without treatment on seed germination and early growth of maize. Surface-sterilized seeds were exposed to nanoparticles at 1, 5 and 10 mg/L, with distilled water as control. Germination was assessed after four days; followed by hydroponics in KNOP/2 medium containing 1 mg/L of nanoparticles, for eight days. Root and shoot length and fresh and dry biomass were determined. Statistical analysis was performed using the Mann-Whitney U test ($P < 0.05$). Studied nanoparticles had no effects on germination. Both treatments significantly promoted root elongation, with a stronger effect in thermally treated nanoparticles. Shoot length was unaffected. Non-thermally treated nanoparticles significantly increased the shoot DW/FW ratio, whereas this effect was not detected following thermal treatment, suggesting that thermal processing modified nanoparticle's properties (crystallinity, morphology, particle size) and their subsequent effects on above-ground biomass. Overall, the nanoparticles promoted root development, while their effects on biomass depended on applied nanoparticle and plant organ. These findings indicate the potential of Zn-based nanomaterials derived from agro-industrial waste as promoter of early maize growth and development.

KEYWORDS: ZnO nanoparticles; maize; DW/FW ratio; seed germination; root growth

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Wound-Induced Response of Endogenous Phytohormones in *Centaureum erythraea* Rafn Leaves

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The medicinal plant *Centaureum erythraea* Rafn (common centaury) is known for its remarkable morphogenic capacity in both root and leaf explants, which can be readily directed by different environmental drivers under *in vitro* conditions. Without exogenous phytohormone supplementation, centaury leaf explants can undergo spontaneous direct regeneration, forming shoots and roots at the wound site under the regulation of endogenous hormones. In contrast, indirect somatic embryogenesis and shoot organogenesis in centaury leaf cultures require the combined application of exogenous auxin and cytokinin.

Wounding is the initial event in plant regeneration, activating cellular plasticity and reshaping local tissue architecture. This study was undertaken to investigate phytohormone profiles in leaves and freshly cut leaf explants, aiming to link wounding stress to distinct types of regeneration in centaury leaf culture. Phytohormone analyses showed that the mechanical cutting of *C. erythraea* leaves triggers rapid shifts in the endogenous hormonal profile compared with intact control leaves. The results revealed instantaneous increases in both bioactive forms of jasmonates, jasmonic acid (JA) and JA-isoleucine (JA-Ile), immediately after wounding. An early, significant increase in the content of indole-3-acetic acid (IAA) was observed in leaf explants, while no significant changes in the content of three bioactive cytokinins (N6-(Δ^2 -isopentenyl)adenine, trans-zeatin, cis-zeatin) were detected. Our results suggest the involvement of jasmonates (JA, JA-Ile) and IAA in regulating wound-induced developmental pathways in centaury leaf culture. Further investigations will provide a detailed temporal characterization of hormonal changes during direct organogenesis and indirect somatic embryogenesis from centaury leaf explants.

KEYWORDS: wounding; jasmonic acid; indole-3-acetic acid

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Complementary LC/HRMS and MALDI-MSI Metabolite Profiling of *Nepeta nuda* L. Leaves

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Species within the genus *Nepeta* spp. are renowned for their high accumulation of specialized metabolites, dominated by monoterpene iridoid and phenolic compounds, which impart significant biological and chemotaxonomic importance to the taxon. Specialized metabolite profiles in *Nepeta* species are highly dynamic and tightly regulated by internal developmental programs, such as leaf ontogeny. Each leaf developmental stage represents a distinct physiological state defined by characteristic metabolic processes. Integrating analytical techniques, such as liquid chromatography (LC)/high resolution mass spectrometry (HRMS) and Matrix-Assisted Laser Desorption/Ionization (MALDI)/mass spectrometry imaging (MSI), enabled qualitative profiling of methanol-soluble metabolites and determination of their spatial distribution across *N. nuda* leaves at different developmental stages. This complementary approach provided a more comprehensive insight into the chemical profile, ontogeny-related metabolomic changes in leaves, and spatio-functional organization of metabolites, thereby overcoming the limitations of individual techniques. An analysis of cotyledons and leaves excised from potted *N. nuda* plants of the uniform developmental stages revealed dynamic spatial and temporal changes in specialized metabolite content. Most notably, caffeic acid and kaempferol demonstrated pronounced accumulation in the oldest true leaves, indicating a clear quantity – leaf maturity dependence. In contrast, glucosyringic acid preferentially accumulated in the embryonic cotyledons, while ascorbic acid showed maximal accumulation in the youngest apical leaves. In conclusion, leaf ontogeny shapes both the abundance and spatial organization of specialized metabolites in *N. nuda*, which, most likely, reflecting the physiological state of leaves. The integrated LC-HRMS/MALDI-MSI approach provides a powerful platform for comprehensive monitoring of developmental metabolomic dynamics.

KEYWORDS: *Nepeta nuda*; Mass Spectrometry Imaging; Leaf ontogeny; Spatial metabolomics

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Seed Germination of *Armoracia macrocarpa* – Preliminary Study

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Armoracia macrocarpa is a rare, endangered endemic species morphologically similar to *A. rusticana*, native to the marshes of the Central and Eastern Danube Basin. Seed germination is an important aspect of its biology, yet previous attempts of germination *in vivo* have been unsuccessful.

Due to the limited number of available seeds, a shortened experimental procedure (primarily proposed by Grubišić and coworkers) was used to identify optimal germination conditions. Aerial parts were collected at the Sava river wetlands near Belgrade (Serbia), kept in darkness at room temperature for two days, after which seeds were extracted and stored at 9 °C in darkness for two additional weeks.

Batches of 3×10 seeds were placed in solutions of KNO_3 (10^{-2} M) or GA_3 (10^{-3} M) and in distilled water at 15 °C or 22 °C and exposed to 15-minute white-light pulses every 2 days. Final germination was recorded after two weeks.

No germination occurred in distilled water. Nitrate produced only 3 % germination (at 15 °C in light). GA_3 induced 20 % germination at 22 °C and up to 30 % at 15 °C.

These results indicate that *A. macrocarpa* seeds are recalcitrant and must remain moist from collection until testing. They are positively photoblastic and nitrate-sensitive; gibberellin is the most effective germination stimulant among the treatments tested.

KEYWORDS: *Armoracia macrocarpa*; seed germination; gibberellic acid, nitrate; temperature

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How Does Growth Environment Influence the Long-Term Response of Lettuce to Cold Plasma Priming?

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Cold atmospheric plasma (CAP) is increasingly recognized as a sustainable seed-priming technology capable of enhancing plant performance without chemical inputs. While numerous studies have demonstrated positive effects of CAP on seed germination and early seedling development, considerably less is known about its long-term physiological consequences under different cultivation environments.

In this study, lettuce (*Lactuca sativa* L.) seeds were treated with radiofrequency cold plasma generated in ambient air at discharge powers of 50, 100, and 150 W. Germination kinetics, flowering dynamics, biomass accumulation, chlorophyll fluorescence parameters (OJIP analysis), and reflectance-based vegetation indices were evaluated during plant development. Plants were cultivated under two contrasting environments: a controlled phytotron and a semi-controlled greenhouse with naturally fluctuating conditions. Cold plasma treatment consistently accelerated germination compared with untreated controls, with only minor differences among discharge powers. However, the long-term physiological responses strongly depended on the cultivation environment. Under stable phytotron conditions, plants originating from plasma-treated seeds exhibited delayed flowering, increased shoot biomass, and improved photosynthetic performance, particularly at higher plasma powers (100–150 W). Enhanced chlorophyll fluorescence and reflectance characteristics indicated improved acclimation and prolonged vegetative growth. In contrast, greenhouse-grown plants showed weaker and more variable responses. High-power plasma treatments promoted earlier flowering, while biomass accumulation remained comparable to controls. Physiological measurements suggested transient stress responses followed by recovery rather than sustained stimulation.

Our results demonstrate that environmental stability is a key factor determining the outcome of cold plasma priming. Although CAP effectively enhances germination across conditions, its long-term benefits for plant growth and photosynthetic performance are primarily expressed under stable cultivation environments. These findings highlight the importance of considering environmental context when evaluating the agronomic potential of plasma-based seed treatments.



METABOLISM AND BIOCHEMISTRY

How Light and Temperature Affect Glandular Trichome Development and Metabolism in Tomato

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Trichomes are epidermal differentiations that contribute to the protection of plants against biotic and abiotic aggressions. Tomato (*Solanum lycopersicum* and related wild species) constitutes a nice model to study trichomes, because of the multiple types of trichomes present, both glandular and non-secretory, and the role they play for example in the resistance against herbivores. Type I and IV capitate trichomes produce and secrete esters of sucrose and short to medium chain fatty acids, also known as acylsugars, which are sticky and help trapping insects on the leaf surface. Type VI trichomes produce terpenes that are stored in an intercellular cavity, which releases its content upon contact. Some of the terpenes produced were shown to protect the plant against white flies. In cultivated tomato, type I/IV trichomes are a juvenile trait and are practically absent from the third and fourth leaves onwards. While growing tomato plants at high temperature (37°C), we observed that type IV trichomes start developing even on newly developing leaves, partially replacing the non-glandular type V trichomes. Phenotyping of various hormone and light signaling mutants show that this developmental switch is dependent on jasmonate signaling. Furthermore, during seed germination and early seedling development the hypocotyl and the cotyledons develop a high density of type IV trichomes. Interestingly in the dark, very few trichomes develop. Using known mutants in light signaling genes (phytochrome, HY5, PIFs) we show that phytochromes and HY5 play a positive role while PIFs act as repressors of trichome development. We then asked the question whether the light signaling genes also played a role in trichome development and metabolism in adult leaves. Contrasting effects were observed on type VI trichomes and the biosynthesis of terpenes. Our results provide new insights on the regulation of trichome development and metabolism by abiotic factors and provide opportunities to breed tomato plants with improved surface defense via glandular trichomes.

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Unveiling the Molecular Complexity of Plant Biopolymers With Ultrahigh-resolution FT-ICR-MS

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Plant-based biopolymers, such as lignins and hemicelluloses, are highly abundant, renewable resources vital for sustainable material applications. However, their efficient utilization is hindered by pronounced chemical complexity and structural heterogeneity, which strongly depend on their specific plant origins and isolation processes. To overcome this limited structural understanding, advanced analytical approaches are required. For that purpose, biopolymers obtained from diverse plant sources via various extraction and fractionation strategies were analyzed using ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). To capture both soluble (polar) and insoluble (less polar) molecules, complementary ionization techniques were applied, including electrospray ionization (ESI) and graphite-assisted laser desorption/ionization (GALDI). The mass spectral data were further evaluated for molecular formula distributions, heteroatomic composition, oligomeric patterns, and dominant structural motifs. Additional resolution was achieved via tandem MS and chemometric data fusion with complementary datasets. Ultimately, FT-ICR-MS enabled a highly detailed, molecular-level comparison, revealing distinct compositional fingerprints that reflect variations in monomer composition, polymerization degree, and functionalization. Combining multiple ionization techniques and tandem MS significantly expanded the accessible chemical space. Overall, this approach highlights the immense potential of FT-ICR-MS as a powerful tool for the advanced structural analysis and comparative assessment of complex plant-based biopolymer systems.

KEYWORDS: lignin; hemicellulose; FT-ICR-MS; ESI; GALDI

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Basil (*Ocimum* L.) Tissue Culture: A Tool for the Production and Characterization of Secondary Metabolites

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The utilization of basil (*Ocimum* L.) plants is mostly associated with the production of biologically active secondary metabolites, in particular essential oils, phenolic acids, and flavonoids. Still, the accumulation of these compounds varies depending on genotype and cultivation conditions. Experiments with several basil cultivars demonstrated that manipulation of mineral nutrition in seedling culture can significantly enhance phenolic acid accumulation and antioxidant capacity. Callus and cell suspension cultures established from different basil cultivars using various plant growth regulators exhibit distinct growth and biochemical responses, demonstrating that culture type and cultivation conditions influence the production of secondary metabolites. Raman spectroscopy was additionally investigated as a rapid, non-invasive and non-destructive approach for detecting changes associated with secondary metabolism. Phenolic compounds represented major contributors to the Raman spectra and were associated with the highest peak intensities, highlighting the potential of Raman spectroscopy for detecting changes in basil phenolic metabolism under *in vitro* conditions. Untargeted metabolomic analysis revealed clear metabolic differences between callus culture and cell suspension cultures. A total of 1949 metabolites were detected, including 60 phenolic compounds, with distinct profiles of hydroxycinnamic acids, phenolic glycosides, benzofuran derivatives, triterpenoids, and methylated flavonoid derivatives. Overall, the results demonstrated that basil tissue culture represent a promising platform for studying and enhancing secondary metabolite production, in particular phenolic acids and flavonoids. The integration of *in vitro* cultivation and manipulation, Raman spectroscopy and metabolomic profiling provide a comprehensive approach for the enhanced production, rapid screening and detailed characterization of basil bioactive compounds.

KEYWORDS: basil; callus culture; cell suspension; Raman spectroscopy; untargeted metabolomic; phenolic profile

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Exploring the Bioactive Profile of Elicited *Morus alba* Cell Cultures

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Methyl- β -cyclodextrin, alone and combined with methyl jasmonate, is an effective elicitor of stilbene phytoalexin production in grapevine cell cultures, leading to the extracellular accumulation of high amounts of resveratrol. Besides Vitaceae, Moraceae is another of the approximately twenty botanical families able to produce stilbenes that display multiple pharmacological properties. Here, we applied this elicitor treatment to *Morus alba* liquid cell cultures and separated the cells from the culture medium to characterize their respective stilbene metabolite profiles. Both fractions were studied using a combination of metabolite analysis techniques. Cell extracts were profiled via LC-MS-based metabolomics, while the culture medium was first processed by liquid-liquid extraction and flash chromatography, followed by MS (Precursor Ion Scan, Single Reaction Monitoring, and Product Ion Scan) and NMR (¹H and ¹³C) experiments. The results showed the presence of the glycosylated stilbenes mulberroside A and piceid exclusively in the intracellular medium, whilst stilbene aglycones (resveratrol and oxyresveratrol) were detected in both the cells and the extracellular medium, with their identity being confirmed using authentic standards. Moreover, MS and NMR data provided evidence of the presence of their respective prenylated (isopentenyl/dimethylallyl) derivatives, as well as chalcones, moracins, and their prenylated derivatives. The use of commercially available authentic compounds confirmed the presence of isoliquiritigenin, isobavachalcone, moracin M, and an isomer of morachalcone A, etc. The identification of other unknown or unconfirmed compounds is currently underway.

KEYWORDS: bioactive profile; cell elicitation; metabolomics; *Morus alba*; stilbenes

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Chemical Composition and Antioxidant Potential of *Raphidiopsis raciborskii* Strains

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Cyanobacteria are important producers of diverse primary and secondary metabolites with potential biological activities. This study aimed to evaluate the chemical composition and antioxidant potential of eight strains of the cyanobacterium *Raphidiopsis raciborskii* isolated from freshwater habitats. Hydromethanolic (75% MeOH) extracts were analysed for total phenolic compounds (TPC), proteins (TPR), and polysaccharides (TPS), while antioxidant properties were evaluated using DPPH and ABTS radical-scavenging assays and the ferric reducing antioxidant power (FRAP) assay. Samples collected in the exponential and stationary growth phases under laboratory conditions were analysed, allowing for a comparison of the biochemical and antioxidant profiles between the two cultivation periods. Considerable variability was observed among the analysed extracts. TPC ranged from 3.41 to 7.80 mg gallic acid equivalents/g extract, with the highest value detected in the UAM/DH-MRr strain. TPR reached 41.51 mg BSAE/g extract, while the highest TPS was recorded for CS-505, reaching 45.17 mg glucose equivalents/g extract. Phenolic content and DPPH-scavenging capacity were generally higher in exponential phase, whereas polysaccharide content and ABTS-scavenging capacity generally increased in stationary growth phase. The highest DPPH-scavenging capacity was observed for CS-506, with 6.37 mg Trolox equivalents/g extract, while the highest ABTS value was recorded for UAM/DH-PnRr, reaching 105.08 mg Trolox equivalents/g extract. The highest FRAP value was detected for CS-506 (31.07 mg ascorbic acid equivalents/g extract), with no consistent relationship with growth phase. Overall, the divergent phase-dependent patterns suggest changes in metabolic allocation during cultivation and highlight the strain-dependent biochemical and antioxidant potential of *R. raciborskii*.

KEYWORDS: *Raphidiopsis raciborskii*; cyanobacteria; phenolic compounds; antioxidant activity; bioactive compounds

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Transcriptomic and Metabolomics Insights into the Flower Color Polymorphism of Two *Centaureum* Species

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Two closely related species of the genus *Centaureum*, *C. pulchellum* (Sw.) Druce and *C. tenuiflorum* (Hoffmanns. & Link) Fritsch, exhibit flower color polymorphism with pink- and white-flowered individuals occurring in natural populations. Following comprehensive metabolomics profiling, cyanidin- and delphinidin-type anthocyanins were recognized as important contributors to the pigmentation of petals of the two *Centaureum* species. To investigate the flower color variation of *C. pulchellum* and *C. tenuiflorum* at the transcriptional level, RNA-seq of flowers was performed. *De novo* transcriptome assembly was searched for differentially expressed genes (DEGs) using DESeq2 package and a Benjamini-Hochberg procedure adjusted to $p < 0.05$, which resulted in the identification of 8 candidate DEGs in *C. pulchellum* and 20 in *C. tenuiflorum*, annotated to the biosynthesis pathways of flavonoids and anthocyanins. Among the eight candidate DEGs identified in *C. pulchellum*, three were annotated as structural genes of the flavonoid biosynthetic pathway, encoding chalcone synthase (CHS), flavanoid 3-hydroxylase (F3'H), and UDP-glycosyltransferase (UGT), all showing higher expression in pink-flowered individuals. A MYB-related regulatory gene encoding a MYB62-like protein, associated with repression of anthocyanin accumulation, was significantly upregulated in white flowers, suggesting its potential contribution to reduced pigmentation. In contrast, in *C. tenuiflorum* gene ontology annotation identified seven DEGs encoding MYB transcription factors (TFs), eleven encoding transporter proteins, including ABC transporters and glutathione S-transferases which showed statistically significant lower expression in white-flowered individuals. Overall, results suggest species-specific transcriptional regulation of biosynthesis, transport, and vacuolar accumulation of flower pigments, and highlight MYB TFs as important regulatory points determining flower color in *C. pulchellum* and *C. tenuiflorum*, thus establishing a valuable foundation for further investigation of the molecular basis of floral polymorphism in *Centaureum* species.

KEYWORDS: *Centaureum*; flower polymorphism; anthocyanin biosynthesis; differential gene expression; RNA-Seq

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Trace Element Profile and Health Risk Assessment of Organic and Conventional Buckwheat Grains

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Sustainable food production has gained increasing importance worldwide due to the environmental and health concerns associated with intensive conventional agriculture. Buckwheat (*Fagopyrum esculentum* Moench), valued for its nutritional properties, has attracted increasing attention in both organic and conventional production systems. The aim of this study was to compare the concentrations of selected trace elements and to evaluate the potential health risks associated with the consumption of buckwheat grains produced under different cultivation practices. The concentrations of As, Ba, Co, Li, Sb, and Se were determined by using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) following previous digestion of samples, and the results were expressed as $\mu\text{g g}^{-1}$ of dry weight. Lithium was the predominant trace element in both production systems, accounting for 93.39% and 92.55% of the total trace element content in organic and conventional buckwheat, respectively. The total concentration of trace elements was approximately 10.2% higher in conventionally produced grains. Conventional buckwheat contained higher concentrations of Ba, Li, and Sb, whereas organic grains showed higher levels of Co and Se. Arsenic was detected only in organic grains, while its concentration in conventional samples was below the detection limit. Estimated Daily Intake and Health Risk Index values calculated for arsenic and antimony were below the corresponding reference doses. HRI values lower than 1 indicated that buckwheat consumption does not pose a significant health risk with respect to these potentially toxic elements. The obtained results suggest that both production systems provide safe grains regarding the investigated trace elements and their dietary exposure.

KEYWORDS: *Fagopyrum esculentum*; trace elements; ICP-OES; organic production; health risk assessment

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Phenolic Profiling and Response Surface Optimisation of *Laser trilobum* Extracts Obtained With Ultrasound Assisted Extraction

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Laser trilobum is an Apiaceae species whose phenolic composition and the factors affecting the levels of individual constituents remain insufficiently characterised. In this study, the phenolic profile of ethanolic extracts obtained by ultrasound-assisted extraction (UAE) was characterised using UHPLC(–)HESI–QqQ–MS/MS, while response surface methodology (RSM) was applied to evaluate the effects of extraction parameters on the yields of selected phenolic compounds. Eighteen extracts were prepared using different ethanol concentrations (50%, 70%), extraction temperatures (20°C, 30°C, 40°C), and extraction times (5 min, 15 min, 25 min). UHPLC–MS/MS analysis revealed a diverse phenolic profile, with variation in the concentrations of individual phenolic compounds depending on the extraction conditions, highlighting the importance of solvent composition and UAE parameters in determining the chemical composition of *L. trilobum* extracts. RSM analysis further demonstrated compound-specific responses to the extraction variables. The extraction yields of 5-*O*-caffeoylquinic acid and quercetin 3-*O*-glucoside were predominantly affected by ethanol concentration, with increasing yields observed as ethanol concentration increased from 50% to 70%. In contrast, extraction temperature and time had comparatively limited effects on the recovery of these compounds. The obtained response models described the relationships between extraction parameters and individual phenolic yields and provided a basis for defining extraction conditions aimed at maximising the yield of selected compounds. Overall, the results demonstrate that combining UHPLC–MS/MS profiling with RSM is an effective approach for characterising and optimising the selective extraction of phenolic compounds from *L. trilobum*.

KEYWORDS: *Laser trilobum*; ultrasound extraction; RSM

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Identification of a Novel Ascorbate Transporter in the Chloroplasts

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Plants require light, but excess light can generate reactive oxygen species (ROS). Ascorbate (Asc) is vital for ROS scavenging, photoprotection via non-photochemical quenching (NPQ), and acts as an alternative electron donor for photosystem II (PSII). Currently, only two ascorbate transporters are known: *AtDTX25* in vacuoles and *AtPHT4;4* in chloroplasts. Interestingly, *Arabidopsis thaliana* mutants lacking *PHT4;4* do not exhibit severe phenotypes, suggesting that other transporters may facilitate Asc transport across the chloroplast envelope.

Our previous experiments showed that *PHT4;2* expression increases under high-light stress in both *pht4;4* and low-Asc *vtc2-4* mutants, suggesting that *PHT4;2* may contribute to chloroplastic Asc transport. To explore this, we examined *Arabidopsis* wild-type (Col-0), *vtc2-4*, *pht4;4*, and *pht4;2* single mutants, and generated *pht4;4* x *pht4;2* double mutants. We estimated the relative chloroplastic Asc content by measuring the rate of Asc-dependent electron donation to PSII via fast chlorophyll *a* fluorescence kinetics and characterized photosynthetic activity under various light conditions.

NPQ, which is highly sensitive to the availability of chloroplastic Asc, was reduced in the *pht4;2* and double mutants. The total cellular Asc, quantified using HPLC, was similar in the wild-type and the *pht4* mutants, whereas it was severely reduced in the *vtc2-4* mutants, as expected. Total foliar phosphate content was similar across genotypes, while the *pht4;2* and double mutants showed a reduced violaxanthin de-epoxidation index.

Our results suggest that *PHT4;2* acts as a chloroplast Asc transporter. We are validating its activity via heterologous yeast expression and examining the relative expression of other *PHT4* genes under various light conditions.

KEYWORDS: Ascorbate transporter; Chloroplast; Photoprotection; *PHT4;4*; *PHT4;2*

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Aliphatic Glucosinolate Metabolism is the Main Distinctive Feature Between Kale (*Brassica oleracea* var. *acephala*) and its Wild Relative (*Brassica incana*)

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The wild Brassica representative *Brassica incana*, which encompasses several taxa formerly described as local endemic species, has been identified as the predominant wild *Brassica* species in the Adriatic coastal region (Croatia). All these populations inhabit sea cliffs and sandy, barren limestone rocks, and are capable of surviving under extremely harsh environmental conditions, including high salinity, elevated temperatures, and prolonged drought. Wild Brassica species are morphologically similar to leafy kale (*Brassica oleracea* var. *acephala*) and share the same type of genome (C) as domesticated *B. oleracea*. Here, we aim to identify fundamental, genetically determined differences in the metabolomic and transcriptomic profiles of kale (*B. oleracea* var. *acephala*) and its close wild relative, wild cabbage (*B. incana*), when grown under the same control conditions.

The underlying differences in the metabolomic and transcriptomic profiles of kale and wild cabbage accessions highlighted glucosinolate (GSL) metabolism as the most distinctive feature. At the metabolomic level, wild cabbage is particularly rich in aliphatic, long-chain (4C and 5C) GSLs compared with kale. Wild cabbage accessions are therefore enriched in upregulated genes involved in GSL biosynthesis. The majority of upregulated GSL genes in wild cabbage accessions function in aliphatic GSL biosynthesis, particularly in side-chain elongation and the early stages of aldoxime formation. Furthermore, genes involved in side-chain modification of aliphatic GSLs are significantly upregulated in wild cabbage accessions compared with kale. Further research will show whether these specialized metabolites are involved in abiotic stress tolerance.

KEYWORDS: Brassicaceae; wild cabbage; kale; glucosinolates

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Integrated Kinetic and Computational Characterization of Phloretin-Mediated Inhibition of Horseradish Peroxidase

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Horseradish peroxidase (HRP) is widely used in biotechnology, diagnostics, biosensing, and bioremediation, making the mechanistic characterization of compounds that modulate its activity of considerable interest. Phloretin, a naturally occurring dihydrochalcone, may modulate enzyme function, yet its interaction with peroxidases remains insufficiently characterized. Here, we investigated the inhibition of HRP by phloretin using an integrated approach combining enzyme kinetics, native PAGE, molecular docking, and molecular dynamics simulations. Phloretin inhibited HRP activity in a concentration-dependent manner, with an IC_{50} of 342.8 μ M. Michaelis-Menten and Lineweaver-Burk analyses showed a progressive increase in apparent K_m , from 6.19 to 20.15 mM, while V_{max} remained essentially unchanged. Global nonlinear fitting yielded a K_i of 255.2 μ M, supporting a competitive inhibition mechanism. Native PAGE confirmed a dose-dependent reduction in HRP activity. Molecular docking identified the most favorable phloretin-binding pose within the catalytic heme-containing active site, with a binding energy of -8.31 kcal mol⁻¹ and hydrogen-bond interactions involving Arg38 and Ala140. During the 250 ns molecular dynamics simulation, the HRP backbone RMSD stabilized at approximately 1.5–2.0 Å, while phloretin conformational RMSD remained below 1.0 Å. A stable radius of gyration and persistent hydrophobic and hydrogen-bond interactions further supported complex stability. The convergence of kinetic and computational data supports a coherent inhibitory mechanism in which phloretin forms a stable complex within the catalytic site and competes with the substrate for binding. Together, these findings identify phloretin as a direct competitive inhibitor of HRP and provide molecular insight into the structural determinants of this enzyme–inhibitor interaction.

KEYWORDS: competitive inhibition; enzyme kinetics; molecular docking; molecular dynamics; heme active site.

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Novel Cardenolide-type Compound From *Digitalis grandiflora* Mill.

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Digitalis grandiflora belongs to the genus *Digitalis* (foxgloves), whose representatives have been reported to exhibit a wide range of biological activities, including antibacterial, antiviral, antioxidant, anti-inflammatory, anticarcinogenic, and are well known for their effects in the treatment of cardiovascular disorders. Cardenolides, the major group of bioactive metabolites present in the genus *Digitalis*, are steroidal compounds within the cardiac glycosides class which exert cardiotonic effects on myocardial cells primarily by binding to the Na⁺/K⁺-ATPase pump, thereby inhibiting this essential ion-transport enzyme. A phytochemical investigation of the butanolic extract of aerial parts of *D. grandiflora*, collected in several localities within the Central Balkans, resulted in the isolation of a novel cardenolide, gitoxigenin 3-O-[α-L-glucopyranosyl-(1→2)-β-D-3-O-methyl-fucopyranoside]. The separation procedure involved fractionation on a Sephadex® column, followed by silica gel column chromatography, and further purification by preparative reverse-phase high-performance liquid chromatography (prep RP-HPLC). A total of 7.14 mg of this compound was finally obtained as small white block crystals from methanol. The structure of the isolated compound was elucidated by high resolution mass spectrometry (HRMS), 1D- and 2D NMR spectroscopic data, and further confirmed by single-crystal X-ray diffraction (SCXRD) analysis. Considering the potent biological activities reported for this type of compounds, the newly isolated compound should be further evaluated for its effects on myocardial cells.

KEYWORDS: yellow foxglove; cardenolide; column chromatography; prep RP-HPLC; SCXRD

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Dual Activity of Versatile Peroxidase on Lignin Model Compounds

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Lignocellulosic biomass depolymerization using enzymes offers an environmentally friendly pathway to produce biofuels, bioplastics, and other value-added products. Versatile peroxidase (VP) is a ligninolytic enzyme that can directly oxidize complex aromatic polymers without a mediator. Its hybrid structure combines the catalytic properties of both manganese and lignin peroxidases. This study investigates the interaction between VP from *Bjerkandera adusta* and a synthetic lignin model, dehydrogenate polymer (DHP), synthesized from coniferyl alcohol. We treated DHP with VP for 96 hours in an acetate buffer solution with 5% DMSO, adding H₂O₂ every 24 hours. The reaction mixture was separated by ultrafiltration and analyzed by liquid chromatography-mass spectrometry (RP-HPLC-MS). While VP did not significantly change the total yields of the major fractions, detailed chromatographic analysis revealed dynamic shifts in oligomeric composition. We identified the peaks of higher-mass intermediates that were systematically cleaved into fundamental monomers. The results suggest that VP works in simultaneous, two-way catalytic reactions, acting on both oligomer synthesis and breakdown. This dual radical-driven polymerization/depolymerization loop highlights the complex kinetic equilibrium of VP. These findings provide valuable biochemical insights into lignin depolymerization, offering a green pathway to enhance target-oriented lignin valorization without adding harsh chemicals.

KEYWORDS: versatile peroxidase; lignin model compounds; enzymatic depolymerization

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The Influence of Different Priming Methods on the Phenolic Profile of Barley

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Barley (*Hordeum vulgare* L.) is an important cereal crop with diverse applications in food and agricultural production and a valuable source of bioactive compounds. Phenolic compounds, including phenolic acids and flavonoids, contribute to antioxidant capacity and plant responses to oxidative stress, and their content and composition may change during germination and early plant development. Seed priming enables controlled activation of metabolic processes prior to sowing and may influence the accumulation and profile of bioactive compounds. Therefore, this study investigated the effects of different seed priming methods on the total content and qualitative and quantitative composition of phenolic compounds during the early stages of barley growth and development. The results showed that the applied priming treatments stimulated the accumulation of total phenolic compounds and flavonoids and significantly modulated the qualitative and quantitative profiles of individual phenolic constituents. In control seedlings, gallic, sinapic, and chlorogenic acids predominated, while 4-HBK acid was additionally detected following halo- and hormoprimering. Redox priming with H₂O₂ increased p-coumaric acid content 20-fold, whereas hydropriming increased ferulic acid content 10-fold and H₂O₂ treatment threefold. Among flavonoids, naringin, epicatechin, and catechin predominated in control seedlings, while rutin was additionally detected following priming. GA₃ had the strongest effect on catechin and epicatechin contents, hydropriming on naringin content, and hormoprimering altered naringenin content. Overall, these findings demonstrate the potential of seed priming as an effective strategy for modulating phenolic metabolism and enhancing the accumulation of selected bioactive compounds during early barley growth and development.

KEYWORDS: *Hordeum vulgare* L.; seed priming; phenolic acids; flavonoids

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Effects of Tree Age, Sex and Location on the Biflavonoid Profile of Ginkgo (*Ginkgo biloba* L.) Leaves

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Biflavonoid accumulation in ginkgo (*Ginkgo biloba* L.) leaves varies considerably among individual trees, but the contributions of tree age, sex, and growing location to this variability remain insufficiently understood. To identify its major determinants, five characteristic ginkgo biflavonoids—amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin – were quantified in leaves collected from 90 trees differing in age, sex, and geographical origin. Sciadopitysin was the predominant compound (1532.89 ± 544.13 µg/g dry weight), followed by isoginkgetin, ginkgetin, bilobetin, and amentoflavone. Principal component analysis explained 71.5% of the total variance and distinguished an overall gradient of biflavonoid accumulation from variability associated with individual compounds and local growing conditions. Piecewise linear regression models explained 75–83% of the observed variation and identified tree age as the most consistent predictor of biflavonoid accumulation. Concentrations were generally highest in trees aged 10–30 years and declined in older individuals, indicating an age-dependent shift in secondary metabolism. Tree sex and growing location also contributed to the variability of individual biflavonoids, although their effects were nonlinear and less consistent than the effect of age. Overall, the results demonstrate that biflavonoid accumulation in ginkgo leaves is strongly influenced by ontogenetic factors and suggest that younger trees may represent a particularly valuable source of biflavonoid-rich plant material.

KEYWORDS: biflavonoids; ginkgo; tree age; sexual dimorphism; secondary metabolism

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Metabolomic Changes in *Solanum lycopersicum* L. Seedlings Induced by *Nepeta nuda* L. Extract and Its Major Iridoid Glycoside

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Species of the genus *Nepeta* L. are recognized as rich sources of structurally and stereochemically diverse monoterpenoid iridoids, particularly nepetalactones, which exhibit pronounced pharmacological and biopesticidal properties. However, the occurrence and bioactivity of iridoid glycosides within *Nepeta* species remain insufficiently explored, despite their considerable biological potential. In this study, the iridoid glycoside 1,5,9-*epi*-deoxyloganic acid (1,5,9-eDLA) was isolated and characterized from the aerial parts of *Nepeta nuda* L. (Lamiaceae). The dried plant material was subjected to maceration with methanol. The resulting crude extract was suspended in water and successively partitioned with chloroform and *n*-butanol. The *n*-butanol fraction was purified using a combination of Sephadex LH-20 gel chromatography, dry flash chromatography, and semi-preparative HPLC. The chemical structure of the isolated 1,5,9-eDLA was unequivocally confirmed by 1D (¹H and ¹³C) and 2D (COSY, HSQC, HMBC, and NOESY) NMR spectroscopy. Furthermore, effects of various concentrations of the *N. nuda* methanol extract and isolated 1,5,9-eDLA were evaluated on seed germination and seedling growth of *Solanum lycopersicum* L. (Solanaceae). The *N. nuda* extract at a concentration of 0.1 mg/mL stimulated radicle elongation, whereas 1,5,9-eDLA simultaneously stimulated radicle growth and inhibited hypocotyl growth when applied at a concentration of 0.3 mg/mL. Metabolomic changes in the *S. lycopersicum* seedlings were investigated using high-performance liquid chromatography coupled with Orbitrap mass spectrometry. Alterations in the polyphenolic profile of *S. lycopersicum* seedlings suggested the activation of antioxidant defense mechanisms in response to *N. nuda* constituents, further suggesting possible phytotoxic effects of the tested extract and iridoid glycoside.

KEYWORDS: *Nepeta nuda* L.; iridoid glycosides; 1,5,9-*epi*-deoxyloganic acid; phytotoxicity; *Solanum lycopersicum* L.

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Steroid Receptor-Binding Profiles of the Cyanobacterium *Raphidiopsis raciborskii*: Effects Of Strain Origin and Growth Phase

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Cyanobacteria are recognized as producers of chemically diverse metabolites with the potential to interact with hormone-responsive molecular targets. In this study, the steroid receptor-interacting profiles of eight strains of the cyanobacterium *Raphidiopsis raciborskii*, isolated from freshwater habitats in Australia (CS-506, CS-505), Poland (UAM/DH-KmRr, UAM/DH-BiRr, UAM/DH-MRr, UAM/DH-BoRr, UAM/DH-PnRr), and Israel (KLL07), were investigated using a yeast-based fluorescent assay. Samples collected in exponential and stationary growth phase were analysed, allowing comparison of receptor responses in relation to the growth phase. Relative responses were evaluated toward the ligand-binding domains (LBDs) of estrogen receptor α (ER α), estrogen receptor β (ER β), androgen receptor (AR), and glucocorticoid receptor (GR). Pronounced differences were observed among strains and between growth phases, with receptor-specific response patterns. The highest ER α responses were recorded for UAM/DH-KmRr collected in both growth phases whereas samples UAM/DH-BiRr and KLL07 showed particularly pronounced responses toward ER β . AR responses were generally lower, with both Australian strains exhibiting the highest values. In contrast, several strains showed pronounced responses toward GR, particularly KLL07 and UAM/DH-PnRr. Overall, the results demonstrate substantial variability in the steroid receptor-interacting profiles of *R. raciborskii* strains and indicate that both strain origin and growth phase may influence the observed receptor-related responses. These findings highlight *R. raciborskii* as a relevant source for further investigation of cyanobacterial metabolites with endocrine-related molecular activity.

KEYWORDS: *Raphidiopsis raciborskii*; cyanobacteria; steroid receptors; receptor binding; culture age

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PLANT STRESS PHYSIOLOGY

Seed Priming as a Strategy to Improve Maize Resilience to Hypoxia

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Hypoxia is an important environmental factor that negatively affects metabolism, cellular redox homeostasis, and ultimately plant growth and yield. Low oxygen concentration interrupts mitochondrial respiration and, consequently, energy metabolism, and can cause excessive production of reactive oxygen species (ROS), leading to oxidative stress. Seed priming is a promising strategy for improving plant tolerance to environmental stress by enabling more efficient activation of defence mechanisms. In this study, we investigated whether priming with menadione sodium metabisulfite (MBS), hydrogen peroxide and chitosan can modulate antioxidant responses and improve maize (*Zea mays* L.) tolerance to three days of hypoxia.

Maize plants were grown under controlled and low-oxygen conditions after seed treatment for 24 h with three priming agents. To assess the oxidative status of the samples, we measured the concentrations of hydrogen peroxide and malondialdehyde in maize leaves and roots. At the same time, antioxidant defence was characterised by determining the activities and isoform profiles of superoxide dismutase, catalase, and class III peroxidases. Hypoxia induced an oxidative response in maize, increasing the concentration of H₂O and malondialdehyde and enhancing the activity of major antioxidant enzymes. However, the intensity and nature of responses strongly depended on the priming treatment and plant tissue. MBS provides the strongest protective effect by increasing antioxidant activity, limiting oxidative damage, and helping maintain antioxidant enzyme isoform diversity under hypoxia. H₂O₂ and chitosan also modified the antioxidant response, although their effects were more variable and tissue-dependent. These results provide new insights into the biochemical basis of priming-induced stress tolerance and highlight the potential of priming as a strategy for improving crop resilience to oxygen-limited environments.

KEYWORDS: menadione sodium metabisulfite; H₂O₂; chitosan; superoxide dismutase; peroxidase class III

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Decoding Chilling Resilience: Molecular, Biochemical, and Phenotypic Adaptations During Early Maize Development in Standard and Quality Protein Maize

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Escalating climate change has heightened environmental instability—marked by rising temperatures, erratic rainfall, and extreme weather events—that threatens global maize production by disrupting reproductive phases through heat stress and water scarcity. To counter this, farmers increasingly rely on early-sowing practices for crops to complete sensitive flowering stages before peak summer heat and drought arrives. However, this exposes early-stage seedlings to cold stress and delayed germination, making the identification of cold-resilience mechanisms vital for developing resilient genotypes. Addressing this challenge, two distinct research approaches to investigate maize cold tolerance were applied: the first approach focuses exclusively on standard lines at a molecular level, examining the transcriptomic profiles (mRNA and small RNAs) and short-term physiological-biochemical traits of 5-day-old seedlings at 6 and 24 hours post-stress, while the second approach evaluates four QPM inbred lines under dynamic low-temperature stress—tested in the growth chamber up to the V3 stage and in the field through to final harvest—across morphological, physiological, biochemical, and yield as well as yield components, with these evaluations currently underway. By combining the established molecular insights of standard lines with upcoming findings from the multi-stage chamber and field evaluations of QPM lines across varying sowing dates, this research framework could comprehensively illuminate how early-season low-temperature stress impacts crop development, generating crucial insights that could eventually support marker-assisted breeding of cold-tolerant germplasm tailored for heat-avoidance, early-sowing cropping systems. The current findings from these studies will be presented.

KEYWORDS: Maize; cold resilience; molecular traits; biochemical traits; phenotypic adaptations

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Illuminating Microalgae With Synchrotron Light

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Investigations of microalgae using synchrotron-based methods have contributed to a comprehensive understanding of cellular processes and responses to environmental stress. Microalgae exhibit remarkable tolerance to diverse stressors, and synchrotron techniques enable these responses to be investigated through complementary imaging and spectroscopic approaches. Element-specific methods can reveal detoxification mechanisms, including the uptake, transformation, and intracellular storage of potentially detrimental metals such as Cu, Mn, and Ni.

The combination of microscopy and spectroscopy using highly brilliant X-ray sources provides complementary information on elemental distribution, chemical speciation, and local structure. X-ray fluorescence microscopy, using both soft and hard X-rays, enables spatially resolved elemental mapping, while X-ray absorption and scattering techniques provide insight into chemical speciation and local structure. Careful experimental design and sample preparation, including physical fixation and cryogenic measurements, are essential for preserving cellular integrity and native chemical states, thereby enabling reliable visualization of elemental distributions and speciation under stress.

Chlorella sorokiniana represents an excellent model for X-ray studies due to its pronounced metabolic response to metal exposure. Under Cu stress, Cu undergoes partial reduction within a chemically heterogeneous cellular environment. In contrast, exposure to high Mn concentrations induces the formation of multivalent Mn–O clusters with magnetic and redox properties resembling those of the oxygen-evolving complex. Acidophilic *Chlamydomonas* species may employ a strategy characterized by limited Mn internalization and sequestration in vacuolar compartments, potentially associated with phosphate-based ligands. A similar mechanism occurs in neutrophilic *Chlamydomonas reinhardtii*, although Mn uptake is more intensive, enabling accumulation of essentially all available Mn.

KEYWORDS: synchrotron; microalgae; x-ray fluorescence microscopy; x-ray absorption spectroscopy

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Secret Messages in Small Packages – Extracellular Vesicles in Inter-Kingdom War And Peace

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Pathogenic, commensal, and plant-beneficial microbes that constitute the root microbiota interact with one another and the host plant in complex, interconnected ways. Multifaceted communication involves direct cell-to-cell contact and the exchange of diffusible signaling and effector molecules. Another means of communication between cells from different organisms and kingdoms has increasingly been recognized. Extracellular vesicles (EVs), nanoparticles containing cell-derived biomolecules enclosed by a lipid membrane, enable the protected transport and exchange of selected molecules between distant cells.

Although the role of EVs in plant-fungal interactions has been reported, data on their involvement in bacterial-plant relationships remain scarce. To investigate the involvement of EVs produced by plant-beneficial bacteria in communication with plant cells, we isolated EVs from *Paraburkholderia phytofirmans* PsJN, a well-known plant growth-promoting strain. We monitored their interactions with *Arabidopsis thaliana* and *Solanum lycopersicum* roots. Using fluorescent lipid-binding dyes and confocal laser scanning microscopy (CLSM), we showed that PsJN-EVs directly interact with epidermal cells of *A. thaliana* and *S. lycopersicum*. Moreover, we showed that bacterial EVs fuse with plant cell membranes, indicating delivery of their contents to plant cells.

To explore the RNA content of EVs, we sequenced small RNAs (sRNAs) from PsJN-EVs and compared them with sRNAs from bacterial cells. The analysis showed that packaging of bacterial RNAs into EVs is not random but the result of selection. Prediction of bacterial and plant target genes indicated possible involvement of EV-sRNAs in intercellular and inter-organismal communication, encouraging further efforts to understand the roles of EVs and extracellular RNAs in plant-bacteria interactions.

KEYWORDS: extracellular vesicles; inter-kingdom communication; plant-beneficial bacteria; small RNA

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Desiccation-driven Structural Transitions of Lea4 Proteins From *Ramonda serbica*

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Resurrection plants, such as *Ramonda serbica* Panč., can withstand almost complete desiccation and rapidly resume metabolic activity upon rehydration. A key mechanism involves the accumulation of late embryogenesis abundant proteins (LEAPs), intrinsically disordered proteins that protect cellular structures during water loss. Comparative transcriptomic and proteomic analyses have identified LEA4 family proteins as the predominant desiccation-responsive LEAPs in *Ramonda serbica*. In this study, LEA4 proteins were recombinantly produced in *E. coli* and characterized using circular dichroism spectroscopy under conditions mimicking different hydration states. Trifluoroethanol (TFE) was applied to simulate a low-water environment, while sodium dodecyl sulfate (SDS) served as a membrane-mimicking system. Additionally, *E. coli* expressing LEA4 proteins were subjected to abiotic stress to assess whether their presence enhances stress tolerance of the bacterial host. All tested LEA4 proteins were confirmed to be intrinsically disordered under native conditions and underwent a gradual structural transition to amphipathic α -helical conformations upon increasing TFE concentrations and in the presence of SDS micelles. Notably, some LEAPs exhibited signs of coiled-coil formation at high TFE concentrations. This higher-order structural organization may facilitate the formation of larger protective complexes, potentially enhancing the shielding of cellular membranes and macromolecules from desiccation-induced damage. Also, *E. coli* cells expressing several LEA4 proteins exhibited enhanced tolerance to abiotic stress, providing preliminary functional evidence for their protective role *in vivo*.

Future work will be focused on the generation of transgenic tomatoes overexpressing selected LEAPs, to evaluate their potential to enhance drought tolerance as a step toward broader agricultural applications.

KEYWORDS: plant desiccation tolerance; resurrection plants; LEA proteins; intrinsically disordered proteins

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Deciphering the Role of bZIP Transcription Factor Family E Under Heat Stress Conditions

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Precise regulation of gene expression is vital for plant growth, development, and environmental adaptation. Following nuclear export, cytoplasmic post-transcriptional mechanisms determine protein levels by governing mRNA translation, storage, and degradation. Under abiotic stress, global translation is transiently repressed, causing untranslated mRNAs and associated RNA-binding proteins to condense into membraneless RNA granules—primarily processing bodies (PBs) and stress granules (SGs). These dynamic compartments act as hubs that determine whether transcripts undergo decay, persistent storage, or re-entry into translation once favorable conditions return.

While basic leucine zipper (bZIP) transcription factors are classically recognized as conserved nuclear regulators—with *Arabidopsis thaliana* (At) encoding 78 bZIP family members classified into 13 phylogenetic groups (A–M)—novel AtbZIP Group E members display striking non-canonical features. Unlike conventional bZIPs that function in the nucleus, novel Group E bZIPs localize exclusively to the cytoplasm and dynamically aggregate into distinct foci in response to heat stress. Co-localization of these cytoplasmic foci with established PB and SG markers suggests a direct involvement in RNA granule dynamics. Structural analyses further reveal intrinsically disordered regions (IDRs) within these proteins, which are essential for driving liquid-liquid phase separation and granule assembly. Together, these findings indicate that novel Group E bZIPs deviate from traditional nuclear transcription factor paradigms. We therefore hypothesize that these proteins function as non-canonical bZIP regulators operating directly in cytoplasmic RNA metabolism and stress-induced RNA granule dynamics.

KEYWORDS: transcription factor; bZIP; heat stress

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Moderately Elevated Temperatures Make Potato More Susceptible to *Pseudomonas syringae* Infection

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Elevated global temperatures can influence the growth and development of plants and reduce the yields of agricultural crops such as potato (*Solanum tuberosum* L.). Additionally, heat stress could inhibit plant response to pathogens making plants more susceptible to infection. One avenue through which this happens could be disruption of plant hormone balance. We investigated the heat-related effects of a range of temperatures (22°C to 42°C) in wild-type potatoes and potato lines with impaired salicylic acid (SA) and jasmonic acid (JA) metabolism. Exposure to temperatures above 38°C for 24 hours increased levels of stress biomarkers in all potato lines (proline content, chlorophyll content and reactive oxygen species). We have measured expression of genes involved in heat stress and pathogen response, as well as JA- and SA- signalling, in potato plants exposed to 32°C and 42°C for 24h. Expression of heat-shock proteins and heat stress factors in all three potato lines was elevated at 42°C but not at 32°C, while SA and JA-related genes were downregulated already at 32°C. To examine the effect of elevated temperature on potato pathogen response system, we infiltrated *Pseudomonas syringae* in leaves of plants previously exposed to 24h of 32°C. Efficiency of infection was increased at 32°C vs 22°C by a factor of 0.5-1.2 fold. The temperature effect was most profound in SA-deficient potato, although JA-deficient potato was more sensitive to infection overall. In conclusion, exposure to moderately elevated temperature of 32°C does not cause heat stress in potato plants, but weakens defense to pathogens.

KEYWORDS: heat stress; plant hormones; potato

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Cold Stress Effects on Physiological Traits in Adapted High-Quality Protein Maize Seedlings

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Maize is among the most widely cultivated and economically significant cereal crops worldwide. High-temperature stress followed by drought during the flowering and grain-filling stages reduces grain yield and quality. Early sowing, a widely used mitigation strategy, can expose plants to cold stress during early growth and development. This study examined the effects of cold stress on photosynthesis (Ft, YII, and Fv/Fm), transpiration (resistance and conductance), and relative water content (RWC) in maize seedlings. Four high-quality protein maize lines (L1-L4) were grown in a growth chamber (photoperiod 12/12 h, humidity 60%, light intensity 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$) under early spring optimal (19 °C/11 °C) and cold median conditions (19 °C/11 °C for two days, followed by 20 °C/13.7 °C, 9.4 °C/6.3 °C, 6.5 °C/3.5 °C, and 19 °C/11 °C, three days each) for 14 days. Measurements were performed after the stress period (day 11) and after recovery (day 14). Photosynthetic parameters were significantly ($p < 0.001$) reduced under cold stress, but improved after recovery. A statistically significant ($p < 0.001$) reduction in transpiration was observed in L3 and L4 on day 11 and in L2 on day 14, suggesting the onset of active transpiration regulation following the formation of the first leaf. Across all lines, RWC decreased significantly ($p < 0.001$) on days 11 and 14 under cold conditions, indicating that plant water status recovery lagged behind transpiration regulation. Together with germination, morphological, and biochemical traits, these results contribute to a better understanding of cold-tolerance mechanisms and help identify promising maize lines for early sowing.

KEYWORDS: maize; early sowing; cold stress; photosynthesis; transpiration

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Abiotic Stress Markers and Antioxidants in Leaves of Field-Grown *Cucurbita* sp. Subjected to Drought and Limited Mineral Fertilization

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The main aim of this study was to evaluate the impact of suboptimal mineral fertilization and drought conditions (combinations of fertilization - F and irrigation - I: 0F+0I, 0F+I, F+0I, F+I), on the most commonly tested markers of abiotic stresses: lipid peroxidation intensity (LP, membrane integrity), total phenolics (TP, antioxidants), proline (Pro, drought stress) and reduced glutathione contents (GSH, abiotic stress tolerance) in leaves of 25 genotypes from three *Cucurbita* species (*C. maxima* - 9, *C. moschata* - 9, and *C. pepo* - 7) grown in an open field. Intensity of LP was markedly higher in samples that were not irrigated (1.5- to 2.1-times the value of irrigated treatments). Total phenolics content was mostly higher in plants that were both irrigated and fertilized – F+I (17-35% higher than in other treatments) reaching the highest values in *C. pepo* genotypes (38.4 mg/g). Accumulation of Pro was higher in non-irrigated treatments, however there was an exception in each species where the highest content was recorded in both irrigated and fertilized treatment. In the majority of cases, plants that were fertilized had slightly higher accumulation of GSH. The results show that, when it comes to tested biochemical markers indicative of abiotic stress tolerance, drought contributes to oxidative stress to a greater extent than limited mineral fertilization in early generative developmental phase of these species.

KEYWORDS: antioxidants; abiotic stress; *Cucurbita* species; drought; osmoprotectants

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Analysis of Zearalenone Contamination in Maize Seed Genotypes

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Zearalenone (ZON) is a secondary metabolite produced predominantly by *Fusarium* species and represents one of the most important mycotoxins contaminating cereal crops worldwide. Due to its estrogenic activity, zearalenone can cause reproductive disorders, hormonal imbalances, reduced fertility, and impaired productive performance in livestock, especially in swine. In humans, prolonged exposure to contaminated food may disrupt endocrine function and adversely affect reproductive health. Therefore, monitoring zearalenone contamination is essential for ensuring food and feed safety. The aim of this study was to determine the zearalenone content in maize seed samples at the Maize Research Institute Zemun Polje in 2024. The analyzed material included yellow Osmak, white Osmak, the blue popcorn population, ZP Rumenka, ZP 611K, and ZP 552B. Zearalenone determination was performed using the DML 1.29:2017 method based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), while the blue popcorn population was analyzed using the IHM-03-ELISA-09 method, based on enzyme-linked immunosorbent assay technology for rapid detection of zearalenone. The results revealed low contamination levels in all tested samples. Zearalenone concentrations were below 20 µg kg⁻¹ in yellow Osmak, white Osmak, ZP Rumenka, ZP 611K, and ZP 552B, while the concentration detected in the blue popcorn population was below 50 µg kg⁻¹. All measured values were in accordance with the limits prescribed by the Regulation on maximum levels of certain contaminants in food, published in the Official Gazette of the Republic of Serbia. The obtained results confirm the safety, quality, and suitability of the analyzed maize seed.

KEYWORDS: zearalenone; maize seed; LC-MS/MS; ELISA; mycotoxin contamination

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Variegated *Pelargonium zonale* as a Model to Elucidate Nitrogen Remobilization for Crop Improvement

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Variegated *Pelargonium zonale* features photosynthetically active green (vG) and non-photosynthetic white (vW) sectors co-existing under identical micro-conditions. Understanding internal nitrogen (N) allocation in such systems is vital to improve plant N-use efficiency and reduce fertilizer dependence. Hydroponically grown variegated and plain green (G) plants were exposed to high (HL) or low light (LL) combined with N sufficiency (+N) or deficiency (-N), followed by non-invasive photosynthetic and epidermal flavonoid measurements, coupled with proteomic profiling. The combination of nitrogen deficiency and high light (HL-N) exerts high metabolic pressure relative to low light (LL-N), driven by significant photo-oxidative stress and nitrogen remobilization. Under LL-N, non-photosynthetic vW tissue relies on basic nutrient import without structural photo-damage, while under HL-N, vW upregulates the ubiquitin-proteasome system for internal protein recycling, enhances nitrogen transporter activity to import amino acids from vG, and accumulates antioxidative enzymes (ascorbate peroxidases, superoxide dismutases) and flavonoids/anthocyanins to maintain homeostasis. In variegated leaves under HL-N, vG Rubisco and light-harvesting complexes breakdown accelerates, triggering a steep reduction in chlorophyll and driving early senescent yellowing. Unlike vG, which faces the additional burden of supplying the sink tissue with nitrogen, plain morphed G leaves initiate nitrogen recycling and photoprotective mechanisms while maintaining significantly more stable chlorophyll levels. Overall, variegated leaves reveal a spatially decoupled source-sink mechanism where green sectors sacrifice photosynthetic machinery to export nitrogen, while white sectors rely on imported metabolites and non-photosynthetic photoprotection offering key molecular targets to engineer resilient crops.

KEYWORDS: Antioxidant defence; Nitrogen remobilization; Protein turnover; Source-sink dynamics; Variegated *Pelargonium zonale*

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Variegated *Pelargonium zonale* as a Model for Uncovering Transferable Mechanisms of Plant Stress Resilience

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Pelargonium zonale cv. Frank Headley is a periclinal chimera whose leaves contain photosynthetically active green, source, sectors (GL) and non-photosynthetic white, sink, sectors (WL). Their co-existence within the same organ provides an excellent model for distinguishing sink/source-specific responses while minimizing environmental influence. We integrated ecophysiological and enzymatic analyses with transcriptomic and metabolomic profiling to characterize the functional specialization of the two leaf sectors and test whether variegation enhances stress resilience relative to fully green morphs. Under optimal conditions, 8,896 genes were differentially expressed between the two leaf sectors. Photosynthesis, pigment biosynthesis and Calvin-Benson cycle pathways predominated in GL. WL showed enhanced nitrogen and protein metabolism, genome maintenance and stress responses, and accumulated more free amino acids, flavonol glycosides, Fe, Cu, Mn and Zn. Under excess excitation energy caused by high irradiance and low temperature, GL accumulated cyanidin glycosides and reinforced its cell wall, while WL increased amino acids and flavonol glycosides, functioning as an alternative sink that helped maintain photosystem II performance. Stress resilience was further tested under Zn deficiency and hypoxia-reoxygenation. Zinc deficiency did not impair growth of variegated plants, whereas fully green morphs showed reduced shoot and root growth and chlorosis. Preliminary hypoxia-reoxygenation experiments indicated greater resilience of variegated plants. Together, these results demonstrate that variegated *P. zonale* is a versatile system for discovering coordinated source-sink interactions important in abiotic stress tolerance. Further work will identify and validate candidate genes and proteins involved in stress resilience, aiming to translate these findings into crop-resilience improvement strategies.

KEYWORDS: *Pelargonium zonale*; leaf variegation; source-sink interactions; abiotic stress tolerance

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Lithium Accumulation, Antioxidant Responses and Nutrient Homeostasis in Edible Radish Tissues

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The increasing use of lithium (Li) in Li-containing products, coupled with limited Li recycling, has raised concerns about environmental contamination. Consequently, the effects of Li exposure on plants have recently received increasing scientific attention, yet Li accumulation patterns and their ecological and physiological implications remain insufficiently understood. Moreover, characterizing Li accumulation in edible plant tissues is of particular importance from a food safety perspective and for public health within the One Health framework. Radish (*Raphanus sativus* var. *sativus*) (Brassicaceae) was cultivated in soil amended with Li at 25, 50, and 100 mg Li kg⁻¹ for 30 days under controlled conditions. Besides dose-dependent Li accumulation in roots and leaves, radish showed predominant Li accumulation in leaves, with concentrations reaching 17- to 36-fold higher values than in the corresponding soil. In the roots, elevated Li concentrations were associated with accumulation of K, Ca, Mg, Cu, and Mn. In the leaves, Li had antagonistic effects on Ca, Mg, Na, Zn, and Fe concentrations, but a strong synergistic effect on Cu concentration. In the leaves, the highest applied Li concentrations increased total soluble protein content, induced minor alterations in antioxidant enzyme activity and slightly reduced membrane lipid peroxidation. In the roots, only transient changes in enzyme activities were detected. Although the data indicate that even extreme Li concentrations caused only moderate nutrient imbalance and limited membrane oxidative damage, leaf chlorosis and reduced shoot and root biomass indicate metabolic disturbance provoked directly or indirectly by elevated Li.

KEYWORDS: radish plants; lithium toxicity; oxidative stress

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Upper Leaf Morphology and Physiology Reveal Phenotypic Markers of Drought Adaptation in Rapeseed

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Increasing drought frequency and heat waves pose significant challenges for sustainable rapeseed (*Brassica napus* L.) production. Identification of traits associated with adaptation to abiotic stresses represents an important approach for selecting genotypes adapted to changing environmental conditions. The aim of this study was to identify leaf traits suitable as phenotypic markers for breeding drought-adapted genotypes through the analysis of morphological, micromorphological and physiological characteristics of upper leaves in two rapeseed genotypes (G1 and G2). The study was conducted under field conditions at the full flowering stage using ten plants per genotype. Leaf morphological traits were analyzed using Digimizer software, stomatal characteristics were assessed by light microscopy, while stomatal conductance, conductance to water vapor and photosystem II efficiency were determined using the LI-600 PF system. Both genotypes exhibited amphistomatic leaves with anisocytic stomata. G1 was characterized by narrower and more elongated leaves with less pronounced lobing, as well as longer stomata on the adaxial epidermis. In contrast, G2 developed larger and more distinctly lobed leaves with greater assimilatory surface, higher stomatal density on the abaxial epidermis and prolonged maintenance of high photosystem II efficiency, accompanied by more pronounced changes in gas exchange regulation. The results indicate the presence of different adaptive strategies associated with leaf morphology, stomatal distribution and physiological regulation. G1 showed a potentially more stable water-conservation strategy through more efficient control of stomatal conductance, whereas G2 maintained greater photosynthetic capacity. These traits could be incorporated into phenotyping protocols for early selection of drought-adapted rapeseed genotypes.

KEYWORDS: *Brassica napus*; leaf; morphology; micromorphology; drought

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Low-Temperature Stress Induces Differential Expression of a Vacuolar Invertase Gene in Roots and Leaves of V3-Stage Maize

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Carbohydrate metabolism regulation and soluble sugars accumulation are steps essential for an efficient low-temperature (LT) stress response. Maintaining proper balance between sucrose synthesis and degradation was shown to be important for this process. Invertases catalyze the cleavage of sucrose to glucose and fructose, and have been shown to play a role in the LT response. As early sowing is one of the most promising strategies for mitigating climate change-induced maize yield losses in temperate regions, elucidating the mechanisms underlying the establishment of LT tolerance during the early developmental stages becomes essential. In the present study, two maize genotypes (LT-susceptible - Lsus, and LT-tolerant - Ltol) were treated with low temperatures for 24h (8/10° C) after reaching the V3 developmental stage, and the roots and leaves were sampled at two time-points (6h and 24h). Paired-end transcriptome sequencing was performed after RNA extractions and cDNA library preparations. The same was done for control plants grown in optimal conditions (25°/20°C). After the sequencing-data quality check, mapping and read quantification, the differential expression analysis was performed. A gene encoding a vacuolar invertase (Zm00001eb229460) was upregulated after 6h of LT-treatment in all samples, but its expression varied after 24h. In LS, it was upregulated in roots, but not in leaves; while it was downregulated in both organs in LT. This suggests that the initial LT-response involved accumulation of glucose and fructose as osmoprotectants, regardless of the genotype and tissue. However after 24h, sucrose appeared to be more important for the stress response and carbohydrate metabolism.

KEYWORDS: maize; low-temperature stress response; osmoprotection; carbohydrate metabolism; invertase

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Biochemical Responses of Wild Annual *Helianthus* Species to Drought Stress

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Drought is a major environmental factor restricting plant growth and productivity. Early physiological responses of plants drought stress comprise changes in antioxidant defence and osmotic regulation, which contribute to the maintenance of cellular homeostasis and tolerance to drought. Wild *Helianthus* species represent a valuable genetic resource for improvement of drought tolerance in cultivated sunflower species due to their diverse adaptive mechanisms. In this study, we assessed the biochemical responses of two wild annual sunflower species, *Helianthus annuus* and *Helianthus argophyllus*, grown under controlled greenhouse conditions. Plants were exposed to either well-watered (control; 70% substrate moisture) or drought stress (40% substrate moisture) conditions for ten days. Following the experimental period, leaf and root tissues were collected for biochemical analyses.

Biochemical responses were assessed by measuring ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPX) activities, as well as glutathione (GSH), malondialdehyde (MDA), and proline contents. Drought stress significantly altered biochemical responses in both species, although the magnitude and pattern of responses differed between them. In *Helianthus annuus*, drought stress significantly increased APX, CAT, and GPX activities in leaves and GPX activity in roots. Significant increases in GSH and proline contents were observed in roots, while MDA content increased significantly in both tissues. In *Helianthus argophyllus*, drought stress significantly increased CAT activity in both tissues, while GSH and proline contents increased significantly in leaves and MDA and proline contents in roots. Overall, the two species showed contrasting biochemical responses to short-term drought stress: in *Helianthus annuus*, more pronounced changes in antioxidant enzyme activities were observed, whereas *Helianthus argophyllus* maintained a more stable biochemical response.

KEYWORDS: wild sunflower; drought stress; antioxidant enzymes; proline; oxidative stress

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Antioxidant and Pigment Responses of Basil (*Ocimum basilicum*) to Mercury and Arsenic Exposure

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Potentially toxic elements (PTEs) are among the major soil contaminants, posing significant risks to environmental and human health. Arsenic (As) and mercury (Hg) are particularly toxic and can disrupt essential biological processes in plants, animals, and humans. Their accumulation in soils is associated with various anthropogenic activities. In the present study, *Ocimum basilicum* L. was cultivated for two months in soil amended with 25 and 50 mg As kg⁻¹ and 10 and 15 mg Hg kg⁻¹ to assess its physiological and biochemical responses to As and Hg exposure. Guaiacol peroxidase (GPX) and glutathione reductase (GR) activities, membrane lipid peroxidation (LP), total antioxidant capacity (TAC), and photosynthetic pigment concentrations were evaluated. GPX activity exhibited a concentration-dependent response to As, increasing at low As but declining at high As, whereas exposure to both Hg concentrations resulted in enhanced GPX activity throughout the plant. Root GR activity showed a similar dose-dependent response, increasing at low and decreasing at high PTE concentrations. In the shoots, however, GR was inhibited by both PTEs. Root LP remained unchanged, whereas shoot LP increased with increasing PTE concentrations, indicating greater susceptibility of the shoots to oxidative membrane damage. TAC showed contrasting responses: increasing with As but decreasing under Hg. Alongside the antioxidant system, chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations decreased with increasing PTE concentrations, suggesting impairment of photosynthesis. Overall, *O. basilicum* exhibited distinct and organ-specific responses to As and Hg exposure, with differences between the two PTEs evident in enzyme activities, TAC, LP and photosynthetic pigments.

KEYWORDS: *Ocimum basilicum*; arsenic; mercury; antioxidant system

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Citric Acid Alleviates Cadmium Stress in Willows Through Species-Specific Physiological and Metabolic Adjustments

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Heavy metal contamination of soils remains a major environmental challenge, underscoring the need for sustainable phytoremediation strategies. Combining biodegradable chelating agents with metal-tolerant, fast-growing tree species is a promising approach to improve phytoremediation efficiency. We asked whether citric acid (CA) alleviates Cd stress in willows and whether that alleviation is species- and dose-specific. *Salix viminalis*, *S. matsudana*, and *S. alba* were grown in Cd-contaminated soil (3 and 6 mg Cd kg⁻¹) with or without CA (20 mmol kg⁻¹ soil). We tracked responses from metal uptake to metabolic adjustment: how much Cd reached the leaves and what it cost the plant (growth and photosynthetic performance), the oxidative damage it caused (H₂O₂ and lipid peroxidation), the defenses mobilized against it (antioxidant and thiol metabolism), and shifts in central carbon metabolism (TCA-cycle intermediates). The most pronounced protective response occurred in *S. matsudana*, where CA reduced H₂O₂ accumulation by 61–67% and lipid peroxidation by 83–87% under Cd stress. At lower Cd doses, CA also increased net photosynthetic rate by 28% and leaf Cd concentration by 52% in this species, indicating improved physiological performance despite enhanced Cd accumulation. *S. viminalis* exhibited moderate CA-mediated physiological adjustment, whereas *S. alba* showed a less uniform response. HPLC profiling revealed species-specific changes in tricarboxylic acid cycle intermediates, including citrate, malate, succinate, and α-ketoglutarate, suggesting reprogramming of carbon metabolism. Overall, CA alleviated Cd stress in a species- and dose-specific manner, most effectively in *S. matsudana*, through reduced oxidative damage, maintained photosynthetic performance, and shifts in TCA-cycle intermediate pools.

KEYWORDS: *Salix* spp.; citric acid; oxidative stress; TCA cycle; phytoremediation

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Phloretin Alters Antioxidant Defense and Induces Oxidative Damage in *Capsella bursa-pastoris* (L.) Medik.

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Phytotoxic effects of plant-derived compounds are frequently associated with disturbances in cellular redox regulation. Phloretin, a naturally occurring dihydrochalcone particularly abundant in apple species, has emerged as a promising allelochemical candidate for bioherbicide development, but its effects on the oxidative physiology of weed species remain insufficiently understood. Here, we investigated the oxidative response of *Capsella bursa-pastoris* (L.) Medik. seedlings to phloretin, with particular emphasis on lipid peroxidation and antioxidant defense mechanisms. Following a two-week exposure to 500 μ M phloretin, malondialdehyde (MDA) content increased by 29.9%, indicating enhanced lipid peroxidation and oxidative membrane damage. Antioxidant enzyme activities and isozyme profiles were assessed spectrophotometrically, accompanied by native-PAGE analysis. Total superoxide dismutase activity increased, together with a pronounced increase in the band intensity of the Mn-SOD isoform, suggesting enhanced detoxification of superoxide anion radicals and the involvement of mitochondrial antioxidant defense. In contrast, catalase activity decreased, whereas peroxidase activity showed no significant alteration. The contrasting responses of SOD and H₂O₂-scavenging enzymes indicate that activation of the antioxidant defense was not fully coordinated and was insufficient to prevent oxidative damage. In addition, proline content increased by 51.9%, suggesting activation of a broader stress-adaptive response that may contribute to cellular protection under phloretin-induced stress. Collectively, our findings demonstrate that phloretin treatment leads to a redox imbalance, accompanied by increased lipid peroxidation despite activation of antioxidant defenses, identifying oxidative stress as an important, species-dependent component of its phytotoxic mode of action.

KEYWORDS: allelochemicals; hydrogen-peroxide; malondialdehyde; proline; redox homeostasis

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Species-Specific Morphogenetic Responses of *Physcomitrium patens* and *P. eurystomum* to NaCl Stress

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Habitat salinization is an increasing worldwide problem that adversely affects plants growth and development. Although responses to salt stress have been extensively investigated in vascular plants and to some extent also in the model species, *Physcomitrium patens*, comparable information for other bryophytes, even closely related, remains limited. This study compared the morphogenetic responses of two congeneric species of similar biology, ecology and strategies, namely *P. patens* and *P. eurystomum*, to increasing NaCl concentrations (0, 10, 50 and 250 mM) under *in vitro* conditions. Morphogenetic traits were monitored after 7, 14, 21 and 28 days, including multiplication index (IM), diameter of secondary protonemal patch (DSPP), number of secondary protonemal buds (NSPB) and bud distance from the initial explant (DSPB). Both species maintained 100% survival across all treatments, indicating that NaCl primarily affected development rather than viability. However, distinct species-specific responses were observed. *Physcomitrium patens* maintained IM and DSPP under low and moderate NaCl concentrations, whereas *P. eurystomum* showed more restricted development, particularly in NSPB. Temporal analysis further revealed that *P. patens* maintained the development of all investigated morphogenetic traits under 0–50 mM NaCl, whereas *P. eurystomum* exhibited reduced development of almost all researched morphogenetic parameters under NaCl exposure. At 250 mM NaCl, IM and secondary protonemal bud formation were completely inhibited in both species, although viable protonemal tissue persisted. These findings demonstrate contrasting developmental responses between two closely related *Physcomitrium* species and highlight species-specific developmental plasticity as an important component of moss responses to salt stress.

KEYWORDS: bryophytes; morphogenesis; salt stress; model organism; *in vitro* cultivation

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Photosynthetic Responses of the Two Closely Related Moss Species *Entosthodon hungaricus* and *E. pulchellus* to NaCl Stress

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Salt stress is a major environmental constraint affecting plant growth and development, yet the physiological mechanisms underlying salt tolerance in bryophytes remain poorly understood. This study compared changes in photosynthetic pigments and chlorophyll fluorescence in the closely related mosses *Entosthodon hungaricus*, a facultative bryo-halophyte, and *E. pulchellus*, a non-halophytic counterpart species, exposed to 0, 10, 50 and 250 mM NaCl under controlled *in vitro* conditions. Photosynthetic pigments were quantified spectrophotometrically, whereas chlorophyll fluorescence parameters, including maximum quantum yield of photosystem II (QYmax), photochemical quenching (qP), and non-photochemical quenching (NPQ), were measured using imaging fluorometry after four weeks of treatment. Relative to *E. pulchellus*, *E. hungaricus* maintained higher chlorophylls a, b and carotenoid contents under moderate (50 mM) stress, with chlorophylls a and b declining only under severe salinity (250 mM). In contrast, pigment contents in *E. pulchellus* remained relatively stable but consistently lower at moderate stress. Chlorophyll fluorescence analyses revealed distinct photoprotective responses between the species. In *E. hungaricus*, qP remained stable across all NaCl concentrations, whereas NPQ increased significantly under moderate NaCl concentration and remained stable under severe stress, indicating efficient photoprotective energy dissipation. Conversely, *E. pulchellus* exhibited a marked decline in both qP and QYmax at 250 mM NaCl together with consistently lower NPQ values, suggesting impaired PSII function and a lower capacity for photoprotection. These findings demonstrate pronounced interspecific differences in photosynthetic acclimation to NaCl stress and indicate that *E. hungaricus* possesses a more effective photoprotective strategy than *E. pulchellus*, consistent with its adaptation to saline habitats.

KEYWORDS: bryo-halophyte; photosynthetic pigments; chlorophyll fluorescence; salt stress; *in vitro* cultivation

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Organ-Specific Metabolic and Non-Enzymatic Antioxidant Responses of *Miscanthus × giganteus* (Poaceae) to Lithium Stress: Implications for Phytomanagement

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The expanding use of lithium (Li), particularly in battery technologies, together with intensified extraction, processing, and disposal of Li-containing materials, is increasing the risk of Li inputs into terrestrial environments. Owing to its relatively high mobility, Li can readily enter the soil–plant system, emphasising the need to understand plant responses and identify species suitable for sustainable management of Li-affected soils. *Miscanthus × giganteus*, a high-biomass perennial grass tolerant to abiotic stress, is a promising candidate for phytomanagement. This study characterised concentration-dependent metabolic and non-enzymatic antioxidant responses of *M. × giganteus* to Li exposure (20–1000 mg Li kg⁻¹ dry substrate). Leaf and root metabolite profiles were analysed by UHPLC/Orbitrap-HRMS, while antioxidant capacity was evaluated using DPPH, ABTS, and FRAP assays. Hierarchical clustering revealed organ-dependent responses to Li, with the clearest treatment-related separation at high concentrations, particularly in roots. In leaves, Li induced extensive remodelling of phenolic metabolism, including marked accumulation of dihydroxybenzoic acid hexoside, gallic acid hexoside, naringenin, and luteolin, whereas antioxidant capacity remained unchanged. Roots displayed a biphasic antioxidant response: capacity increased significantly at 40 mg kg⁻¹ Li, accompanied by increased naringenin, eriodictyol, and luteolin levels, whereas reduced capacity at the highest Li concentrations coincided with pronounced flavonoid depletion. High Li exposure also reorganized organic acid metabolism, with increased aconitic and itaconic acids and decreased malic acid. Overall, these findings demonstrate pronounced organ-specific and concentration-dependent metabolic and non-enzymatic antioxidant responses to Li exposure and provide insight into the potential use of *M. × giganteus* for phytomanagement of Li-affected soils.

KEYWORDS: metabolite profiling; antioxidant capacity; phenolic metabolism; flavonoids; organic acids

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Li-Induced Antioxidant Enzyme Responses in *Miscanthus × giganteus* (Poaceae) and Their Significance for the Phytoremediation Process

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Lithium (Li) contamination is an emerging concern, highlighting the need for sustainable remediation. This study evaluated antioxidant enzyme responses of *Miscanthus × giganteus* following six months of Li exposure. Plants were grown outdoors on substrates containing 0–1000 mg Li kg⁻¹. Activities of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) were assessed spectrophotometrically in leaves and roots, while enzyme profiles were characterised by native PAGE with activity staining and by immunoblotting. Responses were organ- and concentration-dependent. CAT responded at higher Li concentrations, notably at 320 mg kg⁻¹ in leaves and 640 mg kg⁻¹ in roots. POD showed the strongest leaf response at 320 mg kg⁻¹ and fluctuating root activity at higher treatments. Leaf APX declined at 320–640 mg kg⁻¹, whereas root APX increased at 160 and 640 mg kg⁻¹. Leaf SOD reached its highest activity at 320 mg kg⁻¹ and remained elevated, while root SOD displayed a non-linear response. GR was stimulated at low-to-intermediate concentrations in leaves and peaked at 320 mg kg⁻¹ in roots. Activity staining revealed weaker POD, APX, and GR bands in roots than in leaves. Partial correspondence among spectrophotometric activity, native PAGE band intensities, and immunoblot signals suggests differential isoenzyme modulation and post-translational regulation. Enzyme responses at 160–320 mg kg⁻¹ suggest tolerance to moderate Li contamination, while responses at 640 mg kg⁻¹ indicate acclimation to higher exposure. However, the Li concentration range suitable for phytoremediation should be evaluated together with plant growth, biomass production, and Li accumulation and translocation.

KEYWORDS: lithium tolerance; oxidative stress; isoenzyme profiles; protein abundance; redox regulation

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Influence of Moderate Drought on Lettuce Growth and Quality Traits

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Drought represents one of the key environmental factors that significantly influence crop yield and quality. Understanding its impact on the growth parameters and biochemical composition of lettuce plants enables the optimization of irrigation in modern agriculture. This study aimed to investigate the effect of moderate drought on lettuce growth parameters and quality attributes. The experiment was conducted in a growth chamber, where lettuce (*Lactuca sativa* L.) cultivar 'Red Romaine' was grown under control (optimal water availability) and moderate water-deficit conditions (water application reduced by 50% relative to control) from transplanting to harvest stage).

Moderate drought had a significant impact on growth parameters and lettuce vegetative biomass. Significant reductions were noticed in rosette height and diameter, stem height and leaf area, as well as in aboveground biomass related to rosette, leaf and stem. Drought affected biomass, where the reduction was more expressed at the leaf and stem levels than at the root. Also, higher reduction in aboveground biomass indicated that drought predominantly affects biomass rather than growth parameters.

Biochemical analysis shows enhancement in nutritional quality related to secondary metabolites in response to water deficit and significantly increase of antioxidant capacity. Drought significantly induced the increase of total phenolic, total flavonoid content as well as carotenoids compared to control plants. On the other hand, total soluble solids and vitamin C content showed no significant variations between treatments. Overall, moderate water stress limits growth and productivity in 'Red Romaine' lettuce, but stimulates key antioxidant defense pathways, substantially enhancing functional health-promoting compounds.

KEYWORDS: *Lactuca sativa* L.; moderate drought; morphological parameters; crop quality

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Dose-Dependent Effects of Green-Synthesized Silver Nanoparticles on Antioxidant Responses of Winter Wheat Under Heat Stress

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The application of green-synthesized silver nanoparticles (AgNPs) may modify plant responses to abiotic stress, although their effects depend strongly on concentration and environmental conditions. In this study, AgNPs synthesized using *Agrimonia eupatoria* L. extract were investigated for their influence on non-enzymatic antioxidant metabolism and heat-stress responses in winter wheat. Surface-sensitive analysis showed a predominantly metallic Ag core surrounded by a phytochemical-derived organic corona, while phytochemical profiling revealed a diverse polyphenolic composition dominated by epicatechin, chlorogenic acid, myricetin, and rutin. Density functional theory (DFT) calculations using chlorogenate as a representative corona component indicated possible interaction with surface-accessible Ag(I)-like sites and showed that Ag(I) coordination altered the energetics of electron- and proton-transfer steps without substantially improving the overall net hydrogen-transfer process toward ABTS. Three wheat cultivars were exposed to AgNPs under optimal and heat stress conditions. Heat stress reduced phenolic and flavonoid contents, radical-scavenging capacity, and overall antioxidant capacity, with these effects becoming more pronounced as AgNP concentration increased. The applied AgNPs therefore did not protect the non-enzymatic antioxidant system, but instead caused a concentration-dependent disruption of antioxidant homeostasis. The combined experimental and computational results indicate that the redox-active organic corona was insufficient to compensate for the oxidative burden associated with heat stress and relatively high AgNP exposure. Together, these findings link nanoparticle surface chemistry with plant physiological responses and emphasize the concentration-dependent role of green-synthesized nanomaterials in plant stress biology.

KEYWORDS: antioxidant metabolism; dose-dependent effects; heat stress; silver nanoparticles (AgNPs-H₂O); *Triticum aestivum* L.

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Deficiency of the DSS1(V) Protein Enhances Susceptibility to Water Deficit in *Arabidopsis thaliana*

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Drought represents a major environmental threat to global agriculture, frequently causing severe declines in crop yield and harvest quality. Unraveling the molecular pathways and cellular components that dictate plant resilience to water scarcity is essential for both fundamental plant biology and sustainable food security. In this study, we investigated the role of intrinsically disordered protein DSS1(V) in mitigating the effects of mild to moderate drought stress in *Arabidopsis thaliana*. Using a comparative approach, we evaluated wild-type (WT) plants alongside *dss1(v)* knockout mutants and overexpressing lines. To comprehensively assess the physiological and biochemical impact of drought, we monitored a wide array of stress indicators, including lipid peroxidation, total phenolic compounds, chlorophyll degradation, and protein oxidation. Our analysis was extended to key enzymatic and non-enzymatic defense markers, specifically tracking the activities of catalase and peroxidases, along with free proline accumulation. The results revealed that the absence of *DSS1(V)* triggered a profound biochemical collapse; the knockout mutants exhibited a sharp increase in macromolecular damage, altered antioxidant enzyme kinetics, and compromised osmoprotectant levels. Collectively, all measured parameters consistently demonstrated that the *dss1(v)* mutants are highly sensitive to drought conditions. Conversely, overexpressing the *DSS1(V)* gene failed to provide enhanced protection or alter the stress phenotype compared to the WT. Taken together, these findings provide compelling evidence that the *DSS1(V)* protein is an important component of the core machinery required for cellular homeostasis during water deficit.

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Mitigating Salt Stress in Basil Plants Through Salicylic Acid Application

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Salt stress may affect the growth and metabolism of plants negatively, but at the same time it can stimulate the biosynthesis of secondary metabolites involved in the stress response. Basil (*Ocimum basilicum* L.) is a widely cultivated and globally utilized aromatic plant, valued for its distinctive flavour and fragrance as well as its applications in medicinal and pharmaceutical industries. We tested the potential protective effect of salicylic acid (SA) on basil exposed to salt stress caused by NaCl. Starting from seeds, plants were grown under salt stress conditions (150 mM NaCl) for one month. When first pair of leaves appeared, different concentrations of SA (0.29, 1, and 1.7 mM) were applied by foliarly spraying. Non-stressed plants were treated as a control. At the end of the experiment, MDA content, photosynthetic pigments, osmolite content, enzymatic (SOD, CAT, and different groups of peroxidases) and non-enzymatic defence components, together with the content of basil phenolic compounds were analyzed. The results showed that the salt stress significantly altered the antioxidant defence mechanism of basil. Furthermore, changes can be seen in both photosynthetic activities and osmolite concentration. Application of SA under salinity conditions modified basil's response to salt stress in a concentration-dependent manner. Among the tested SA concentrations, 0.29 mM SA had the greatest impact in eliminating the effects of stress caused by NaCl. It can be concluded that the application of SA can alleviate the adverse effects of salt stress in basil plants, although its protective effect is strongly dependent on the concentration applied.

KEYWORDS: salt stress; *Ocimum basilicum* L.; salicylic acid; phenolic compounds; lipid peroxidation; antioxidant enzymes

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Phenolic and Physiological Responses Of Cabbage to *Xanthomonas campestris* pv. *campestris* Infection and Preventive *Bacillus velezensis* Treatment

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Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), poses a major threat to cabbage production and creates a need for effective and sustainable disease management strategies. Microbial biocontrol strains such as *Bacillus velezensis* offer an environmentally sustainable alternative to conventional chemical-based disease management, potentially reducing reliance on synthetic pesticides. In this study we evaluated the effects of Xcc infection and preventive treatment with *Bacillus velezensis* RD-FC 88 on phenolic composition, fluorescence profiles (excitation emission matrices - EEMs), antioxidant activity, leaf chlorophyll content (Chl), epidermal flavonoid content (EpFlav), and nitrogen balance index (NBI) of cabbage cv. Futoški. Plants were grouped as: untreated control (NB), *Bacillus*-treated (Bac), Xcc-inoculated (Xcc), and *Bacillus*-pretreated followed by Xcc inoculation (Bac+Xcc).

Xcc infection was associated with lower levels of most of the detected hydroxybenzoic and hydroxycinnamic acid derivatives, while Bac+Xcc plants showed increased accumulation of selected hydroxybenzoic acids, hydroxycinnamic acids, and kaempferol derivatives compared with Xcc. PCA of the fluorescence profiles clearly separated the Bac+Xcc samples from control (PC1: 52.03%; PC2: 41.43% of total variance). Antioxidant activity was significantly lower in Xcc-infected plants than in NB and Bac, whereas Bac+Xcc showed a partial recovery compared to Xcc. Flav was lower in Xcc and Bac+Xcc plants, while NBI was higher in both. Chl did not differ significantly among treatments.

Overall, *B. velezensis* RD-FC 88 modulated cabbage responses to Xcc infection by increasing phenolic levels and antioxidant capacity, suggesting its potential as a priming biocontrol agent.

KEYWORDS: black rot; *Bacillus velezensis*; priming; phenolic compounds; antioxidant capacity

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Impact of *Bacillus velezensis* RD-FC 88 on the antioxidative Metabolism of Cabbage cv. Futoški During Infection With *Xanthomonas campestris* pv. *campestris*

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The objective of this study was to investigate the effect of preventive treatment with *Bacillus velezensis* RD-FC 88, a strain with biocontrol potential, on the antioxidative response of cabbage (*Brassica oleracea* var. *capitata* cv. Futoški) to the pathogenic bacterium *Xanthomonas campestris* pv. *campestris* (Xcc). In this experiment we analysed the metabolism of ascorbate (Asc), glutathione (GSH), enzymes involved in Asc-GSH cycle, and the activities of antioxidant enzymes and their isoform profiles in the leaves of control (NB), *Bacillus*-treated (Bac), Xcc-inoculated (Xcc) and *Bacillus*-treated Xcc-inoculated (Bac+Xcc) cabbage plants. Xcc infection led to a decrease in the Asc content and an increase in the GSH content, which was accompanied by enhanced activities of monodehydroascorbate reductase and glutathione reductase. Pathogen inoculation induced catalase activity and decreased peroxidase activity, implying that infection changed the balance between different pathways involved in hydrogen peroxide elimination. In Bac+Xcc plants, several responses were altered: the activities of monodehydroascorbate reductase and glutathione reductase were lower than in Xcc plants, and the ascorbate and glutathione pools showed a different pattern compared with infected plants. IEF analysis showed treatment-dependent changes in class III peroxidase isoforms. Regarding superoxide dismutase (SOD), total activity was unchanged, but the isoform pattern changed after infection: the Cu/Zn-SOD band at pI 4.65 was absent in Xcc, while Bac+Xcc showed a distinct Mn-SOD band at pI 4.35. Overall, pretreatment with the biocontrol strain RD-FC 88 modified the redox regulation of cabbage during Xcc infection, suggesting its potential to mitigate the negative effects of Xcc by enhancing plant defence responses.

KEYWORDS: cabbage cv. Futoški; biocontrol; ascorbate; glutathione; antioxidant enzymes

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ORCID ID: 0009-0006-0008-3100

Distinct Adaptation Strategies of Three Basil Varieties Under Different Light Intensities: Trade-Offs Between Growth, Cell Wall Remodelling, and Secondary Metabolism

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Light intensity is a key factor in shaping plant growth, morphology, and metabolic profiles. This study aimed to compare the adaptive and metabolic trade-off strategies of three basil varieties – Genovese (*Ocimum basilicum* var. Genovese), lemon (*Ocimum × citriodorum*), and purple (*Ocimum basilicum* var. *purpurascens*)—during acclimation to low, medium, and high light intensities (LL – 100, ML – 300, and HL – 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). Plants were grown in controlled conditions in a growth chamber, and their morphological traits, photosynthetic performance, chlorophyll concentration, leaf epidermal flavonoids, individual phenolic compounds, total antioxidant capacity, and cell wall structural modifications were comprehensively evaluated.

Photosynthetic efficiency (electron transport rate) indicated that ML was optimal for all cultivars, whereas maximum biomass accumulation occurred under HL. Under HL conditions, all varieties exhibited increased dry biomass and a lower SLA, indicating leaf thickening. FTIR spectroscopy revealed contrasting cell wall remodelling strategies: higher light intensity led to a reduction of cell wall components in Genovese, whereas in purple basil their accumulation was induced. The content of secondary metabolites was highly light-dependent. Purple basil exhibited the highest baseline phenolic content under LL while in lemon basil, rosmarinic acid content was 6-fold higher under HL compared to LL.

The results show distinct, cultivar-specific trade-off mechanisms during light acclimation: Genovese exhibits high sensitivity to light variations, primarily investing in leaf area expansion and transpiration; lemon basil balances photosystem protection via non-photochemical quenching with structural investment in stem tissue; and purple basil prioritizes early activation of the phenylpropanoid pathway and structural cell wall reinforcement.

KEYWORDS: *Ocimum basilicum*; light acclimation; phenolic compounds; FTIR spectroscopy; cell wall

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Physiological Response and Spectral Changes in *Solanum nigrum* L. Leaves Under Waterlogging and Subsequent Recovery

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The aim of this study was to investigate the effects of waterlogging and the subsequent recovery period on the physiological characteristics of *Solanum nigrum* L., a plant known as a hyperaccumulator of heavy metals. In this experiment, plants at the six-leaf stage were subjected to 9-day waterlogging (W-waterlogging, CW-control during waterlogging), followed by a 9-day recovery period (R-recovery, CR control during recovery). Non-invasive measurements were conducted to determine the chlorophyll and epidermal flavonoid content, as well as the photosynthetic performance, while the fluorescence emission spectra were analysed from leaf methanolic extracts. Waterlogging affected the accumulation of epidermal flavonoids, whose level remained elevated during the recovery period, suggesting their importance in the plant's defence strategy, while reduced chlorophyll content was noticed during the recovery period. Also, the noticeable reduction in the nitrogen balance index implies an altered nitrogen status during waterlogging and recovery periods. Effective quantum yield of photosystem II was significantly altered by treatment, whereas the electron transport rate did not differ among treatments. Principal component analysis of the fluorescence spectral data showed a clear separation between the control groups (CW and CR) and the treatment groups (W and R). While the control samples showed stronger associations with the UV-A spectral region, W and R plants were more closely associated with wavelengths around 440–450 nm. Obtained results suggest that waterlogging induces significant changes in the physiological response of *S. nigrum*, while the recovery period does not lead to a complete return to control values.

KEYWORDS: *Solanum nigrum*; waterlogging; fluorescence; principal component analysis

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Pharmacological Analysis of Phloretin-Induced Membrane Depolarization in *Arabidopsis thaliana* Root Epidermal Cells

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Phloretin, a phytotoxic dihydrochalcone abundant in apple trees, has recently been identified as an allelochemical capable of disrupting cellular and hormonal homeostasis in plants. Despite its recognized effects, the mechanisms underlying its action at the root plasma membrane level remain poorly understood. Here, we dissected early ion-transport processes at the root epidermal plasma membrane of *Arabidopsis thaliana* using a pharmacological approach combined with electrophysiological measurements of membrane potential. Root epidermal cells were impaled with an intracellular microelectrode, and a stable membrane potential was recorded for several minutes before the onset of phloretin treatment. Addition of phloretin to the bathing solution at final concentrations of 250 and 500 μM induced rapid membrane depolarization in a concentration-dependent manner. Pharmacological analysis using three plasma membrane ion-transport inhibitors (vanadate, an inhibitor of plasma membrane H^+ -ATPase; LaCl_3 , a blocker of Ca^{2+} -permeable channels; and tetraethylammonium chloride (TEA), a blocker of voltage-gated K^+ channels) was conducted to assess the contribution of ion transport processes to the generation of early electrical signaling events in the root response to phloretin. Among the tested inhibitors, only LaCl_3 significantly reduced the amplitude of phloretin-induced depolarization compared with phloretin treatment alone, whereas vanadate and TEA had no significant effect. When applied alone, none of the inhibitors significantly altered membrane potential relative to their corresponding controls. These findings identify the root plasma membrane as an early target of phloretin action and indicate the involvement of La^{3+} -sensitive Ca^{2+} -permeable channels in the generation of phloretin-induced membrane depolarization in *A. thaliana* root epidermal cells.

KEYWORDS: Ca^{2+} -permeable channels; ion transport inhibitors; lanthanum chloride; membrane potential; plasma membrane H^+ -ATPase

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Photosynthetic Pigment Responses of *Chlamydomonas acidophila* PM01 to Reduced Light Intensity: Comparison of Normalization Approaches

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Light intensity is an important factor influencing microalgal growth, biomass production, and photosynthetic pigment content. However, less is known about the effects of low light conditions. *Chlamydomonas acidophila* is a microalga that thrives in environments and exhibits adaptations in its photosynthetic processes. This study investigated the effect of reduced light intensity on biomass, cell number, and pigment concentration in *C. acidophila* strain PM01 during the early stationary growth phase. After 15 days of cultivation under conditions, cultures were exposed to two-fold lower light intensity for 1 and 24 h. Microscopic cell counts revealed no differences between control and treated cultures, indicating no effect on cell proliferation. Gravimetric analysis showed a significant decrease in biomass after 1 h, followed by recovery after 24 h. Photosynthetic pigment analysis revealed a significant increase in chlorophyll *b* and total carotenoid concentrations after 1 h when normalized to biomass, with pigment levels tending to return toward control values after 24 h. In contrast, no significant differences were observed when pigment concentrations were normalized to cell number. The significant increase observed after 1 h when normalized to biomass may therefore reflect the decrease in biomass rather than a true increase in pigment content, as no changes in cell number were detected. These findings highlight the importance of the normalization approach when interpreting pigment responses. Reduced light intensity induced transient changes in biomass and photosynthetic pigment content, while cell proliferation remained unaffected, suggesting a rapid acclimation response of *C. acidophila* PM01 to short-term light reduction.

KEYWORDS: *C. acidophila* PM01; photosynthesis; pigments; normalization

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Extremophilic Microalga *Chlamydomonas acidophila* PM01: Effective Photoacclimation to Reduced Light Intensity

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The intensity, duration, and quality of light significantly influence the growth and biomass composition of microalgal cultures, with acclimation to different light intensities involving changes in the organization and function of the photosynthetic apparatus. *Chlamydomonas acidophila* is an extremophilic microalgal species for which previous studies have shown that cultures cultivated under low light from the beginning can maintain cell viability and photosynthetic activity even at light intensities nearly 20 times lower than the optimal level. This study investigated the short-term response of *C. acidophila* strain PM01 to reduced light intensity during the early stationary growth phase. After 15 days of cultivation under the optimal light conditions, the cultures were exposed to a two-fold lower light intensity for 1 and 24 h and analyzed using Pulse-Amplitude-Modulated (PAM) fluorometry. Short-term exposure of *C. acidophila* PM01 to reduced light intensity induced transient changes in photosynthetic performance. F_v/F_m remained statistically unchanged, while $Y(II)$ and ETR were significantly reduced after 1 h, followed by an increase toward control values after 24 h. This indicated the successful acclimation of the photosynthetic apparatus rather than permanent impairment of PSII. NPQ also showed an initially higher value after 1 h, followed by a decrease after 24 h, and exceeded control levels. These changes suggest temporal adjustments in photoprotective energy dissipation in response to altered light availability. Overall, the stable F_v/F_m and recovery of $Y(II)$ and ETR, together with dynamic NPQ responses, indicate that *C. acidophila* PM01 can rapidly adjust photosynthetic activity to reduced light conditions.

KEYWORDS: *C. acidophila* PM01; photosynthesis; PAM; ETR; NPQ; $Y(II)$; F_v/F_m ; photoacclimation

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Antioxidative Metabolism of Yellow Toadflax (*Linaria vulgaris* Mill.) in Response to Herbivory and Gallogenesis

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Plant galls represent specialized insect-induced structures that reprogram host development, requiring fine-tuned antioxidant metabolism for tissue hypertrophy. This study compares the effects of insect herbivory and gallogenesis induced by the weevil *Rhinusa pilosa* on antioxidant enzyme activities and the ascorbate-glutathione redox status in leaves and stems of *Linaria vulgaris*. The activities of ascorbate-glutathione cycle enzymes (ascorbate peroxidase, APX; dehydroascorbate reductase, DHAR; monodehydroascorbate reductase, MDAR; glutathione reductase, GR) and class III peroxidases (soluble peroxidases, solPOD, and ionically bound peroxidases, ionPOD) were analyzed spectrophotometrically and via isoelectric focusing (IEF).

Gallogenesis triggered a distinct redox strategy compared to stem herbivory. During gall development, APX activity significantly decreased in leaves and gall-bearing stems, likely facilitating localized accumulation of reactive oxygen species needed for cell wall loosening and tissue hypertrophy. Conversely, stem herbivory substantially increased solPOD and ionPOD activities, correlating with defensive cell wall lignification. IEF analysis revealed two solPOD isoforms exclusive to gall tissues. Furthermore, herbivory increased stem DHAR activity while MDAR activity dropped 3.4-fold compared to controls. GR activity was unaffected by either herbivory or gallogenesis. Gall development was accompanied by a decreased ascorbate redox state in stems and ascorbate depletion in leaves, along with a shift toward oxidized glutathione, contrasting with the elevation of reduced glutathione observed under herbivory alone.

In conclusion, while stem herbivory activates peroxidase pathways to drive defensive cell wall lignification in response to tissue damage, *Rhinusa pilosa* suppresses APX activity and alters ascorbate-glutathione homeostasis to reprogram host redox status for gall morphogenesis in *Linaria vulgaris*.

KEYWORDS: *Linaria vulgaris*; *Rhinusa pilosa*; gallogenesis; insect herbivory; antioxidant enzymes

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Correlations Between Oxidative Stress and Antioxidant Responses in Tomato Under Waterlogging

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Waterlogging is an increasingly important environmental constraint for agricultural production, particularly under changing climatic conditions characterized by more frequent and intense precipitation events. Tomato (*Solanum lycopersicum* L.) is highly sensitive to waterlogging, which restricts oxygen availability in the root zone and induces a complex cascade of physiological, biochemical, and molecular responses. Root hypoxia can promote reactive oxygen species accumulation, leading to oxidative damage, while plants activate enzymatic and non-enzymatic antioxidant mechanisms to maintain redox homeostasis. In this study, the relationship between oxidative stress markers and antioxidant response in tomato plants exposed to 24h waterlogging was investigated. Hydrogen peroxide (H₂O₂), malondialdehyde (MDA), reduced glutathione (GSH), protein concentration, peroxidases class III (POX) activity, and total phenolic compounds were analysed, and correlations among these parameters were determined. H₂O₂ and MDA concentrations showed highly significant positive correlations with total phenolic compounds ($r = 0.862$ and 0.573 , respectively), protein concentration ($r = 0.912$ and 0.645), and GSH concentration ($r = 0.939$ and 0.717). H₂O₂ and MDA were also strongly positively correlated with each other ($r = 0.760$). Total phenolic compounds were positively correlated with protein concentration ($r = 0.735$), while POX activity showed a significant positive correlation with total phenolic compounds ($r = 0.432$). These correlations indicate that higher levels of oxidative stress were associated with enhanced accumulation of GSH, proteins, and phenolic compounds, suggesting their involvement in the tomato response to waterlogging. The coordinated enzymatic and non-enzymatic responses appear closely linked to the tomato response to waterlogging stress.

KEYWORDS: hydrogen peroxide; malondialdehyde; glutathione; phenols and peroxidases class III

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Analysis of Uncharacterized Transcripts Involved in the Potato Transcriptome Response to French Marigold Essential Oil Volatiles

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Plants communicate with neighbouring organisms through volatile organic compounds (VOCs), however the molecular mechanisms underlying plant responses to VOC-mediated signals remain poorly understood. To investigate plant–plant communication via VOCs, potato (*Solanum tuberosum* L.) plants were exposed to French marigold (*Tagetes patula* L.) essential oil (EO). Among 2,796 differentially expressed (DE) transcripts ($|FC| \geq 2$; $p \leq 0.05$) identified by cDNA microarray analysis, 445 sequences (15.92%) lacked functional annotation in the available databases. PFAM analysis provided putative functional information for 46.96% of the uncharacterized transcripts and revealed diverse domains mainly associated with transcriptional regulation, signaling, stress responses, and cellular and metabolic processes. Six domains were associated with transcription factors, including GATA-type transcriptional activator, MYB-CC-type transcription factor, Myb-like DNA-binding, helix-loop-helix DNA-binding, GRF zinc finger, and BED zinc finger domains. The GATA-type transcriptional activator was among the most differentially expressed uncharacterized transcripts ($\log_2FC = -6.74$) and was therefore selected for further *in silico* characterization. Comparison with the corresponding genomic sequence showed 98.43% sequence identity, confirming the predicted potato gene identity. The encoded protein showed high similarity to GATA-type proteins from closely related Solanaceae species and was associated with chloroplast organization and positive regulation of transcription. These findings highlight uncharacterized transcripts as potential components of VOC-induced signaling and transcriptional regulation in potato and identify the GATA-type transcriptional activator as a promising candidate linking EO-induced signaling with transcriptional and chloroplast-associated responses.

KEYWORDS: transcriptomics; microarray; plant communication; VOCs; *in silico* analysis

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Transcriptomic Insights Into Desiccation Tolerance in the Resurrection Fern *Asplenium ceterach* L.

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Asplenium ceterach L. (family Aspleniaceae) is a perennial herbaceous fern belonging to the group of homoiochlorophyllous desiccation-tolerant plants, capable of withstanding prolonged periods of severe dehydration and resuming metabolic activity upon rehydration. To investigate the molecular mechanisms underlying the dehydration response and desiccation tolerance, cDNA libraries were constructed in triplicate from mRNA isolated across *A. ceterach* fronds at fresh, dry, and rehydrated (8 h) states and sequenced using Illumina next-generation technology. *De novo* transcriptome assembly and filtering using Trinity and CORSET yielded a high-quality unigene set, validated by BUSCO for completeness, while principal component and hierarchical cluster analyses revealed a well-defined separation of gene expression patterns according to water status. To characterize the transcriptomic landscape, Gene Set Enrichment Analysis (GSEA) via the *fgsea* algorithm and Gene Set Variation Analysis (GSVA) followed by linear modeling with the *limma* package were performed. These analyses identified significant transcriptomic shifts in translation and photosynthesis, alongside the upregulation of genes involved in water deprivation and osmotic and oxidative stress to mitigate dehydration stress. Concurrently, targeted GSVA profiling revealed a robust and synchronized activation of antioxidant response specifically in the dry state, characterized by the marked upregulation of antioxidant hub genes. Importantly, as a homoiochlorophyllous species, photosynthesis-related pathways were downregulated in the dry state and remained suppressed during early rehydration (8h). These comparative profiles provide new insights into the molecular mechanisms underlying desiccation tolerance in *A. ceterach* and provide a valuable foundation for understanding resilience in resurrection plants.

KEYWORDS: RNA-Seq; Dehydration tolerance; *Asplenium ceterach*

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Induction of Antioxidant Enzyme Activities in Potato (*Solanum tuberosum* L.) Triggered by Aqueous Extracts of Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) Larvae

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Plant responses to herbivory rely on recognizing insect-derived signals that trigger physiological and biochemical defence pathways, including the modulation of the antioxidant system. In this study, we aim to investigate the potential of aqueous extracts obtained from whole Colorado potato beetle (*Leptinotarsa decemlineata* Say) larvae to act as elicitors of biochemical defence responses in potato (*Solanum tuberosum* L.) leaves. Potato plants were grown in chamber under controlled climate conditions. Larvae at different developmental stages were collected from chemically untreated field. To prepare the stock extract, larvae were ground in liquid nitrogen, extracted in distilled water, and filtered after 5 days. The resulting stock extract was then serially diluted to prepare 1:10, 1:20, 1:40, 1:60 (v/v) solutions. Fully developed potato plants were sprayed daily with 100 mL of the respective larval extract dilutions for four continuous days, alongside a water-treated control group. Leaf samples were collected at five time points (24, 48, 72, 96 h, and 5 days after the final application) for biochemical analysis. To assess potential changes in key antioxidant enzymes, spectrophotometric assays will be used to determine catalase (CAT) and peroxidase (POX) activities, while native gel electrophoresis (Native PAGE) will be employed for in-gel isoenzyme detection and Western blot analysis for protein-level assessment. The results revealed that repeated treatment with 1:10 and 1:20 dilutions of the larval extract significantly modulated POX activity ($F = 4.21$, $p < 0.01$), inducing the strongest response compared to controls, whereas CAT activity did not differ significantly among treatments ($p > 0.05$). These findings demonstrate a differential regulation of antioxidant enzymes, advancing our understanding of plant-insect interactions for sustainable crop protection strategies.

KEYWORDS: *Solanum tuberosum* L.; *Leptinotarsa decemlineata* Say; plant defense; elicitors; catalase; peroxidase

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Characterization of Gamma-Irradiated Wheat M5 Lines Based on Agronomic, Physiological, and Grain Quality Traits

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Induced mutations represent a useful approach in plant breeding for generating novel variation that can be explored through subsequent selection and evaluation. The identification of promising genotypes with favourable characteristics may provide valuable material for further breeding and crop improvement. In this study, a set of 68 wheat M5 lines derived from gamma-irradiated seeds of the wheat (*Triticum aestivum* L.) cultivar NS40S were characterized based on agronomic, physiological, and grain quality traits. Among agronomic traits, spike length (cm), number of spikelets per spike, and thousand-grain weight (g) were analysed. Using a Dualex leaf-clip meter the following physiological parameters were assessed: chlorophyll, flavonol, and anthocyanin contents, as well as the nitrogen balance index (NBI). Grain quality was evaluated based on protein, starch, and gluten contents using a FOSS InfratecTM Near-Infrared (NIR) analyzer. Considerable variation in the investigated traits was observed among the analysed lines, with some lines exhibiting pronounced deviations from the corresponding trait values of the cultivar NS40S. The highest coefficients of variation were recorded for anthocyanin content (23.1%), spike length (15.5%), NBI (14.1%), thousand-grain weight (10.3%), and gluten content (9.1%), whereas starch content showed the lowest coefficient of variation (2.3%) among the analysed traits. The observed variability and the identification of lines exhibiting pronounced deviations from the cultivar NS40S provide a valuable basis for further investigation of their physiological responses, phenotypic stability, and agronomic performance under different environmental conditions, with potential applications in wheat breeding.

KEYWORDS: *Triticum aestivum* L.; gamma irradiation; yield related traits; quality traits; physiological parameters

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Photosynthetic Performance of Tomato Plants in Response to *Trichoderma* spp. During Short-Term Water Deficit

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Tomato cultivar Narvik was previously found to show enhanced seed germination under *Trichoderma* treatment. Therefore, it was used to test whether this effect extends to the photosynthetic apparatus under water deficit. Seedlings were grown in pots in a growth chamber and exposed to six treatments: optimal watering (37% soil volumetric water content), cessation of watering, and both regimes combined with *T. harzianum* or *T. brevicompactum*. *Trichoderma* suspension (8×10^6 CFU mL⁻¹) was applied near the root zone at the four fully developed leaf stage. Soil water content (Theta probe, Delta-T, Cambridge, UK), linear electron flow (LEF), non-photochemical quenching (NPQt), PSI active centres, relative chlorophyll content (PhotosynQ, East Lansing, USA), and chlorophyll *a* fluorescence (Handy PEA, Kings Lynn, UK) were recorded together with leaf temperature (FLIR, Oregon, USA) 6, 24 and 48 hours after inoculation.

Fv/Fm remained ≈ 0.83 across all treatments, indicating intact PSII. Under drought, *T. brevicompactum* raised leaf temperature after 6 and 24 h, suggesting reduced transpiration and potential stomatal closure. Both strains increased LEF at 6 h and PSI active-centre values at 24 and 48 h in optimally watered plants. NPQt was consistently lowest under drought with *T. brevicompactum*, while relative chlorophyll content remained unchanged. Photosynthetic performance index (PI-ABS) tended to be higher with both strains after 24 h and was highest with *T. harzianum* after 48 h under drought. These strain and time dependent responses occurred without a decline in PSII maximum quantum efficiency, indicating regulatory adjustment of energy use rather than impairment of photochemistry.

KEYWORDS: plant–microbe interactions; *Solanum lycopersicum*; drought; thermal imaging; chlorophyll *a* fluorescence

ACKNOWLEDGEMENT: This research was funded by the Provincial Secretariat for Higher Education and Scientific Research through the project "A Biological Approach to Improving Germination, Growth, and Nutrient Use Efficiency in Wheat", Project No. 002496898 2026 09418 002 000 000 001.

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Structural and Interaction Dynamics of RsLEA301 Protein With Thylakoid Membranes

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Late Embryogenesis Abundant (LEA) proteins play a crucial protective role in plants under abiotic stress conditions, such as drought and extreme temperatures, by stabilizing cellular structures and chloroplast thylakoid membranes. This is particularly important during vegetative desiccation in resurrection plants such as *Ramonda serbica* Panc. Understanding the molecular mechanisms of LEA-membrane interactions remains challenging due to the dynamic and disordered nature of these proteins. In this study, we investigated the conformational behavior and membrane-binding dynamics of the RsLEA301 protein interacting with a complex thylakoid lipid bilayer. The initial structural model of RsLEA301 was generated using the CALVADOS framework (Cao et al., 2024), while the molecular dynamics simulations were performed using the GROMACS software package.

Trajectory analysis revealed that the system successfully reached equilibration, with the Root Mean Square Deviation (RMSD) stabilizing at approximately 0.14-0.15 nm. The radius of gyration (Rg) decreased slightly from around 1.68 nm to 1.64 nm, indicating protein compaction upon surface adsorption. Furthermore, distance profiling and contact analysis demonstrated persistent interactions between LEA301 and lipid headgroups, with minimum distances consistently remaining below 0.2 nm and high contact numbers being maintained throughout the simulation. Root Mean Square Fluctuation (RMSF) analysis highlighted specific flexible regions, particularly around residue 25, acting as key interaction sites facilitating adaptation to the membrane. Additionally, these computational findings were complemented by Circular Dichroism (CD) spectroscopy measurements. Together, these insights reveal how intrinsically disordered LEA proteins protect thylakoid membranes under stress, supporting future developments in plant resilience engineering.

KEYWORDS: disordered proteins; LEA proteins; coarse-grained simulations; membrane-protein interactions

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Dual Effect of Rugulosin A: Inhibition of *Botrytis cinerea* and *Alternaria alternata* Growth and Protection Against Mold Disease in Grapevine Plantlets and Berries

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Rugulosin A (RugA), a bis-anthraquinone, was isolated and purified from the *Coniochaeta velutina* endolichenic fungus. To test RugA potential effects on phytopathogenic fungi such as *Botrytis cinerea* and *Alternaria alternata*, RugA was firstly evaluated on mycelial growth and spore germination of both fungi species. Whereas RugA inhibited *Botrytis cinerea* mycelium growth with an IC50 of 2.5 μ M, four times more RugA was necessary to inhibit *Alternaria alternata* mycelial growth (IC50 of 10 μ M). Nevertheless, considering spore germination inhibition, RugA was slightly more efficient on *Alternaria* than on *Botrytis* with a MIC of 75 μ M for *Alternaria alternata* and 100 μ M for *Botrytis cinerea*. The expression of genes involved in *Botrytis cinerea* development and infection decreased in the presence of 25 μ M of RugA. Confocal microscopy, paired with fluorochromes such as propidium iodide, DCFH DA, and 2-NDBG, indicated that RugA likely compromised membrane integrity, triggered oxidative stress, and decreased sugar uptake, all of which led to cell death. Furthermore, RugA effect was monitored during spore infection of grapevine leaves, plantlets, and berries that indeed altogether, showed a significant decrease in disease propagation. Moreover, the expression of some defense genes, such as PR1, PR3, PR4, STS, HSR1, and EDS1 involved in pathogenicity, was not induced on infected leaves treated with RugA. Furthermore, RugA did not show any toxicity onto housefly maggots and grapevine plantlets. Taken together, these data suggested that RugA could be a promising candidate for combating phytopathogenic fungi for further applications in agriculture.

KEYWORDS: *Alternaria alternata*; *Botrytis cinerea*; Phytopathogen fungi; Rugulosin A; Grapevine

Exploration of Physiological and Genetic Characteristics of Desert-Dwelling Fabaceae (Leguminosae)

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Desert-grown legumes have naturally evolved over the years and acquired specialized survival strategies, including physiological and genetic adaptations, like other plant species. Most legumes meet consumers' preferences because of their nutritional value, oil-producing properties, and therapeutic applications. Additionally, legumes' rhizobial symbiosis, which enables interactions with soil microbes and results in atmospheric N₂ fixation in root nodules, is advantageous for improving plant growth and soil fertility. Nonetheless, due to abrupt climatic changes caused by extensive human activity, the soil quality of both vegetated and desert lands is deteriorating, directly affecting crop nutrition and human health. Despite their significant importance, research on desert legumes is somewhat neglected. Therefore, in the present study, we focused on exploring the physiological and genetic characteristics of legumes grown in desert and dry/arid, drought-prone climatic conditions. This is the first review to compile the most relevant information available on the physiological and genetically resilient features of desert legumes, including the genetic correlation between rhizobacterial symbiosis and improved desert soil quality and plant vigor. Further, interventions using advanced biotechnological omics approaches that target improved endurance against severe environmental triggers are comprehensively discussed. The presented work highlights the prerequisites for incorporating legumes into desert land intercropping systems to facilitate crop improvement. Strategic research initiatives that associate reference genetic databases of desert legumes to explore distinct adaptive features could assist in the functional characterization of novel stress-resilient genes/alleles, thereby enhancing compromised legumes and non-legume crops from both desert and non-desert regions and addressing global food insecurity.

KEYWORDS: Fabaceae; Desert legumes; Soil microbiome; Abiotic stress tolerance; Genetic advancements

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Dissecting Boron Tolerance in *Puccinellia distans*: Physiological and Genetic Variation Among Diverse Accessions

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Boron (B) toxicity is an important constraint to plant growth and agricultural productivity, particularly in arid and semi-arid regions where B can accumulate to phytotoxic levels. Naturally occurring plant populations exposed to B-rich environments provide valuable material for exploring the physiological and genetic basis of tolerance to excessive B. In this study, 11 geographically diverse *Puccinellia distans* accessions were evaluated under increasing B concentrations (0, 250, 500, and 1000 mg L⁻¹). Boron responses were characterized through root and shoot growth, stress tolerance indices, H₂O₂ accumulation, and genetic diversity analysis using Start Codon Targeted (SCoT) markers. The accessions displayed pronounced variation in their responses to B toxicity. While increasing B concentrations progressively inhibited root and shoot growth in most accessions, the Kirka ecotype, originating from a B-rich mining region in Türkiye, showed exceptional tolerance. At 500 mg L⁻¹ B, its shoot tolerance index exceeded 100%, while root growth was nearly fully maintained. Even at 1000 mg L⁻¹ B, Kirka retained more than 90% of its shoot growth. This superior growth performance was accompanied by remarkably limited oxidative stress. At 500 mg L⁻¹ B, H₂O₂ accumulation increased by up to 2.43-fold in sensitive accessions, whereas Kirka showed virtually no increase (1.01-fold). SCoT analysis revealed substantial genetic diversity among the accessions, with 90.46% polymorphism detected across 15 markers. UPGMA clustering and principal component analysis further demonstrated genetic structuring among the accessions; however, the observed patterns of B tolerance were not fully explained by overall genetic similarity. Overall, the integration of physiological, oxidative stress, and molecular marker data revealed considerable intraspecific variation in B tolerance within *P. distans*. The exceptional performance of the Kirka ecotype under severe B stress highlights naturally adapted populations as valuable resources for investigating the mechanisms underlying B tolerance and for identifying genetic material with potential applications in the development of B-tolerant crops.

KEYWORDS: *Puccinellia distans*; boron toxicity; stress tolerance; SCoT markers; genetic diversity; oxidative stress



ECOLOGY, GENETICS AND EVOLUTION

Tiny Plants, Big Impact: Recent Needs and Modern Solutions in Bryophyte Conservation

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Classical approaches to plant conservation are fading with contemporary environmental challenges. Novel and modern approaches are needed urgently. This is especially evident in bryophytes, which are less well-known and highly specialized compared to other plants. Modern bryophyte conservation is based on an integrated understanding of their ecological importance, biological characteristics, and responses to environmental change. Bryophytes, including mosses, liverworts, and hornworts, are important components of many ecosystems, contributing to water regulation, soil formation, nutrient retention, and the provision of microhabitats for numerous organisms. However, habitat loss and fragmentation, pollution, climate change, and alterations in moisture regimes pose increasing threats to their survival.

Contemporary conservation strategies combine *in situ* protection of natural populations with *ex situ* approaches, biological features studies including cultivation under controlled conditions, propagation, genetic resource banking, and the preservation of reproductive structures. Molecular techniques provide valuable tools for accurate species identification, assessment of genetic diversity, and analysis of population structure. Biotechnic and biotechnological approach combining with prior stated, are promising tool in saving bryophytes and enable self-sustainable survival.

In addition, remote sensing, geographic information systems, and advanced monitoring methods improve habitat assessment and facilitate long-term population monitoring. Effective bryophyte conservation requires the integration of field research, experimental approaches, and ecological modelling, together with the involvement of many laboratory testings with aim to development of species specific protection strategies to overcome future negative climate scenarios. This interdisciplinary approach i.e. integrative conservation provides an important foundation for conserving bryophyte diversity and maintaining the ecological functions of the ecosystems they inhabit.

KEYWORDS: mosses; liverworts; conservation physiology; integrative conservation; protection

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Progress in Oat DH technology and Functional Characteristics of Oat × Maize Hybrids

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The development of various biotechnological methods for generating homozygous plants has significantly influenced modern agriculture. Among them, the production of doubled haploids (DH) provides an efficient tool for accelerating improvement and enhancing breeding progress.

Production of oat (*Avena sativa* L.) DH lines remains highly challenging, with efficiency strongly dependent on the donor genotype and culture conditions. Studies demonstrate that oat DH are predominantly produced through wide hybridization with maize (*Zea mays* L.), not by anther or microspore cultures. Wide hybridization derived oat × maize addition (OMA) lines frequently retained maize chromatin, including whole chromosomes or their fragments, resulting in embryo development, as well as morphological and physiological changes of plants.

Extensive characterization of OMA lines showed that introgressed maize chromatin affects nuclear architecture, photosynthetic performance, and drought tolerance. Studies using chlorophyll *a* fluorescence, biochemical markers, and yield components demonstrated that OMA plants exhibit distinct physiological responses under soil drought stress. Compared with conventional oat cultivars, OMA plants exhibited lower excised leaf water loss and maintained higher yield, stem biomass, and grain production under drought. They also accumulated more soluble sugars and phenolic compounds, which contribute to osmotic adjustment and antioxidant defense. Additional studies highlighted the roles of metallothioneins in improving oat stress resilience and enhancing the seed quality.

Together, these studies provide a comprehensive understanding of DH lines production, embryo developmental biology, and the functional consequences of maize chromatin introgression, supporting improved breeding strategies for enhancing drought tolerance and yield stability of oat.

KEYWORDS: androgenesis; *Avena sativa* L.; gynogenesis; *Zea mays* L.; wide crossing

Integrating Genetic Resources, Phenomics, Genomics and Transcriptomics to Enhance Wheat Drought Resilience

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Climate change is significantly altering the conditions in which crops are grown, while the increasing frequency and severity of drought episodes across agricultural regions worldwide threaten yield stability and food security, particularly in rainfed crops such as bread wheat. Traditional breeding approaches have significantly contributed to wheat improvement; however, they have achieved only limited success in enhancing drought tolerance due to its complex genetic architecture, low heritability, strong genotype-by-environment interactions, and genetic erosion resulting from intensive breeding. Wheat landraces have been maintained independently across diverse geographic regions and have been subjected to less intensive selection than modern cultivars, thereby preserving unique genes and alleles, especially those associated with tolerance to abiotic and biotic stresses. Hence, their use in breeding programs has been limited due to their lower yield potential compared with elite cultivars and the lack of comprehensive genotypic and phenotypic data required for their effective exploitation.

In this talk, I will present the latest strategies implemented at the Institute of Field and Vegetable Crops for in-depth characterization of wheat genotypes at the morphological, physiological, and molecular levels. These approaches, including remote sensing, controlled-environment high-throughput phenotyping, SNP-based genotyping, and gene expression analysis, enable the identification of key traits, genes, and alleles associated with wheat drought tolerance throughout different stages of plant development. This talk will also focus on the latest techniques for the efficient introgression of novel alleles from landraces into modern breeding germplasm to improve wheat yield stability under water-deficit conditions.

KEYWORDS: *Triticum aestivum* L.; DNA sequencing; genome-wide association study; root architecture; water-use efficiency

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From Field to Genome: UAV Phenotyping and SSR Markers Reveal Drought-Tolerance Signatures Across Maize Hybrid Environments

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How do maize hybrids behave when pushed across contrasting environments – and can we predict their resilience from molecular markers alone? This presentation previews results from a large-scale multi-environment trial evaluating ten maize hybrids across five locations. Using a dual approach that combines UAV-based remote phenotyping with manual field measurements, we characterized agronomic performance for yield, and yield components. In parallel, we screened the same material with ten SSR markers linked to these traits, aiming to uncover associations relevant to drought tolerance. The integration of drone-based phenomics, fieldomics and marker-assisted evaluation across diverse environments provides both a methodological blueprint and early insights into which hybrids – and which markers – hold the greatest promise for stress adaptation. The dual-platform approach – combining remote sensing efficiency with targeted molecular profiling – offers a scalable framework for accelerating the selection of stress-resilient maize genotypes in breeding pipelines.

KEYWORDS: drought stress; maize; multienvironment trials; multienvironment trials; molecular breeding

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Intercropping Modifies Species-Specific Responses to Multiple Metal Stress in Flotation Tailings

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Flotation tailings are challenging environments characterised by elevated metal concentrations and unfavourable physicochemical properties that can severely limit plant establishment and growth. Successful colonisation of such substrates depends on the ability of plants to adjust their physiological responses to multiple metal stress. This study investigated species-specific physiological responses of *Lotus corniculatus* L. (Fabaceae) and *Festuca arundinacea* Schreb. (Poaceae) grown on Pb-Cu-Zn mine flotation tailings and uncontaminated soil, either in monoculture or intercropped. Oxidative stress and antioxidant responses were assessed through electrolyte leakage (EL), lipid peroxidation (LP), antioxidant enzyme activities (superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase), total antioxidative capacity, phenolic compounds content, total soluble protein content and photosynthetic pigment quantification, while biomass was used as an indicator of plant performance. Increased EL and LP, coupled with reduced soluble protein content in both species, suggest that tailings induced pronounced oxidative stress, particularly in monoculture. Intercropping increased both free and bound phenolics in shoots and reduced EL, while effects on LP and antioxidant enzyme activities depended on the species and plant organ. Photosynthetic pigments and pigment ratios were strongly altered, particularly in *L. corniculatus*. Intercropping increased *L. corniculatus* biomass on both substrates, and significantly enhanced *F. arundinacea* biomass on tailings, with limited effects on uncontaminated soil. Overall, the results indicate that *L. corniculatus* and *F. arundinacea* employ distinct, organ-dependent physiological strategies to cope with multiple metal stress. Intercropping modifies these responses and may enhance plant performance on flotation tailings, suggesting that species interactions can influence physiological tolerance to complex metal stress.

KEYWORDS: oxidative stress; antioxidant response; metal tolerance; *Lotus corniculatus*; *Festuca arundinacea*

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Exploring the Genomic Basis of Seed Protein Accumulation in Confectionery Sunflower

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Seed protein content is an important quality trait in confectionery sunflower, yet the molecular processes underlying its variation remain insufficiently understood. In this study, we combined *de novo* genome sequencing and whole-genome resequencing of confectionery sunflower inbred lines differing in seed nitrogen and protein content to explore genomic variation associated with this trait. A high-quality reference assembly was generated using PacBio sequencing, while additional genotypes were re-sequenced using Illumina technology for comparative variant analysis. Integrated gene-level and functional analyses identified genomic variation associated with processes involved in nitrogen and amino acid metabolism, transport, and protein processing. Overall, the results suggest that seed protein accumulation is a complex trait involving multiple interconnected metabolic and regulatory processes. The identified genomic regions and candidate processes provide a basis for further investigation of the mechanisms involved in seed protein accumulation and their potential use in breeding of confectionery sunflower with improved seed quality.

KEYWORDS: *Helianthus annuus* L.; amino acid metabolism; nitrogen remobilization; seed quality; whole-genome resequencing

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Does an Increase in Ploidy Level Affect Resilience to Copper Stress in the Rare and Endangered Funaroid Moss *Physcomitrium eurystomum*?

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Increased ploidy level is generally associated with enhanced plant resilience to environmental stresses, mostly through mechanisms of gene dosage and genetic redundancy, although the specific effects depend strongly on the species, stressor, and experimental conditions. In this study, we investigated whether ploidy level influences the response to copper acetate stress in the rare and endangered moss *Physcomitrium eurystomum* Sendt. under axenic conditions. In mosses, the dominant life stage is the haploid, photosynthetically active gametophyte. To obtain diploid gametophytes, controlled apospory was induced through injury-driven redifferentiation of diploid sporophyte seta tissue. Haploid and diploid lineages of the target species were exposed for 2 or 24 h to copper acetate solutions at different concentrations (0, 200, 700, and 1200 μM), simulating transient *in situ* contamination as species inhabits ephemeral muddy deposits near water bodies subject to frequent water-level fluctuations. Survival, morphological, and photosynthetic responses were assessed after five weeks of recovery on KNOP minimal medium following stress exposure. Diploid plants exhibited a lower survival percentage than their haploid counterparts after 24 h of exposure to copper acetate. Stressor reduced multiplication in diploid plants at 700 and 1200 μM after 2 h, whereas haploid plants were unaffected. After 24 h, all concentrations strongly inhibited new shoot formation, although surviving plants developed secondary protonemata. Short-term exposure did not affect photochemical (qP) or nonphotochemical quenching (NPQ), while diploids exposed to 1200 μM for 24 h maintained control-like qP values. These findings suggest a complex role of ploidy in copper stress resilience and emphasize need for further mechanistic research.

KEYWORDS: apospory; photosynthesis; morphogenesis; *in vitro*; bryophytes

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Genetic Variability Screening of Five *Digitalis* species in the Balkan Peninsula

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The genus *Digitalis* counts 20 species, some of which being recognized as producers of valuable pharmacologically active specialized metabolites, primarily cardenolide glycosides. The aim of the research was to analyse the genetic diversity of selected Balkan Peninsula populations. The microsatellite study was performed using 6 newly developed EST-SSR markers and included all five Balkan species belonging the genus *Digitalis*, originating from 42 locations. The analysed loci showed excellent polymorphism, up to 100% across the populations. Interpopulation genetic variation contributed to around ¼ of total variation, whereas within-population variation represented the predominant source of overall genetic variation, indicating that the studied populations of five *Digitalis* species have maintained a high level of genetic heterogeneity. The observed heterozygosity of ~0.25–0.6 also supports this standpoint. Population differentiation was mostly pronounced in *D. lanata* and the lowest was recorded in *D. ferruginea*. The lowest number of private alleles was found in *D. grandiflora* and the highest in *D. laevigata*. Although being endemic for the Balkan Peninsula, *D. viridiflora* has retained high level of allelic variation, with the *F_{ST}* values between the four investigated population varying from 0.112 to 0.417. It is generally estimated that species of the genus *Digitalis* on the Balkan Peninsula represent a significant reservoir of genetic diversity.

KEYWORDS: Foxglove; disjunct populations; fixation index; genetic differentiation

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If it is an Adjoining Neighbor, and if it Has Exactly the Same Flower Color, Then it Must be From the Same *Iris pumila* Clone... Not Necessarily!

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Iris pumila L. is a clonal species with huge flower colour polymorphism. In previous research on this species, adjoining flowers with an exact match in flower colour were considered parts of the same clone with the same genotype, and were used as such in many experiments and studies. In the IRIS Project (PRISMA programme) we are testing this assumption by analysing five microsatellite loci with more than 40 alleles, and detecting the so-called multilocus allele phenotype for each analyzed flower. If a difference between individuals is detected at any locus of a microsatellite marker, we can conclude that the individuals are genetically different and do not represent the same clone. Furthermore, to eliminate any subjectivity in determining flower colour, in the IRIS Project it was assessed precisely using UV-enhanced reflectance spectroscopy. In this contribution we present preliminary findings from a pilot analysis performed only on sampled yellow flowers. In this pilot test we have already detected adjoining flowers of similar yellow colour (determined visually and confirmed by spectroscopic analysis) that had different multilocus allele phenotypes, i.e. possessed different genotypes and consequently were not parts of the same clone.

In further analyses of all sampled flowers with different colours in the IRIS Project, we will attempt to determine how often nearby flowers that appear to be exactly the same colour truly belong to the same clone, and to evaluate possible reasons for the presence of different clones with the same color phenotype at such small distances.

KEYWORDS: *Iris pumila*; natural populations; clone identification

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Effects of Genotoxic Agents on Growth and Homologous Recombination-Related Responses in *Arabidopsis thaliana* *dss1(l)* Mutants

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A small intrinsically disordered protein DSS1 is known for numerous roles in cell. The participation of protein DSS1 in the maintenance of genome stability and the response to oxidative stress has been well studied. Due to the prevalence of gene duplication in plant species, *Arabidopsis thaliana* has two highly homologous *DSS1* genes, *DSS1(l)* and *DSS1(V)*. To investigate the role of DSS1 in genome stability, we examined *Arabidopsis thaliana dss1(l)* mutant seedlings treated with the genotoxic agent 1,2,3,4-diepoxybutane (DEB) at a concentration of 0.000375%. Expression analysis of genes involved homologous recombination, including *BRCA1*, revealed a significant increase in expression levels under the influence of DEB. On the 11th day of DEB treatment, the primary root length of *dss1(l)* mutant seedlings was significantly reduced compared with wild-type seedlings, indicating that *dss1(l)* mutants are more sensitive to DEB-induced DNA damage than wild-type plants. In addition, we examined the subcellular localization of a YFP-tagged DSS1(l) fusion protein in *Nicotiana benthamiana* leaves using agroinfiltration. DSS1(l)-YFP fluorescence was predominantly localized in the nuclei. While these results suggest that DSS1(l) is involved in maintaining genome stability and the DNA damage response, our previous findings demonstrate that DSS1(V) specifically contributes to protection against oxidative stress. Together, our findings highlight a clear functional divergence between these two DSS1 homologs.

KEYWORDS: DSS1(l); DEB treatment; *Arabidopsis thaliana*

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Seed viability of Three *Dactylorhiza* (Orchidaceae) Species: Implications for *Ex Situ* Conservation

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The genus *Dactylorhiza* (Orchidaceae) is taxonomically complex, with representatives distributed mainly across temperate and boreal regions. Because species of this genus predominantly occupy wet habitats, climate change is expected to cause habitat loss, leading to the decline and disappearance of numerous populations. The aim of this study was to determine seed viability in *Dactylorhiza cordigera* subsp. *cordigera*, *D. incarnata* subsp. *incarnata* and *D. sambucina* in relation to altitude, vegetation type, geological substrate, population size, area occupied by the subpopulation and flowering period, in order to identify the most favorable source populations for developing an *ex situ* conservation protocol for these species. Seeds were collected from Kopaonik Mountain (Serbia) in July 2026. To determine seed viability, a triphenyltetrazolium chloride (TZ) test was conducted. The procedure involved soaking the seeds in distilled water for one hour, followed by incubation in a 1% TZ solution in Petri dishes in complete darkness at 28 °C for 20 hours. Viable seeds, identified by their red staining, were subsequently quantified under a stereo-microscope. The results showed significant differences in viability, ranging from 0% in *D. cordigera* subsp. *cordigera* to 44% in *D. sambucina*. Statistical analysis revealed that the onset of flowering and the area occupied by the populations had the strongest effects on seed viability. These findings indicate that higher-altitude *Dactylorhiza* populations require later sampling due to delayed phenological dynamics. This study highlights that earlier-flowering populations occupying smaller areas exhibited the highest seed viability, identifying them as optimal source populations for developing *ex situ* conservation protocols.

KEYWORDS: orchids; *Dactylorhiza*; ecology; seeds; conservation

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Exploring Flower Color Polymorphism in *Iris pumila* L. Through Pigment Analysis by Ultra-High-Performance Liquid Chromatography (UHPLC)

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Iris pumila L. is a clonal species with huge flower color polymorphism. Maintenance of such polymorphisms is one of the central problems in evolutionary and conservation biology, so studying it in *I. pumila* can help answer important evolutionary questions about the mechanisms that maintain genetic variability maintenance in natural populations. Furthermore, flower color has been previously used to determine different *I. pumila* clones in various experiments and studies. However, this was done by visual identification, which is imprecise and condition dependent, and therefore can lead to inconsistencies in defining color morphs. In the project entitled "Genetic diversity maintenance and population dynamics in natural populations of endangered plant species: Flower color polymorphism in *Iris pumila* L." (Project IRIS in the PRISMA Programme) we are avoiding subjectivity in color determination by utilizing UV-enhanced reflectance spectroscopy as well as through profiling pigments from various pigment classes by Ultra-High-performance liquid chromatography (UHPLC) on 240 *I. pumila* flowers.

In this contribution, we present findings from the Partial Least Squares-Discriminant Analysis (PLS-DA) performed on selected pigments quantified by UHPLC method. The results show that yellow flowers are clearly separated from other color morphs on the first axis, while other color variants show moderate separation on both first and second axes. The most influential secondary metabolites driving the phenotypic separation observed in the PLS-DA model through calculation of Variable Importance in Projection (VIP) scores, were also identified.

In further analyses we will explore the relationship between VIP pigments and spectral quality of *I. pumila* flowers, analyzed in context of both insect and human vision.

KEYWORDS: Flower color polymorphism; *Iris pumila*; natural populations; pigments; UHPLC

ACKNOWLEDGEMENT: This research was supported by the Science Fund of the Republic of Serbia, Grant No. 7003, "Genetic diversity maintenance and population dynamics in natural populations of endangered plant species: Flower color polymorphism in *Iris pumila* L. – IRIS". Research was also financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant 451-03-33/2026-03/ 200007.

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Morphological and Spectroscopic Characterization of *Coeloglossum viride* Pollinia

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The orchid species *Coeloglossum viride* (L.) Hartm. (Frog orchid) is considered relatively rare on the Balkan Peninsula and is mostly confined to mountainous areas with preserved grassland habitats. The aim of the study was to investigate the morphology of the pollen-bearing structures (pollinia) of *Coeloglossum viride* using scanning electron microscopy (SEM) and stereomicroscopy, with special emphasis on tetrad organization and exine ornamentation. Besides that, the chemical composition of the orchid pollen was analyzed by Raman spectroscopy. The pollinarium consists of two pollinia attached to a caudicula and a viscidium, forming a cohesive pollen dispersal unit typical of entomophilous orchids. The pollinia appear distinctly granular, reflecting the dense aggregation of pollen tetrads with numerous massulae that are characteristic of the tribe Orchideae. The ornamentation of exine is rugulate-hamulate, characterized by irregular, winding, and variably thick muri. These muri are frequently anastomosing and associated with vermiform and labyrinthine elements, as well as numerous shallow depressions forming a complex maze-like pattern. The mean spectrum of Raman bands implies that in pollen samples were present different macromolecules such as carbohydrates, carotenoids and lipids. The presence of sporopollenin, a bio-chemical polymer which is found mainly in the outer wall of pollen grains, was also confirmed. The combination of broad distribution and regional rarity makes the species of particular interest from both biogeographical and conservation perspectives, but detailed palynological data of orchid species *Coeloglossum viride* remain relatively limited, especially for populations from south-eastern Europe.

KEYWORDS: pollen-bearing structures; Orchidaceae; Raman; SEM

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Phenotypic Selection on Heat Shock Proteins And Antioxidant Enzymes in *Iris pumila* Under *In Situ* Experimental Warming

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To examine whether warming affects phenotypic selection on biochemical stress-response traits, we conducted an *in situ* open-top chamber (OTC) experiment in two sun-exposed natural populations of the clonal perennial *Iris pumila* in the Deliblato Sands, Serbia. Within each population, one half of each clone was exposed to OTC warming, while the other half remained under ambient conditions. Air temperature inside the OTCs averaged approximately 1.5 °C above ambient levels. Phenotypic selection was assessed for heat shock proteins (Hsp101, Hsp70, Hsp17.6) and antioxidant enzymes (SOD, APX, CAT). Fitness was estimated as seed production per flowering ramet and linear selection gradients were estimated using relative fitness. Absolute fitness was analyzed across 108 flowering clone–treatment units, each representing a unique combination of clonal genotype and temperature treatment. Selection analyses and the mean absolute fitness values were based on the 49 units with biochemical data. Mean absolute fitness was lower under warming in both populations, although the effects of temperature, population and their interaction were not statistically significant. No significant selection gradients were detected for Hsps. In contrast, significant directional selection on antioxidant enzymes was detected only under warming. In Population 1, selection favored lower APX and SOD levels, whereas in Population 2, higher CAT levels were favored. However, formal comparisons provided no evidence that selection gradients differed significantly between temperature treatments or populations, or that treatment-related differences in selection varied between populations. Overall, the differential associations with fitness across biochemical stress-response traits under elevated temperature highlight their complexity as targets of selection.

KEYWORDS: *Iris pumila*; open-top chamber (OTC); phenotypic selection; heat shock proteins, antioxidant enzymes

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Flowering Phenology of *Iris pumila* Under *In Situ* Experimental Warming

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Climate warming may alter flowering phenology, with responses differing among phenological components and populations within a species. To examine the effects of warming on flowering phenology, we conducted an *in situ* open-top chamber (OTC) experiment in two sun-exposed natural populations of the clonal perennial *Iris pumila* in the Deliblato Sands, Serbia. Clonal ramets representing 68 genotypes (34 per population), growing under ambient conditions and in OTCs where air temperature averaged approximately 1.5 °C above ambient levels, were monitored daily throughout a 16-day flowering period. Flowering probability, total flower number, flowering onset, mean flowering date, duration of the central flowering period (T90–T10) and overall daily flowering dynamics were analyzed. Earlier flowering onset was observed under warming in both populations. However, the effect of warming on mean flowering date was population-dependent: in Population 1, flowers opened on average 1.19 days earlier under warming, whereas no significant change was detected in Population 2. Warming did not significantly affect flowering probability or the duration of the central flowering period. Total flower number showed a marginal trend toward a reduction under warming, corresponding to an estimated 23% decrease across populations. Overall daily flowering dynamics did not differ significantly between temperature treatments. These results indicate that mild warming may shift some components of flowering phenology toward earlier dates without uniformly altering the overall temporal pattern of flowering. The population-specific response in mean flowering date further suggests that the phenological consequences of warming may vary among populations context even within the same species.

KEYWORDS: *Iris pumila*; climate warming; open-top chamber (OTC); flowering phenology; population-specific response

ACKNOWLEDGEMENT: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract No. 451-03-33/2026-03/200007. The results presented in this study are in line with Sustainable Development Goal 13 (Climate Action) of the United Nations 2030 Agenda.

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Genetic Factors Affecting Carbohydrate Content in Plant Seeds

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Genetic factors play a central role in determining carbohydrate content and composition in plant seeds, which are critical for nutritional value, germination potential, energy storage, and seed adaptability. The accumulation of carbohydrates, including starch, simple sugars, and oligo-saccharides, is under strong genetic control, although environmental conditions and genotype–environment interactions can also influence their levels. Carbohydrate metabolism is regulated through the coordinated action of structural and regulatory genes. Structural genes encode key enzymes involved in starch biosynthesis, such as ADP-glucose pyrophosphorylase, soluble starch synthases, granule-bound starch synthase, branching enzymes, and debranching enzymes, which determine the rate of synthesis, quantity, structure, and spatial distribution of starch within seed tissues. In contrast, amylases primarily participate in starch degradation during seed germination and early seedling growth. Regulatory genes and transcription factors, including members of the bZIP, NAC, MYB, WRKY, and DOF families, coordinate the expression of carbohydrate metabolism genes and enable dynamic regulation throughout seed development. The aim of this study is to investigate the genetic mechanisms controlling carbohydrate content and composition in seeds, including the role of allelic variation, dominance, epistatic interactions, and the combined effects of maternal and paternal genotypes on endosperm characteristics. Understanding these mechanisms enables the selection of plant lines with improved carbohydrate quantity and composition, optimized starch and sugar profiles, and predictable seed phenotypes. Such knowledge provides a foundation for breeding programs aimed at developing varieties with enhanced nutritional quality, favorable technological properties, and improved end-use characteristics, particularly in maize, wheat, rice, and other starch-rich crops.

KEYWORDS: seed carbohydrates; starch biosynthesis; genetic regulation; allelic variation; endosperm

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PLANT BIOTECHNOLOGY AND APPLICATIONS

Genotype-Driven Regulation of Phenylpropanoid Biosynthesis in *Cistus creticus*: A Combined Metabolomic, Transcriptomic, and Functional Characterization Approach

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Cistus creticus is a Mediterranean shrub rich in phenylpropanoids, however, the molecular basis of their diversity remains poorly characterised. Targeted LC/MS profile of 91 Greek genotypes form seven populations, revealed substantial variability in gallic acid, gallocatechin, catechin, myricitrin, rutin, and quercitrin contents among genotypes with total polyphenol content ranging from 5.210 to 12.285 µg/g fresh leaf weight. Untargeted metabolomic analysis of three genotypes exhibiting differences in total content of the above compounds (C3-rich, C9-medium, C25-poor) led to the identification of 134 bioactive compounds dominated by flavan-3-ol (F3O) derivatives and proanthocyanidins, along with 33 terpenes governed by labdane-related diterpenes. RNA-seq displayed a coordinated upregulation of the genes related to the phenylpropanoid pathway (*PAL*, *C4H*, *4CL*, *CHS*, *CHI*, *F3H*, *F3'5'H*, *FLS*, *DFR*, *ANS*, *LAR*, *ANR*) in C3 genotype as well as of those involved in the biosynthesis of terpenes. qPCR validated significant up regulation of *CcCHS1* and *CcLAR1* in C3 and identified *PAL1* as a putative marker for epigallocatechin accumulation. Co-expression analysis revealed 66 transcription factors with MYB12 and MYB46 as orchestrators of the flavonol and proanthocyanidin branches, B3-domain and Trihelix factors as novel dual gene-metabolite nodes, and a WRKY (CL11491.Contig1) as a putative master regulator linking phenylpropanoid with labdane-related diterpenoid biosynthesis. Lastly, functional characterization of flavonoid 3'-hydroxylase (*CcF3'H*) through heterologous yeast expression confirms its catalytic activity, converting dihydrokaempferol to dihydroquercetrin. Collectively, these combined analyses revealed genotype-driven transcriptional reprogramming, rather than environmental influence in *C. creticus* phenylpropanoid diversity, and identified candidate genes and transcription factors for future breeding and engineering applications.

KEYWORDS: *Cistus creticus*; phenylpropanoids; terpenes; transcriptional factors; functional characterization

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Aeroponics: From the Space Station, Through High-Yield Potato Farming, to a Tool for Studying the Process of Tuberization

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Aeroponics is a modern technology for soilless plant cultivation. In an aeroponic system, the plant's underground parts (roots and underground stems) are located in the module, suspended in the air, and supplied with water and nutrients through a fine mist of dispersed nutrient solution, which then recirculates. This technology developed rapidly in the late 1990s, when the first high-performance aeroponic system was constructed for a NASA experiment on plant growth in microgravity aboard the Mir space station. Today, aeroponics is used for commercial farming of leafy greens, herbs, and tomatoes, as well as for urban agriculture (vertical farms). Another important application is producing high-quality seed potato material, minitubers, from acclimated plants of *in vitro* origin. Aeroponics has significant advantages over other minituber-growing technologies, including higher productivity, successive harvests that enable collecting tubers of uniform size, efficient use of space and water, and minimal environmental pollution. Within the AerPotato project, we designed and constructed a modular aeroponic system with several original features for potato minituber production. Extensive research was conducted on potato plant growth and development, nutrient requirements, and stress-protective treatments. In addition, the possibility of using aeroponics to investigate the process of tuberization, including stolon emergence and growth, tuber initiation, and tuber enlargement, was explored. Aeroponic growing allows a non-invasive approach, without mechanical injury or disturbance, to gather information about or apply treatments to the underground organs of potato plants.

KEYWORDS: aeroponics; potato; minitubers; tuberization

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From Plants to Bioactive Compounds: Metabolomics, Food Applications and Bioaccessibility

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Plants and plant-derived materials are valuable sources of structurally diverse bioactive compounds, but their relevance cannot be assessed solely from their initial phytochemical profile. Our studies combined UHPLC-QToF-MS-based targeted and untargeted metabolomics with biological assays, food formulation and simulated gastrointestinal digestion to follow bioactives from plant sources to potentially absorbable fractions.

Medicinal and edible plants were characterized to reveal how origin, cultivation and extraction shape their metabolomes and biological properties. Studies on *Tanacetum parthenium*, *Hypericum perforatum*, goji berries, wild rocket and highbush blueberry demonstrated variation in phenolics, phenylamides, glucosinolates and other metabolites. Studies of honey further illustrated how botanical origin and production conditions influence the chemical and functional characteristics of natural food matrices. Importantly, a plant extract does not necessarily reflect what is actually consumed, as demonstrated by differences between *Hypericum* methanolic extracts and infusion tea.

Plant and food by-products, including sage waste, okara and grape pomace, were explored as sources of functional ingredients. Their incorporation into beverages, mayonnaise and goat-milk-based products demonstrated that phytochemical profiles are further reshaped by processing, fermentation and food matrix interactions, affecting compound recovery and biological activity. Interactions with food matrix and salivary proteins may additionally modify bioactives even before gastrointestinal digestion.

Finally, simulated gastrointestinal digestion introduced another level of complexity: compound recovery was strongly structure- and matrix-dependent, ranging from extensive losses to values above 100% due to matrix release. Thus, metabolomics, food matrix interactions and bioaccessibility represent interconnected steps required to evaluate the functional potential of plant bioactives.

KEYWORDS: UHPLC-QToF-MS; plant bioactives; functional foods; food matrix; bioaccessibility

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Essential Oils Revisited: Three Decades of Research from Traditional Remedies to Next-Generation Biobased Therapeutics

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Essential oils have been used in traditional medicine since ancient civilizations for the treatment of infections, wound healing, food preservation, and health promotion. Today, modern scientific research increasingly validates these traditional applications while expanding their therapeutic potential.

Over the past decades, we have investigated numerous essential oils isolated from aromatic plant species native to Serbia, the Balkan Peninsula, and other regions. Their chemical composition was comprehensively characterized, revealing complex mixtures of monoterpenes, sesquiterpenes, diterpenes, alcohols, aldehydes, esters, and phenolic compounds that underlie their biological activities. We evaluated their antimicrobial effects against pathogenic microorganisms affecting humans, animals, plants, together with antioxidant, anti-inflammatory, wound-healing, immunomodulatory, and antibiofilm activities, as well as their potential as natural food and feed additives. Individual constituents were also studied to better understand their mechanisms of action.

Essential oils rich in phenolic compounds, particularly thymol and carvacrol, exhibited the strongest *in vitro* antimicrobial activity. However, *in vivo* studies demonstrated that several oils with relatively low phenolic content also showed remarkable efficacy, indicating that their biological effects arise from synergistic interactions among multiple constituents, enhanced bioavailability, and multitarget mechanisms rather than from individual compounds alone.

Our long-term research demonstrates that essential oils are among the most potent natural products tested, exhibiting significant biological activity at very low concentrations. Recent advances in nanoformulations, liposomes, controlled-delivery systems, and multi-omics technologies are creating new opportunities for their application in medicine, pharmacy, medicine, functional foods, cosmetics, and sustainable agriculture, supporting the renaissance of essential oils as scientifically validated natural therapeutics.

KEYWORDS: Essential oils; Bioactive compounds; Antimicrobial activity; Translational medicine

ACKNOWLEDGEMENT: This work has been supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-33/2026-03/200007)

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Optimizing CRISPR/Cas9 Genome Editing Protocols in Sunflower: Overcoming Recalcitrance and Microbial Bottlenecks in Hypocotyl-Derived Explants

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Sunflower (*Helianthus annuus* L.) remains notably recalcitrant to *Agrobacterium*-mediated genetic transformation, severely limiting the use of functional genomics and precision breeding tools. This study outlines recent progress under HEU project HelEx to establish and optimize an efficient, repeatable CRISPR/Cas9 genome editing protocol utilizing hypocotyl-derived tissue culture. Using *Agrobacterium tumefaciens* strain AgRO 3101, experiments targeted the phytoene desaturase (PDS) gene as a visual knock-out marker, which characteristically yields an albino phenotype upon successful mutation. Initial protocol optimizations evaluated three commercial genotypes (PH-BC2-91, Ha-26-PR, and NO-SU-12) under varied pre-conditioning and sterilization regimens. Seedlings subjected to a modified sterilization method featuring a 2-day pre-germination step, followed by culture under full light conditions, exhibited significantly superior growth, elongated hypocotyls, and heightened callus induction. Based on these performance benchmarks, genotype Ha-26-PR was selected for localized transformation trials.

Despite achieving robust initial explant quality, major downstream bottlenecks emerged following the transfer from selection to callus induction media. Approximately two-thirds of the cultured segments developed severe, systemic bacterial overgrowth. Parallel experiments aiming to identify the definitive lethal selection threshold for Hygromycin proved inconclusive due to widespread plate contamination. Surviving explants successfully developed calli, though albino regenerative tissue has not yet been isolated, indicating potential developmental penalties associated with PDS disruption. To overcome these tissue culture bottlenecks, ongoing strategies focus on adjusting co-cultivation durations, evaluating alternative bactericidal agents like Cefotaxime, and integrating the highly visible RUBY reporter plasmid alongside a targeted gene of interest to achieve stable sunflower transformation.

KEYWORDS: sunflower tissue culture; CRISPR/Cas9; *Agrobacterium* transformation; hypocotyl explants; Phytoene desaturase (PDS); Antibiotic selection

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Antioxidant Properties and Hypoglycemic Potential of *Spartium junceum* L. Extracts

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Spartium junceum L. (Spanish broom), a perennial shrub of the Fabaceae family, is a medicinal plant traditionally used in Mediterranean and Middle Eastern countries and is valued for its diverse biological properties. It has been used to treat digestive ulcers, arthritis, bone fractures, and skin warts. This study evaluated the antioxidant potential and α -amylase inhibitory activity of methanol and aqueous extracts prepared from the herba of *S. junceum*. Plant material was collected from three locations in Montenegro (Bar, Budva, and Ulcinj). Among the tested extracts, aqueous extracts made from plants collected in Budva exhibited the highest antioxidant capacity, with DPPH and ABTS IC_{50} values of 1.877 and 1.703 mg/mL, respectively. In contrast, aqueous extracts from the herba collected in Bar and Ulcinj showed weaker antioxidant properties (DPPH: IC_{50} = 2.325 and 2.514 mg/mL; ABTS: IC_{50} = 2.479 and 2.192 mg/mL, respectively). The methanol extracts of plants from all three locations had weaker effects than the aqueous extracts. The strongest α -amylase inhibitory effect was also detected in the methanol and aqueous extracts from the plant material collected in Budva (IC_{50} = 9.30 and 21.246 mg/mL, respectively), compared with those from Ulcinj and Bar (IC_{50} = 10.40 and 22.929 mg/mL; 7.64 and 23.495 mg/mL, respectively). These findings demonstrate that *S. junceum* is a promising natural source of antioxidants and α -amylase inhibitors, and highlight the influence of population origin on its biological activity.

KEYWORDS: *Spartium junceum* L.; antioxidant activity; α -amylase; hypoglycemic potential

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Liposomal Encapsulation of *Cotinus coggygia* (Scop.) Inflorescences Aqueous Extract Improves Sustained Antimicrobial Activity Against Skin Pathogens

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Plant-derived bioactive compounds have attracted increasing attention as sustainable alternatives to conventional antimicrobial agents. *Cotinus coggygia* Scop. (smoke tree) is a medicinal plant rich in polyphenols that has traditionally been used to treat inflammatory skin conditions and promote wound healing. However, the therapeutic application of plant extracts is often limited by poor stability and rapid release of bioactive constituents. Liposomal delivery systems offer a promising approach to improve their stability and enable controlled release. Herein, we evaluated the antimicrobial activity of an aqueous extract of *C. coggygia* inflorescences, as well as extract-loaded and empty liposomes, against clinically relevant skin pathogens isolated from patients, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Citrobacter koseri*, *Proteus mirabilis*, and *Candida albicans*. Antimicrobial activity was determined using the broth microdilution method, while microbial growth was monitored after 24, 48, and 72 h of incubation. The aqueous extract showed the strongest antimicrobial activity after 24 h, particularly against *P. aeruginosa* (MIC 0.125 mg/mL) and *P. mirabilis* (MIC 0.25 mg/mL). Empty liposomes exhibited only limited antimicrobial effects, indicating minimal contribution of the lipid carrier to the overall antimicrobial potential. Although encapsulation initially reduced antimicrobial potency compared to the extract alone, extract-loaded liposomes consistently outperformed empty liposomes during prolonged incubation. They maintained measurable activity after 72 h, with an MIC of 8.00 mg/mL against *S. aureus*, *C. koseri*, and *C. albicans*, whereas empty liposomes completely lost activity. These findings suggest that liposomal encapsulation enables sustained release of plant bioactive compounds, prolonging antimicrobial activity despite lower initial efficacy. Overall, the results highlight the potential of liposomal nanocarriers to enhance the therapeutic applicability of *C. coggygia* inflorescence extract and support its future use in dermocosmetic formulations.

KEYWORDS: *Cotinus coggygia*; liposomes; antimicrobial activity; sustained release; skin pathogens

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The Influence of Thidiazuron and Various Auxins on Bioactive Compounds Accumulation in *Cannabis sativa* L. Callus Cultures

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Plant tissue cultures represent a promising alternative system for the production of valuable bioactive metabolites under controlled *in vitro* conditions. The regulation of secondary metabolite production in *Cannabis* callus cultures by exogenous plant growth regulators is an important tool for enhanced cannabinoid accumulation. This study evaluated the effects of thidiazuron (TDZ) in combination with auxins, including indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA), on cannabinoid and phenolic contents and antioxidant properties of callus extracts.

Calli were cultured on MS/B5 medium supplemented with 1.0 mg·L⁻¹ TDZ and 0.1–1.0 mg·L⁻¹ of IBA, 2,4-D or NAA. Cannabinoids were extracted from lyophilized calli using the German Pharmacopoeia (DAB) method for *Cannabis flos*. Total phenolic (TP) content was determined using the Folin-Ciocalteu method, while antioxidant capacity was evaluated by the CUPRAC assay.

Cannabidiolic acid (1.8–3.1 µg·g⁻¹ CBDA) and Δ⁹-Tetrahydrocannabinolic acid (2.4–4.6 µg·g⁻¹ Δ⁹-THCA) contents varied among NAA treatments. The highest CBDA and Δ⁹-THCA contents (3.1 and 7.3 µg·g⁻¹, respectively) were observed in the presence of 0.5 mg·L⁻¹ IBA. Although 2,4-D resulted in moderate cannabinoid levels, significant positive correlations were found with CBDA and THCA (p<0.01). IBA treatments showed the highest TP accumulation (9.8–11.8 mg GAE·g⁻¹ DW), significantly correlated with CUPRAC antioxidant capacity (p<0.01).

The combination of 1.0 mg·L⁻¹ TDZ and 0.5 mg·L⁻¹ IBA was most effective for enhancing cannabinoid and phenolic production with antioxidant properties, highlighting the potential of cytokinins and auxins as promoters of bioactive metabolite production in *Cannabis sativa* L. callus cultures.

KEYWORDS: *Cannabis*; callus cultures; thidiazuron; cannabinoids; phenolic compounds

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Phytotoxicity and Preliminary Bioremediation Assessment of Cherry-Washing Wastewater Using *Lactuca sativa* Germination Test and *Lemna minor*

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Fruit-processing wastewater contains readily biodegradable organic matter and residues of compounds used during industrial washing. This preliminary study evaluated the phytotoxicity of industrial cherry-washing wastewater using *Lactuca sativa* seeds and explored its suitability for biological treatment with *Lemna minor*.

Initial wastewater characterization showed pH 5.40, electrical conductivity of 1435 $\mu\text{S cm}^{-1}$, dissolved matter of 715 ppm, total dissolved salts of 0.07%, and oxidation-reduction potential (ORP) of +632 mV. Seeds of *L. sativa* were exposed to 0 – 100 % wastewater in three independent replicates. Germination ranged from $44.4 \pm 9.6\%$ to $69.7 \pm 5.2\%$, compared with $63.0 \pm 6.4\%$ in the control, without a significant overall effect on germination probability. Seedling responses were concentration-dependent, tentatively suggestive of a hormetic response. Relative inhibition occurred at 1% and 5%, with relative stimulation at intermediate concentrations (10 and 25%) and slow decrease at 50% and 100%. The apparent maximum was reached at 10% ($154.6 \pm 29.8\%$), accompanied by increased radicle and shoot elongation, whereas shoot fresh weight remained comparable to the control.

In parallel, *L. minor* initially showed limited visible phytotoxicity in diluted wastewater *ex vitro*. Severe phytotoxicity developed by day 5, manifested by floc and microbial biofilm formation and duckweed mortality, and accompanied by ORP decrease to – 80 and – 172 mV in 10% and 25%, respectively. Similar phenomena were observed *in vitro*.

These preliminary findings reveal concentration- and time-dependent plant responses and suggest that duckweed may be more suitable for wastewater polishing following appropriate pretreatment than for direct treatment of raw fruit-processing wastewater.

KEYWORDS: *Lemna minor*; wastewater; phytotoxicity; germination index; phytoremediation

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Maize Chromosomes in the Oat Genome Moderate Leaf Anatomy and Photosynthetic Efficiency

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Oat × maize addition (OMA) lines were produced by crossing oat (*Avena sativa* L., $2n = 6x = 42$) with maize (*Zea mays* L., $2n = 20$), resulting in the insertion of maize chromosomes into the oat genome. OMA lines provide a unique material for studying maize gene expression and intergeneric translocations and, importantly, for introducing maize traits into oat, such as disease and drought resistance.

The aim of the present study was to evaluate how soil drought (20% field water capacity [FWC]) affects chlorophyll *a* fluorescence kinetics and leaf anatomical parameters in four OMA lines carrying different numbers of maize chromosomes in the oat genome compared to the oat cv. Bingo.

Under soil drought conditions, a significant decrease in the quantum efficiency of photosystem II (F_v/F_m) was observed in OMA lines, except for lines 18 and 23, in which this parameter remained at levels similar to those of the control plants. Two-way analysis of variance showed significant effects of lines, treatment, and lines × treatment interaction on the tested anatomical features: thickness of adaxial and abaxial epidermis, thickness of mesophyll, distance between two small vascular bundles, and number of stomata in the adaxial and abaxial epidermis. These results show that maize chromosome introgression affects the physiological and anatomical responses of oat to soil drought. The highest vascular bundle density in line 18, with four maize chromosomes, may indicate enhanced drought tolerance and highlights the potential for further studies on the contribution of maize chromosomes to drought adaptation in oat.

KEYWORDS: chlorophyll *a* fluorescence kinetics parameters; drought stress; leaf anatomical traits; oat × maize addition (OMA) lines

ACKNOWLEDGEMENT: Radenko Radošević for technical help to prepare transverse cross-sections of the leaves.

Cytokinin-Induced Shoot Multiplication in *Ocimum basilicum* L. var. *minimum* Alef. Bioreactor Culture

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Basil plants have gained increasing interest due to high content of metabolites, particularly essential oils and phenolic compounds. Plant tissue culture represents a promising approach for the sustainable and controlled production of basil phenolic compounds, enabling the optimization of biosynthesis independently of season or environmental variations. Therefore, the aims of this study were to optimise shoot multiplication and secondary metabolite production in small-leaved basil (*Ocimum basilicum* L. var. *minimum* Alef.) using the Rita® temporary immersion bioreactor by applying different cytokinins: meta-topolin, zeatin, kinetin, and 6-benzylaminopurine. A cytokinin-free medium was used as the control. Seeds were germinated on Murashige and Skoog (MS) medium supplemented with 6 g/L agar and 30 g/L sucrose at pH 5.8. Subsequently, single-node shoot explants were isolated from four-week-old seedlings and transferred to MS medium supplemented with 5 µM BAP for shoot multiplication. After 12 weeks of multiplication, the resulting axillary shoots were used to establish bioreactor cultures. The tested cytokinins exerted a positive effect on the appearance and growth of *O. basilicum* shoots *in vitro* in the Rita® bioreactor. Basil seedlings cultivated on media supplemented with cytokinins had green leaves, whereas leaves and shoots grown on the control medium without cytokinins were necrotic, turned brown, and eventually died. The type of cytokinin further affected shoot biomass accumulation, as well as the content of photosynthetic pigments, soluble sugars, and phenolic compounds. The resulting plant material will be used in future studies to investigate in detail the specific production of secondary metabolites under the specified conditions.

KEYWORDS: cytokinins; *Ocimum basilicum* L.; phenolic compounds; photosynthetic pigments; temporary immersion bioreactor Rita

Potential Use of *Lavandula angustifolia* Mill. Inflorescences From Urban Green-Space Maintenance for Sustainable Suppression of *Setaria viridis* (L.) P. Beauv.

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Routine maintenance of urban vegetation generates residual plant biomass that may be repurposed for sustainable weed control. Green bristle grass, *Setaria viridis* (L.) P.Beauv., is an annual weed of cultivated and disturbed habitats, including parks, roadsides, lawns, and managed urban sites. Lavender (*Lavandula angustifolia* Mill.) inflorescences removed during green-space maintenance may have limited commercial value, creating opportunities for alternative valorisation. This study evaluated the potential of lavender inflorescences for sustainable suppression of green bristle grass under greenhouse conditions. Treatments included watering with a 25% (v/v) dilution of the aqueous inflorescence extract (LaAE; stock extract prepared from 10 g dry ground biomass/200 mL water), incorporation of dry ground inflorescence biomass into the soil substrate (LaDW; 40 g/2 L substrate) followed by watering, and a combined LaDW+LaAE treatment. Germination, shoot length, shoot fresh biomass, and catalase (CAT) and peroxidase (POD) activities in shoots were assessed, while LaAE was characterised by UHPLC/Orbitrap-HRMS. Compared with the control, germination was inhibited by 34.9% and 23.3% under LaDW and LaDW+LaAE. Shoot length was 30.6%, 61.4%, and 72.1% lower under LaAE, LaDW, and LaDW+LaAE, while shoot fresh biomass was 79.0% and 78.3% lower under LaDW and LaDW+LaAE. CAT activity remained unchanged, whereas POD activity significantly increased by 126% under LaDW+LaAE. Phenolic acids and flavonoids, particularly rosmarinic acid, caffeic and ferulic acid derivatives, and luteolin- and kaempferol-related compounds, may have contributed to growth inhibition and POD responses. These findings support the evaluation of urban lavender biomass for sustainable weed suppression and circular green waste valorisation.

KEYWORDS: green waste valorisation; sustainable weed management; phenolic compounds; antioxidant enzyme activity; bioherbicidal potential

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Assessment of the Phytotoxic And Insecticidal Activities of Yarrow Essential Oil-Based Formulations

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The growing demand for environmentally sustainable alternatives to synthetic insecticides has stimulated research into plant-derived products for the management of the Colorado potato beetle (CPB, *Leptinotarsa decemlineata* Say), a major pest of potato (*Solanum tuberosum* L.). This study evaluated the biological activity of yarrow (*Achillea millefolium* L.) essential oil formulated as a microemulsion, β -cyclodextrin inclusion complex, and hydrosol, assessing their insecticidal efficacy against CPB and potential phytotoxic effects on potato plants. Insecticidal activity was evaluated based on larval survival, body mass, developmental dynamics, and total proteolytic activity, while phytotoxicity was assessed through electrolyte leakage, relative water content, and chlorophyll content in potato leaves.

The results demonstrated that the biological activity of yarrow essential oil depended on the formulation type. The microemulsion showed the strongest insecticidal effect, significantly reducing larval survival to 33.3% compared with 80.0% in the control. However, it also caused the highest electrolyte leakage ($53.85 \pm 3.85\%$ vs. $14.02 \pm 1.61\%$ in the control), indicating a greater risk of phytotoxicity. The β -cyclodextrin inclusion complex showed the most favorable safety profile, with no significant changes in electrolyte leakage ($12.63 \pm 0.92\%$), relative water content ($83.42 \pm 1.41\%$), or chlorophyll content (0.540 ± 0.007 mg/g FW) compared with the control, although its effect on larval survival was not statistically significant. Different formulations showed no significant treatment effect on total proteolytic activity. Overall, the results indicate that formulation strongly influences the biological activity and safety of yarrow essential oil, likely by affecting the bioavailability of its active components.

KEYWORDS: *Achillea millefolium* L; *Leptinotarsa decemlineata* Say; *Solanum tuberosum* L; essential oil, β -cyclodextrin

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Effect of Mealworm (*Tenebrio molitor* L.) Frass on Gene Expression Related to Starch Metabolism in Potato (*Solanum tuberosum* L.)

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The aim of this study was to investigate the effects of frass from *Tenebrio molitor* L. larvae, fed either on wheat bran alone or on wheat bran supplemented with polystyrene, on the growth, development, and metabolism of potato plants (*Solanum tuberosum* L.). Three treatments were applied: control (K), frass from larvae fed wheat bran (FM), and frass from larvae fed wheat bran with polystyrene addition (FS). The results showed that frass from both treatments did not affect potato tuber germination; however, statistically significant effects were observed on plant morphological status and novel tubers yield compared to the control. FTIR analysis did not reveal changes in the chemical composition of frass and tubers, except for the emergence of new functional groups, providing direct evidence of polystyrene degradation. Expression analysis of genes involved in starch metabolism showed treatment-specific adaptive changes. Considering that the larvae can partially degrade polystyrene, frass produced in this way could be used as a sustainable organic fertilizer within a circular economy framework. These findings highlight the potential of *T. molitor* larval frass for sustainable agriculture while emphasizing the need for further research on the chemical composition and possible toxic components of polystyrene.

KEYWORDS: insect rearing; elicitors; starch metabolism; polystyrene; tuber yield

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***In vitro* grown Sporophytes of Golden Fern (*Asplenium ceterach* L.) as a Basis for Producing ivACME, a Promising Immunomodulatory Phytoproduct Against Respiratory Infections**

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In vitro plant propagation techniques enable year-round production of plants under controlled and sterile conditions, while offering opportunities to optimize their phytochemical composition. The fern *Asplenium ceterach* has attracted pharmacological interest due to its diverse bioactive compounds, low toxicity, and traditional use in respiratory diseases. Methanol extracts of *A. ceterach* sporophytes grown *in vitro* (ivACME) display a quantitatively and qualitatively distinct phytochemical profile compared with wild-grown ferns (WF), including a group of flavonoids absent from the methanol WF extract. Analysis showed that ivACME strongly stimulated peritoneal macrophage effector functions, including proinflammatory cytokine production, bacterial uptake, and bactericidal activity, whereas these effects were absent or less pronounced with the WF extract. At the same time, ivACME promoted a balanced immune response, characterized by increased IL-10 production and enhanced antioxidant activity, reflected by reduced ROS generation and increased expression of antioxidant enzymes. Oral administration of ivACME to rats for five days further demonstrated activation of innate immune defence mechanisms, particularly macrophage activity, without signs of systemic toxicity. Together with previously demonstrated antibacterial and antivirulence activity, these findings indicate that ivACME combines immunostimulatory, antioxidant, and antibacterial properties relevant to the prevention and control of respiratory infections. These properties together with its consistent phytochemical profile support ivACME as a promising phytoproduct and potential complement to conventional antibiotic therapy, with possible applicability to respiratory and other infectious diseases.

KEYWORDS: *in vitro* plant culture; *Asplenium ceterach* sporophytes; macrophages; immunomodulatory activity

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Macrophyte-Assisted Treatment of Pig-Farm Wastewater: Establishing a Constructed Wetland Platform Under Real-Environment Conditions

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Intensive livestock production generates wastewater with high and variable organic and nutrient loads, requiring low-energy technologies suitable for decentralized treatment. This study presents baseline characterization and design of a pilot constructed wetland established at the Institute for Animal Husbandry, Belgrade-Zemun, Serbia, emphasizing wetland macrophytes as functional treatment components. Wastewater was characterized along the existing separation lagoon before introduction into vegetated units. Monitored parameters included pH, chemical oxygen demand (COD), total nitrogen (TN), ammonium, total phosphorus (TP), and total organic carbon (TOC). Along the separation lagoon, COD decreased from 3960 to 1800 mg O₂ L⁻¹, TN from 863 to 625 mg N L⁻¹, ammonium from 610 to 350 mg N L⁻¹, TP from 74.5 to 23.7 mg P L⁻¹, and TOC from 1100 to 340 mg L⁻¹, demonstrating substantial natural attenuation before the vegetated stage. A modular treatment train comprising a sedimentation lagoon, two functionally differentiated vegetated lagoons, and a treated-water collector was established. The units were planted with 260 macrophytes: *Phragmites australis* (50), *Juncus effusus* (80), *Carex riparia* (60), *Iris pseudacorus* (50), and *Typha latifolia* (20). The system provides a real-environment platform for investigating plant–substrate–microorganism interactions, nutrient retention, organic matter transformation, and macrophyte-assisted wastewater treatment, supporting development of low-energy approaches to agricultural water management and reuse. To our knowledge, this is the first constructed wetland pilot project in Serbia specifically established for phytoremediation of livestock wastewater.

KEYWORDS: constructed wetlands; macrophytes; rhizosphere; phytoremediation; livestock wastewater

ACKNOWLEDGEMENT: The pilot system was developed within the project "Wetland Solutions for Wastewater Management", implemented under the "EU for Green Agenda in Serbia" initiative with technical and financial support from the European Union and UNDP, in partnership with the Ministry of Environmental Protection of the Republic of Serbia.

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Phyto-Mediated Synthesis and Characterization of CuO Nanoparticles Using *Urtica dioica* L. Extract

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Phyto-mediated synthesis of metal oxide nanoparticles has attracted growing interest as a simple, sustainable, and environmentally friendly alternative to conventional chemical synthesis routes. This study aimed to synthesize copper oxide nanoparticles (CuO NPs) using *Urtica dioica* L. (stinging nettle) aerial part extract as a natural source of bioactive compounds involved in nanoparticle formation and stabilization. The polyphenolic profile of *U. dioica* is characterized by flavonoids such as rutin, kaempferol, and quercetin, along with phenolic acids (e.g. chlorogenic, ferulic, and caffeic acid). CuO NPs were synthesized using $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ as the copper precursor and a 0.2% aqueous nettle extract, with the reaction pH adjusted to 4–5 using NaOH. The synthesis process was monitored by UV–Vis spectroscopy over a 120-minute period, while the resulting material was characterized using Fourier-transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). Time-dependent changes in the UV–Vis spectra, accompanied by a distinct visible color transition of the reaction mixture from blue through greenish to dark green, indicated the progressive evolution of nanoparticle formation. FTIR analysis revealed characteristic shifts in the functional groups of the plant extract, suggesting the active involvement of phytochemicals in nanoparticle nucleation and stabilization, with absorption bands in the lower wavenumber region attributed to Cu–O bond vibrations. TEM imaging revealed heterogeneous particles with predominantly irregular, polygonal, and plate-like morphologies, along with a pronounced tendency toward agglomeration. These findings highlight the potential of *U. dioica* as a natural, low-cost agent for the successful synthesis of CuO-based nanomaterials, supporting the broader development of green nanotechnology approaches.

KEYWORDS: green synthesis; copper oxide nanoparticles; *Urtica dioica*; plant extract; nanoparticle characterization

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Synthesis, Structural, and Morphological Characterization of Copper Oxide Nanoparticles Obtained via *Melissa officinalis* Extract

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The growing demand for sustainable nanotechnology has positioned green synthesis of metal oxide nanoparticles as a compelling alternative to conventional chemical routes. In this work, copper oxide nanoparticles (CuO NPs) were synthesized through an eco-friendly, plant-mediated approach using an aqueous extract of *Melissa officinalis* L. (lemon balm), which served simultaneously as a reducing and stabilizing agent. The synthesis was carried out using CuSO₄·xH₂O as the copper precursor combined with a 0.2% plant extract solution, under mild conditions of pH 5–6 and 50–60°C. Reaction progress was monitored in real time using UV–Vis spectroscopy, while the structural, chemical, and morphological features of the resulting nanoparticles were examined through Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). UV–Vis spectra displayed characteristic time-dependent shifts in absorbance within the 300–450 nm range, reflecting the gradual evolution of nanoparticle formation. FTIR analysis revealed distinct alterations in the functional groups of the plant extract, pointing to their active participation in nanoparticle nucleation and stabilization, alongside absorption bands in the lower wavenumber region attributed to Cu–O bond vibrations. TEM imaging revealed nanoscale particles with predominantly irregular to quasi-spherical/polygonal shapes, along with a notable tendency toward agglomeration. These findings underscore the promise of *M. officinalis* extract as a natural, low-cost mediator for the green synthesis of CuO nanoparticles, offering a simple, sustainable, and environmentally responsible synthetic pathway.

KEYWORDS: copper oxide nanoparticles; *Melissa officinalis*; plant extract; nanoparticle characterization

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Selection of Elite Diploid *Centaurium erythraea* Rafn Genotypes With Enhanced Regeneration Capacity and Secoiridoid Content

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Centaurium erythraea Rafn is a medicinal plant species characterized by a high content of secoiridoid glucosides and represents a valuable source of bioactive specialized metabolites. The establishment of efficient *in vitro* culture systems and selection of highly regenerative genotypes are essential for obtaining sufficient and genetically uniform plant material for biotechnological applications. In this study, we evaluated the regenerative potential of ten diploid *C. erythraea* genotypes and investigated whether differences in regenerative capacity can be linked to variations in phytochemical profiles. Apical root segments were cultured in liquid half-strength Murashige and Skoog (MS/2) medium, and spontaneous bud regeneration was evaluated after five weeks. Diploid genotype DG1 exhibited the highest regenerative response, producing an average of 30 regenerated buds per flask per subculture, followed by DG7 with an average of 21 buds, whereas the remaining eight genotypes produced fewer than 10 buds per flask. Based on their regenerative performance, DG1 and DG7 were selected for further comparative phytochemical analyses. Targeted quantitative analysis of major metabolites by UHPLC/DAD/MS/MS revealed significantly higher contents of loganic acid, deoxyloganic acid, sweroside and gentiopicroin in DG1 than in DG7 (*t*-test, *p* < 0.05). Swertiamarin was also more abundant in DG1, but the difference was not statistically significant. The results demonstrate considerable genotype-dependent variation in regenerative capacity and phytochemical composition, and emphasize the importance of standardized protocols for rapid propagation of genetically uniform plant material in ensuring experimental consistency, minimizing phenotypic variability in metabolite profiles, and supporting biotechnological application.

KEYWORDS: *Centaurium erythraea* Rafn; genotype selection; *in vitro* multiplication; secoiridoid glucosides

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Cultivation Conditions as a Metabolic Switch: Connecting Biosynthesis and Bioactivity in *Nepeta nuda* L.

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Nepeta nuda is a herbaceous perennial aromatic plant and, together with *N. rtanjenis* and *N. cataria*, is the only representative of the genus *Nepeta* in Serbia. Essential oils and extracts of *N. nuda* possess a range of biological activities due to the presence of various bioactive components, primarily terpenes and phenolic compounds. In order to analyze the influence of cultivation conditions on the metabolism of specialized metabolites, a comprehensive chemical analysis of methanol extracts of clonally propagated *N. nuda* under *in vitro* (IV) and greenhouse (GH) growth conditions was performed using GC-MS and UHPLC/DAD/(±)HESI-MS². The results indicated a higher amount of iridoid compounds in IV extracts and a lower content of phenolic compounds than in GH extracts. In particular, IV extracts contain higher levels of *trans,trans*- and *trans,cis*-nepetalactone and its derivative 5,9-dehydro nepetalactone, the levels of which were eight times higher than in GH. The presence of β -farnesene, phytol and squalene was detected exclusively in IV-grown plants, while 1,8-cineole and pinanediol were identified only in GH-grown plants. In line with these results, the relative expression levels of ten genes involved in the iridoid biosynthetic pathway in *N. nuda* were significantly higher in IV-grown plants compared to GH-grown plants. In addition, the expression of genes involved in the biosynthetic pathway of characteristic phenolic compounds was higher in GH-grown plants, consistent with the enhanced accumulation of these metabolites, primarily rosmarinic and caffeic acids. This metabolic shift was reflected in the biological activity of the extracts, showing stronger antimicrobial activity of IV-grown plants compared to extracts of GH-grown plants against *Staphylococcus aureus* (including MRSA), *Pseudomonas aeruginosa*, and *Candida* species (with the exception of *C. parapsilosis*), while GH extracts exhibited stronger antioxidant activity in the DPHH assay, suggesting contribution of phenolic compounds. The obtained results highlight the functional relevance of growing conditions-induced changes in iridoid and phenolic metabolism in *N. nuda*, which may be exploited to modulate its phytochemical profile and enhance specific bioactive properties for targeted applications.

KEYWORDS: Naked catmint; terpenes; phenolics; antimicrobial activity

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Metabolic Engineering of Secoiridoid Biosynthesis in *Centaurium erythraea* Rafn Leaves Through Transient Expression of *Nepeta rtanjensis* Iridoid Pathway Genes

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The iridoid pathway underlies secoiridoid biosynthesis and comprises several enzymatic steps whose individual contributions to metabolite accumulation remain unresolved. This study builds on the shared iridoid biosynthetic machinery of *Centaurium* and *Nepeta* species, which extends to the formation of nepetalactol. *Centaurium erythraea* leaves were exploited as a heterologous platform to functionally assess the contribution of *Nepeta rtanjensis* iridoid pathway genes to secoiridoid biosynthesis.

Leaves of clonally propagated diploid genotype DG1 were agroinfiltrated with *Agrobacterium tumefaciens* LBA4404 carrying constructs with *NrGPPS*, *NrGES*, *NrMLPL1*, *NrMLPL2*, *NrNEPS1* and *NrNEPS5* mixed in different combinations, together with *Ruby* and *p19*. Leaves infiltrated with *Ruby* + *p19* served as the control. Targeted UHPLC-DAD-MS/MS profiling of major (seco) iridoids was performed 7 and 14 days after agroinfiltration. No significant differences in metabolite contents between engineered and control plants were detected at day 7, whereas significant changes were observed at day 14 (Student's *t*-test, $p < 0.05$). Swertiamarin accumulation increased significantly in all but one gene combination, with the strongest responses observed for combinations containing: 1) *NrMLPL1*, *NrMLPL2*, *NEPS5*, *Ruby*, and *p19* ($p < 0.001$), and 2) the full set of analysed biosynthetic genes ($p < 0.001$). Gentiopicrin showed the strongest increase following the co-expression of *NrGPPS*, *NrGES*, *NrMLPL1*, and *NrMLPL2* ($p < 0.001$). Sweroside, loganic and deoxyloganic acids were significantly increased, particularly following the transient co-expression of *NrGES*, *NrGPPS*, *NrMLPL1*, and *NrMLPL2* ($p < 0.001$). Transient heterologous expression highlighted GES, MLPL, and oxidase-type NEPS enzymes as promising biotechnological targets for further engineering of secoiridoid production.

KEYWORDS: *common centaury*; transient expression; agroinfiltration; *Agrobacterium tumefaciens*; metabolic engineering

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The Effect of Primocane Tipping on the Leaf Physiological Traits and Fruit Cycle Manipulation of Raspberry Cultivar 'Paris'

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The research investigated the effect of cane tipping of a primocane-fruiting raspberry cultivar 'Paris' on plant phenology, leaf nitrogen and chlorophyll content, enzymatic activity, and architecture, and yield-related traits, monitored during primocane and floricanes cropping. The study was conducted in an experimental soilless-grown raspberry orchard of the company 'Floriva Berry' (municipality of Ivanjica) during 2024 and 2025. Tipping, which involves removing 2-5 cm of the apical cane tip, was performed in 2024 when primocanes reached 70 cm in height (T70). Tipping did not affect leaf chlorophyll and nitrogen content, but cropping did, with floricanes showing lower content in June, coinciding with the fruiting period. Although the total protein concentration was not statistically significant regardless of cropping season and tipping, specific activity of ascorbate (APX) and guaiacol peroxidase (POD) in leaves was altered by cropping season, with greater activity observed on floricanes. POD was also affected by the cropping × tipping interaction, with T70 increasing enzyme activity on primocanes and decreasing it on floricanes compared with a corresponding control. While cane height was reduced by 20%, cane diameter, number of lateral branches, and their length were significantly increased in T70. Tipping considerably increased the number of fruits and yield per primocane (267.3 and 1010.7 g, respectively). The results suggest that tipping could be a powerful practice for primocane-fruiting raspberries maintained in an annual production cycle, which, by managing plant physiological status, promotes (bio)chemical processes in fruit and leaf tissue, leading to improved growth and yield performance.

KEYWORDS: *Rubus idaeus* L.; chlorophyll content; enzymatic activity; plant architecture; yield

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First-Year Implementation and Results of Sustainable Cropping Systems in the ClimaPannonia Project

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During the first year of ClimaPannonia project, we tested four sustainable cropping systems designed to improve drought resistance, soil health, nitrogen availability, biodiversity, pest and disease control, and crop productivity. Through natural cropping system optimization we cultivated winter oil flax as an alternative to sunflower in crop rotations, while field pea was grown as a green manure and natural nitrogen source before sunflower. Soil, crop growth, biomass, and nitrogen measurements were collected throughout the experiment. Second system included pea–wheat intercropping where winter pea and wheat were grown both separately and as an intercrop. Monitoring included crop emergence, flowering, pests, diseases, biomass, yield, and soil nitrogen. Next intercropping system involved alfalfa and sunflower. Early observations suggest that the intercrop may help reduce weeds while allowing both crops to establish successfully. The last system referred on strip cropping: wheat, soybean, grain sorghum, and onion were grown in separate strips to increase biodiversity, improve nitrogen balance, and reduce pests and diseases. Crops were successfully established, and no major pest or disease outbreaks were observed during the initial monitoring period. The first results indicate that field pea can feasibly be used as a green manure and nitrogen source before sunflower; pea–wheat intercropping may improve crop stability and reduce disease and lodging compared with sole pea; alfalfa–sunflower intercropping shows potential for reducing weeds without preventing successful crop development and strip cropping successfully established both winter and spring crops and may contribute to better biodiversity, pest and disease management, and soil nitrogen balance.

KEYWORDS: agriculture; crops; sustainability; resistance

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Phenolic Profile and Antioxidant Potential of *Salvia amplexicaulis* Lam. Grown *In Vitro*

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Secondary metabolites represent important components of plant chemical composition and exhibit diverse biological activities, including pronounced antioxidant potential, making them valuable for applications in the pharmaceutical and food industries. *Salvia amplexicaulis* Lam. (Lamiaceae) is a potential source of natural bioactive compounds. The aim of this study was to analyze the phenolic composition and antioxidant properties of *S. amplexicaulis* grown under *in vitro* and *ex vitro* conditions, with particular emphasis on differences in their accumulation among distinct plant organs. The results showed that, under both growth conditions, total content of phenolic compounds was highest in the roots, with *in vitro* plants generally displaying increased levels of bioactive metabolites compared with plants grown *ex vitro* (reaching 2198.05 mmol GAE and 184.62 mmol RE per g DW for phenolics and flavonoids, respectively). These results correlated significantly with the antioxidant activity of different plant extracts, as measured by three different methods (DPPH, ABTS and FRAP assays). Using UHPLC-LTQ Orbitrap MS untargeted metabolomics analysis a total of 40 phenolic compounds were identified in *S. amplexicaulis* methanol extracts, mainly belonging to phenolic acids and derivatives and to flavonoids and their glycosides. Rosmarinic acid and its derivative sagerinic acid were the most abundant phenolic compounds in all samples tested, with significantly higher amounts detected in plants grown *in vitro*. These results demonstrate the potential of *in vitro* culture of *S. amplexicaulis* to support biodiversity conservation and the production of bioactive compounds.

KEYWORDS: *Salvia amplexicaulis*; phenolic compounds; flavonoids; antioxidant activity; *in vitro*

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Plant Part-Dependent Variation in the Chemical Composition of *Cotinus coggygia* Scop. Essential Oil

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The aim of this study was to characterize and compare the chemical composition of essential oils obtained from different plant parts of *Cotinus coggygia* Scop. Essential oils from inflorescences, bark, and leaves were analyzed by gas chromatography–mass spectrometry (GC/MS), and their constituents were identified based on mass spectra and retention indices using authentic standards, mass spectral libraries, and published literature data. The total percentages of identified constituents were 99.0%, 99.8%, and 99.6% for inflorescence, bark, and leaf oils, respectively. The chemical profiles showed pronounced variation among the investigated plant parts. Monoterpene hydrocarbons were the predominant class in all three essential oils, representing 90.0% of the inflorescence, 85.1% of the bark, and 81.7% of the leaf oil. Oxygenated monoterpenes accounted for 4.4%, 12.9%, and 10.5% in inflorescence, bark, and leaf oils, respectively. Sesquiterpene constituents were present in comparatively lower proportions, with the highest combined contribution of sesquiterpene hydrocarbons and oxygenated sesquiterpenes observed in the leaf oil (4.8%). The inflorescence oil was characterized mainly by limonene (51.9%), followed by α -pinene (19.4%) and *cis*- β -ocimene (8.8%). In contrast, bark oil exhibited a markedly different profile, with α -pinene as the dominant constituent (57.4%), followed by β -pinene (14.9%) and limonene (6.1%). Leaf oil contained the highest proportion of limonene (73.0%), accompanied by α -pinene (5.9%) and linalool (3.5%). These results demonstrated substantial plant part-dependent variation in the essential oil composition of *C. coggygia*, particularly in the relative abundance of the major monoterpene constituents. The observed chemical differentiation highlighted the importance of the plant part used in assessing the phytochemical characteristics and potential biological properties of *C. coggygia* essential oils, providing a valuable basis for further investigation of the biological activities of secondary metabolites produced by this species.

KEYWORDS: GC/MS; monoterpene hydrocarbons; limonene; α -pinene; β -pinene; oxygenated monoterpenes; plant parts

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Can Cultivar × Biofertiliser Interactions Provide a Universal Pattern of Secondary Metabolite Accumulation in Lettuce?

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This study aimed to determine whether cultivar × biofertiliser interactions influence the accumulation of secondary metabolites in lettuce. Three green ('Kiribati', 'Aquino', 'Aleppo') and three red ('Murai', 'Gaugin', 'Carmesi') cultivars were evaluated under three biofertiliser treatments: EM Aktiv (EM), Vital Tricho (VT), and their combined application (EM+VT), in anthropogenic soil under unheated greenhouse conditions during autumn. Biofertilisers were applied to the soil before planting and foliarly four times during cultivation. Total phenolic content (TPC) and total antioxidant capacity (TAC), as well as selected secondary metabolites (chicoric acid, quercetin-3-O-(6"-O-malonyl)-glucoside, and lactucin), were determined using spectrophotometric and HPLC/DAD/MS methods, respectively. Two-way ANOVA revealed significant cultivar effects for all evaluated parameters, while biofertiliser effects were significant only for TPC and lactucin. Significant cultivar × biofertiliser interactions were observed for all evaluated parameters, demonstrating cultivar- and metabolite-specific responses. Overall, red cultivars showed broader responses, while green cultivars, especially 'Kiribati', were less responsive. 'Murai' showed enhanced TPC under VT but reduced TAC and chicoric acid under microbial fertilisation. 'Gaugin' responded positively to EM+VT for TPC, while 'Carmesi' showed enhanced TPC and quercetin-3-O-(6"-O-malonyl)-glucoside under EM or EM+VT and the highest lactucin content under EM. 'Aleppo' responded mainly through increased TAC and chicoric acid with EM and VT, whereas 'Aquino' showed a selective increase in chicoric acid under VT. No fertiliser consistently enhanced secondary metabolites across cultivars. These findings demonstrate that biofertilisation produces cultivar- and metabolite-specific responses, highlighting the importance of matching biofertiliser type with cultivar to target specific phytochemical traits and improve lettuce quality.

KEYWORDS: *Lactuca sativa*; biostimulants; spectrophotometric assays; phenolics; sesquiterpene lactones

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Evaluation of the Antioxidant And Antimicrobial Properties of Leaves and Aerial Parts of Traditional Balkan Medicinal Plants

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Centaurium erythraea Rafn, *Helichrysum italicum* (Roth) G. Don, and *Hypericum perforatum* L. are highly valued plant species in the traditional medicine of the Balkans. They are used worldwide in the form of tea, tinctures, oils, and ointments for the treatment of various skin, digestive, respiratory, inflammatory disorders, and depression. The aim of this research is a comparative examination of chemical composition of methanolic extracts of their leaves and aerial parts, and corresponding antioxidant and antimicrobial properties. The chemical composition was determined using both high performance liquid chromatography-mass spectrometry (HPLC/MS) and gas chromatography-mass spectrometry (GS-MS), antioxidant activity was assessed using spectrophotometric methods, while 96 - plate microdilution method was adopted to evaluate antimicrobial activity. The results varied between studied plant species, but also between leaf and aerial parts samples of the same species. Extracts of *H. italicum* showed the highest concentration of phenolic compounds (99.576 ± 0.253 mM GAE/mg DW) (GAE- gallic acid equivalent; DW – dry weight), predominated by hydroxycinnamic acid derivatives, and accordingly demonstrated the strongest antioxidant, and antimicrobial activity. Interestingly, samples of the wild grown *H. italicum* showed greater biological activity than that of cultivated plants, which can also be attributed to higher content of polyphenolics. *H. perforatum* and *C. erythraea* were rich in xanthone-type phenolics, but they displayed significantly lower antioxidant and antimicrobial potential than *H. italicum*. This research confirmed the potent biological activities of studied plant species which are shaped by species- and organ- specific chemical composition and growth conditions.

KEYWORDS: phytochemical composition; antimicrobial activity; antioxidant potential; *Hypericum perforatum*; *Helichrysum italicum*; *Centaurium erythraea*

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***Caenorhabditis elegans* Assays: Lifespan Extension and *In Vivo* Antioxidant Activity**

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The species *Morus alba*, commonly known as the white mulberry, is a medicinal plant used in traditional Chinese medicine. Its root and bark extracts are the richest sources of natural bioactive compounds. Literature indicates that *M. alba* cell cultures can be stimulated with elicitors to trigger the extracellular production of compounds similar to those found in various parts of the plant, such as stilbenes, chalcones, and moracins. The bioactive properties of the extract and its major constituent compounds remain poorly characterized or uncharacterized. We chemically characterized the extract's composition and evaluated its anti-aging properties using the *Caenorhabditis elegans* model; these evaluations included assessing *in vivo* antioxidant activity (using strain TJ375) and lifespan extension (using wild-type strain N2). A total of ten major compounds were structurally identified - oxyresveratrol, resveratrol, moracin M, 2,4,2',4'-tetrahydroxychalcone, isoliquiritigenin, 4-prenyloxyresveratrol, 4-prenylresveratrol, moracin C, morachalcone A, and isobavachalcone – and were enriched in chromatographic fractions to a purity of >90%. The extract, prepared at 0.2 mg/mL in a DMSO vehicle, demonstrated an 81.4% reduction in oxidative stress and an 8.8% extension of *C. elegans* lifespan. Furthermore, all fractions enriched with these constituent compounds - prepared at 0.1 mg/mL - exhibited *in vivo* antioxidant capacity, achieving oxidative stress reductions of up to 88.2% and a maximum lifespan extension of 21.14%.

KEYWORDS: bioactive profile; *Caenorhabditis elegans*; *Morus alba*; lifespan extension; stilbenes

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A Visual Reporter System for Transient Transformation of *Nepeta nuda* L. leaves

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Nepeta species are rich in specialized metabolites, particularly iridoids such as nepetalactones, whose biosynthesis involves several unresolved steps. Efficient *in planta* transformation systems are therefore needed for functional characterization of candidate biosynthetic genes. Here, we established a rapid transient transformation platform for *Nepeta nuda* L. using the betalain-based RUBY reporter system. The 35S::RUBY construct was introduced into *Agrobacterium tumefaciens* LBA4404 and transiently expressed in *N. nuda* leaves by agroinfiltration. Successful transformation resulted in localized, visually detectable betalain accumulation, enabling rapid identification of transformed tissues. Optimization of bacterial density showed that an OD₆₀₀ of 1.0 provided the strongest and fastest reporter response, with visible pigmentation appearing three days after infiltration. Spectrophotometric analysis confirmed substantial betalain accumulation in transformed tissues whereas betalains were undetectable in control leaves. Importantly, RUBY expression did not significantly affect total iridoid or phenolic content, indicating minimal interference with the specialized metabolic profile of *N. nuda*. To our knowledge, this represents the first successful application of the RUBY reporter system for transient leaf transformation in *Nepeta* and the Lamiaceae family. The co-expression of RUBY reporter with targeted biosynthesis-related genes provides a simple, rapid, and visually detectable platform for future *in planta* metabolic engineering approaches to enhance iridoid production in *Nepeta*.

KEYWORDS: *Nepeta nuda*; agroinfiltration; RUBY

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