

Rethinking Plant Systems for the Future



International Conference of the Slovenian Society of Plant Biology

Hotel Habakuk

September 17 – 18, 2026, Maribor, Slovenia



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GENERAL INFORMATION

The conference will start on Thursday, September 17th 2026 at 10 am and will close on Friday, September 18th 2026 at 3 pm.

Venue

The conference will be held in the lecture hall of the Hotel Habakuk, Pohorska ulica 59, 2000 Maribor (for exact location scan the QR code below). The conference reception desk will be open on Thursday from 8.30 to 10 am. Please follow the conference signposts. Parking is available on-site in the hotel's parking garage. Before leaving, please validate and pay your parking ticket at the hotel reception desk. Coffee breaks and lunches will be served at the venue. The conference dinner will be held in the hotel restaurant.



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Scientific Programme

Contributions will be presented as invited lectures (INV), oral presentations (OP) or posters (P).

The speakers are kindly requested to deliver their presentation material in Microsoft PowerPoint (PPT) or PDF format on USB at least 15-20 minutes before the beginning of the session. We recommend using the Standard (3:4) slide size. The length of invited lectures is 30 minutes and of short oral presentations 15 minutes including discussion.

Posters will be displayed in the main corridor outside the lecture hall. Authors are kindly requested to mount their posters at the beginning of the conference and to dismount them at the end of the second day in order to present their work throughout the period of the conference. At least one of the authors is requested to be available for discussion during each poster viewing session.

Organizing committee

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CONFERENCE PROGRAMME

Thursday 17 September

8.30 – 10.00 Registration, welcome coffee, and poster mounting

10.00 Welcome address

Session 1: Plant associated microbiomes (chair: Špela Baebler)

10.30 Šibanc Nataša: Forest trees as holobionts in a stress-prone Karst landscape: drought, fire and host identity shape fungal resilience (INV-1)

11.00 Stopnišek Nejc: The microbiome as a plant trait: from cataloguing to steering plant-microbe systems (INV-2)

11.30 Stražar Martin: Characterisation of potato microbiota for design of successful potatobiome communities (OP-1)

11.45 Vogrinec Lana: Spatiotemporal dynamics of plant viruses across wastewater, river, and wild vegetation (OP-2)

12.00 Pelko Teja: Contrasting effects of biodegradable PBAT and polyethylene microplastics on sunflower root-associated soil microbiomes (OP-3)

12.15 Mravlje Jure: Intergenerational restructuring of the buckwheat grain microbiome following cold plasma seed treatment under field conditions (OP-4)

12.30-13.30 Lunch

Session 2: Understanding plant pathogens and pests (chair: Maja Križnik)

13.30 Štajner Nataša: Small RNAs in plant immunity: Insights from plant-pathogen and plant-virus interactions (INV-3)

14.00 Luskar Lucija: Association mapping of Verticillium wilt response in hop (*Humulus lupulus* L.) using a phased reference genome to support resistance breeding (OP-5)

14.15 Lešnik Mario: Assessment of the possibility of mitigating the disease severity and reducing the incidence of grapevine infections with phytoplasma causing Flavescence dorée disease by treating vines with the endophytic bacterium *P. phytofirmans* (OP-6)

14.30 Nolte Nadjia: LongPolyASE: A framework for allele-specific gene and isoform analysis in polyploid plants using long-read RNA-seq (OP-7)

14.45 Levak Valentina: Gene expression during potato leaf response to wounding is determined through interaction between jasmonic acid and salicylic acid signalling (OP-8)

15.00-16.00 Coffee break and poster viewing

16.00-18.00 Rethinking Plant Systems for the Future (discussions in breakout rooms)

20.00- Conference dinner

Friday 18 September

Session 3: **Microbes as plant's friends** (chair: Nejc Stopnišek)

- 8.30 Zajc Žunič Janja: From emerging threats to biological solutions: Rethinking the role of fungi in plant health (INV-4)
- 9.00 Vozelj Anže: Understanding harmful and beneficial plant interactions through the study of harmful Potato virus Y and beneficial endophytic *Fusarium oxysporum* isolate (OP-9)
- 9.15 Fink Katja: Visualizing fast calcium response during beneficial microbe interactions in potato (OP-10)
- 9.30 Maček Irena: Drivers of arbuscular mycorrhizal communities in plant roots: from community composition to specific taxa (OP-11)
- 9.45 Molnar Zoltan: Application of chitosan and cyanobacterial biomass in agriculture for improving cereal crop productivity (OP-12)

10.00-11.00 Coffee Break and Poster Viewing

Session 4: **New methods in studying plants** (chair: Živa Fišer)

- 11.00 Križnik Maja: Unraveling potato immune signaling in response to potato virus Y using spatially resolved small RNA and transcriptomic profiling (INV-5)
- 11.30 Godec Tim: Integrating chromatin accessibility and transcriptomics to dissect potato responses to climate stress (OP-13)
- 11.45 Strah Rebeka: Subcellular localisation of SAP11-like and SAP54-like effector proteins from 'Candidatus Phytoplasma solani' (OP-14)
- 12.00 Kladnik Aleš: How to look closer at plants? (OP-15)
- 12.15 Režonja Blaž: Mineral element atlas of six underutilised dicot seeds revealed by high-content ion microscopy (OP-16)

12.30-13.30 Lunch

Session 5: **Plants are not alone: Ecology and conservation** (chair: Nataša Šibanc)

- 13.30 Fišer Živa: Beyond the plant: Rethinking plant conservation through ecological interactions (INV-6)
- 14.00 Bočaj Valentina: Evolutionary co-adaptation and functional architecture of the *Noccaea praecox* root-associated microbiome (OP-17)
- 14.15 Ambrožič Dolinšek Jana: Water extracts from cereal weed corn cockle (*Agrostemma githago*) showed plant growth regulator efficiency (OP-18)
- 14.30 Urbanek Krajnc Andreja: Mulberry germplasm diversity as a foundation for European sericulture, biodiversity conservation and cultural landscapes (OP-19)

Closing session

- 14.45-15.00 Concluding remarks

PROGRAMME AT A GLANCE

Thursday 17.09.2026		Friday 18.09.2026	
08.30-10.00	Registration and Welcome Coffee Poster Mounting	Session 3: Microbes as plant's friends (chair: Nejc Stopnišek)	
		8.30-9.00	INV-4: Zajc Žunič J.: From emerging threats to biological solutions: Rethinking the role of fungi in plant health
		9.00-9.15	OP-9: Vozelj A.: Understanding harmful and beneficial plant interactions through the study of harmful Potato virus Y and beneficial endophytic <i>Fusarium oxysporum</i> isolate
		9.15-9.30	OP-10: Fink K.: Visualizing fast calcium response during beneficial microbe interactions in potato
		9.30-9.45	OP-11: Maček I.: Drivers of arbuscular mycorrhizal communities in plant roots: from community composition to specific taxa
		9.45-10.00	OP-12: Molnar Z.: Application of chitosan and cyanobacterial biomass in agriculture for improving cereal crop productivity
10.00-10.30	Welcome Address	10.00-11.00	Coffee Break and Poster Viewing
Session 1: Plant-associated microbiomes (chair: Špela Baebler)		Session 4: New methods in studying plants (chair: Živa Fišer)	
10.30-11.00	INV-1: Šibanc N.: Forest trees as holobionts in a stress-prone Karst landscape: drought, fire and host identity shape fungal resilience	11.00-11.30	INV-5: Križnik M.: Unraveling potato immune signaling in response to potato virus Y using spatially resolved small RNA and transcriptomic profiling
11.00-11.30	INV-2: Stopnišek N.: The microbiome as a plant trait: from cataloguing to steering plant-microbe systems	11.30-11.45	OP-13: Godec T.: Integrating chromatin accessibility and transcriptomics to dissect potato responses to climate stress
11.30-11.45	OP-1: Stražar M.: Characterisation of potato microbiota for design of successful potatobiome communities	11.45-12.00	OP-14: Strah R.: Subcellular localisation of SAP11-like and SAP54-like effector proteins from ' <i>Candidatus</i> Phytoplasma solani'
11.45-12.00	OP-2: Vogrinc L.: Spatiotemporal dynamics of plant viruses across wastewater, river, and wild vegetation	12.00-12.15	OP-15: Kladnik A.: How to look closer at plants?
12.00-12.15	OP-3: Pelko T.: Contrasting effects of biodegradable PBAT and polyethylene microplastics on sunflower root-associated soil microbiomes	12.15-12.30	OP-16: Režonja B.: Mineral element atlas of six underutilised dicot seeds revealed by high-content ion microscopy
12.15-12.30	OP-4: Mravlje J.: Intergenerational restructuring of the buckwheat grain microbiome following cold plasma seed treatment under field conditions	12.30-13.30	Lunch
12.30-13.30	Lunch	Session 5: Plants are not alone: Ecology and conservation (chair: Nataša Šibanc)	
Session 2: Understanding plant pathogens and pests (chair: Maja Križnik)		13.30-14.00	INV-6: Fišer Ž.: Beyond the plant: Rethinking plant conservation through ecological interactions
13.30-14.00	INV-3: Štajner N.: Small RNAs in plant immunity: Insights from plant-pathogen and plant-virus interactions	14.00-14.15	OP-17: Bočaj V.: Evolutionary co-adaptation and functional architecture of the <i>Noccaea praecox</i> root-associated microbiome
14.00-14.15	OP-5: Luskar L.: Association mapping of Verticillium wilt response in hop (<i>Humulus lupulus</i> L.) using a phased reference genome to support resistance breeding	14.15-14.30	OP-18: Ambrožič Dolinšek J.: Water extracts from cereal weed corn cockle (<i>Agrostemma githago</i>) showed plant growth regulator efficiency
14.15-14.30	OP-6: Lešnik M.: Assessment of the possibility of mitigating the disease severity and reducing the incidence of grapevine infections with phytoplasma causing Flavescence dorée disease by treating vines with the endophytic bacterium <i>P. phytofirmans</i>	14.30-14.45	OP-19: Urbanek Krajnc A.: Mulberry germplasm diversity as a foundation for European sericulture, biodiversity conservation and cultural landscapes
14.30-14.45	OP-7: Nolte N.: LongPolyASE: A framework for allele-specific gene and isoform analysis in polyploid plants using long-read RNA-seq	14.45-15.00	Concluding Remarks
14.45-15.00	OP-8: Levak V.: Gene expression during potato leaf response to wounding is determined through interaction between jasmonic acid and salicylic acid signalling		
15.00-16.00	Coffee Break and Poster Viewing		
16.00-18.00	Rethinking Plant Systems for the Future (discussions in breakout rooms)		

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Plant associated microbiomes

Forest trees as holobionts in a stress-prone Karst landscape: drought, fire and host identity shape fungal resilience

Nataša Šibanc^{1*}, Tanja Mrak¹, Hojka Kraigher¹

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Sub-Mediterranean Karst forests are increasingly exposed to recurrent drought, wildfire and shifts in tree species composition. In these shallow, heterogeneous and water-limited soils, forest-tree functioning depends not only on aboveground traits, but also on root-associated and soil fungal communities. Here, we synthesize studies from Slovenian Karst forest systems to examine how drought, fire intensity and host identity reshape fungal communities and their potential functions.

Temporal monitoring of *Quercus pubescens* ectomycorrhiza showed that sequential drought and wildfire strongly reduced the ratio of vital to non-vital ectomycorrhizal root tips, whereas community composition remained relatively stable but locally heterogeneous, suggesting that stochastic processes may be important in fragmented Karst woodlands (Mrak et al. 2021). A rain-exclusion experiment further confirmed that reduced soil water availability can decrease ectomycorrhizal vitality and the number of unique morphotypes, suggesting that prolonged drought may narrow the functional breadth of ectomycorrhizal communities (Mrak et al. 2025a).

At the landscape scale, extramatrical mycelium beneath native *Ostrya carpinifolia* and *Q. pubescens*, and non-native *Pinus nigra*, was shaped by host identity, interannual climatic variability, soil conditions and spatial context (Mrak et al. 2025b). Although many fungal taxa were shared among tree species, distinct mycelial structures and exploration strategies indicate that host identity remains an important ecological filter in Karst woodlands.

Recent post-fire sampling after the 2022 Goriški Kras megafire shows that fire intensity, forest type, substrate and time since fire interact to restructure soil and root fungal communities (Šibanc et al., in prep.). Crown fire caused the strongest reductions in richness, Shannon diversity and evenness, especially in coniferous stands, whereas surface-fire effects were weaker and declined with time. Burned plots remained separated from controls, with more early-successional and pyrophilous Ascomycota and fewer ectomycorrhizal Basidiomycota after severe crown fire.

Together, these studies show that fungal responses to drought and fire are not simple diversity loss. Karst fungal communities combine resilience, reduced symbiotic vitality and compositional reassembly. Integrating host identity, fungal guilds, exploration strategies and disturbance intensity is therefore essential for predicting forest resilience under future climate and disturbance regimes.

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Acknowledgements

This synthesis draws on studies supported by the Slovenian Research and Innovation Agency (P4-0107, J4-7203, J4-9297, V4-2222).

The microbiome as a plant trait: from cataloguing to steering plant–microbe systems

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Plants do not function as autonomous organisms: their phenotypes emerge partly from interactions with the microbial communities they host. If those communities shape nutrition, growth and stress tolerance, can the microbiome be understood—and steered—as a plant trait?

This talk traces the field's shift from describing who is present to identifying the organisms and functions that predictably improve plant performance. Drawing on our work in common bean, tomato and wheat, I will develop a set of concepts rather than catalogue results: that some microbial members persist across geography, season and host genotype while most turn over; that presence is not function, since activity and expression change with context; that host genetics measurably shape which microbes assemble; and that moving from correlation to causation demands manipulative experiments such as synthetic communities, stress-induced recruitment and microbiome-assisted breeding.

Together these expose a productive tension. Plants do influence their microbiota, yet soil, climate, management and microbe–microbe interactions constrain that influence. The microbiome is therefore not a fixed, fully heritable trait but a dynamic, distributed component of plant phenotype, one we can begin to steer only where plant biology and microbiology are brought together. The goal is not a universal optimal microbiome, but resilient plant–microbe systems whose beneficial functions persist under changing conditions.

Characterisation of potato microbiota for design of successful potatobiome communities

Martin Stražar^{1,2,*}, Karmen Pogačar¹, Anže Vozelj¹, Tina Arnšek¹, Klara Šuštar¹, Marko Petek¹, Paloma Sánchez-Bel³, Victor Flors Herrero³, Peter Dolničar⁴, Polonca Štefanič⁵, Ines Mandić Mulec⁵, Kristina Gruden^{1,*}

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The plant microbiota offers key ecosystem services to the plant host, including nutrient exchange, community regulation, production of phytohormones and protection against biotic and abiotic stresses (Lukan et al. 2025; Gruden et al. 2020). To investigate the effects of potato microbiota under field conditions, we sampled eight tetraploid cultivars, grown under conventional and organic agricultural management between 2022 and 2023. Sampling of roots and leaves was performed at the time of tuber initiation and at the end of tuber filling. We split each sample, rigorously sterilising half to select for endophytic microbes, while the remaining samples predominantly included epiphytes. A total of 1016 samples were analysed using V3V4 sequencing and clustered according to alpha and beta diversity. Of these, 24 samples were selected for isolation of bacterial microbes as representatives of identified clusters to maximize the captured diversity. Cultivation was performed on three different media, and isolated bacteria were classified using the MALDI Biotyper mass spectrometer. A total of 550 unique strains were stored in the NIB potato microbiota collection, representing an unprecedented resource for mechanistic studies and community design. Here, we perform deep characterization of the collection at the level of genome, metabolome, and plant growth-promoting and protecting properties.

The mechanistic basis of interaction between plant and bacteria was investigated through untargeted metabolomics of exometabolites — compounds bacteria secrete into media. To this end, we profiled eleven of the endophyte strains and three known growth-promoting strains, detecting at least twofold changes in 31 identified metabolites, including purines and aromatic amino acids, potentially important in natural product synthesis. To shed light on the more than 36,000 increased, unannotated features, we adapt and retrain MS1-Tools (Stražar et al. unpublished)—a machine-learning-based method for molecular fingerprint prediction—for plant and plant-bacterial molecular repertoires. Using experimental and computational techniques, we aim towards comprehensive identification of microbially-derived phytohormones.

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Spatiotemporal dynamics of plant viruses across wastewater, river, and wild vegetation

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Plant viruses are major contributors to crop losses worldwide, affecting both yield and product quality. Although past studies have focused mainly on crop-infecting viruses, recent evidence shows that plant viruses are widely distributed across diverse hosts and environments, including aquatic ecosystems. Similar observations extend to wastewater, where their viral particles can persist and, in some cases, retain infectivity. The release of wastewater into rivers establishes a connected pathway that may facilitate the long-distance movement of plant viruses, however the extent to which these routes contribute to viral spread and establishment in plants remains poorly understood.

We investigated the spatiotemporal dynamics of plant viruses within an anthropogenically impacted ecosystem/ comprising a wastewater treatment plant (WWTP Domžale-Kamnik), its receiving river (Kamniška Bistrica), and the associated riverbank vegetation. We collected samples of treated and untreated wastewater, river water, and wild plants growing along the riverbanks over the span of two years. We concentrated 37 river water and 18 wastewater samples using monolithic chromatography (CIM), followed by total RNA extraction and random preamplification. We extracted total RNA from plant samples representing 1,386 individuals across 245 taxa and combined it into 119 pools containing RNA from 10-17 individuals. All samples were sequenced using Illumina high-throughput sequencing and analyzed with a custom bioinformatic pipeline.

We detected diverse viral communities in river and wastewater samples, including numerous members of plant-infecting viral families. Members of *Virgaviridae* and *Tombusviridae* were among the most abundant, with the latter particularly enriched in effluent samples. We also detected members of families *Aspiviridae*, *Bromoviridae*, *Closteroviridae*, *Kitaviridae*, *Potyviridae*, and *Geminiviridae*. Viral communities in river and wastewater showed pronounced temporal variability, although several persistent taxa were identified across seasons, including members of the *Potexvirus* genus (*Alphaflexiviridae*), which were detected in >85% of river samples. Cross-compartment comparisons revealed overlaps between water and plant viromes; notably, we detected Gorica tombusvirus 1 in river water, wastewater, and wild plants during the spring and summer 2023, suggesting an active exchange between these different ecosystem fractions.

In this study, we characterized plant virus dynamics across interconnected environmental compartments, identifying wastewater and river systems as potential vessels for viral transport within ecosystems. Our findings underscore the ecological relevance of plant viruses, given their diversity, abundance, and potential roles in shaping host dynamics.

Contrasting effects of biodegradable PBAT and polyethylene microplastics on sunflower root-associated soil microbiomes

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Microplastics are increasingly accumulating in agricultural soils through mulch fragmentation, compost application and repeated plastic inputs (Pelko et al. 2026; Gioia et al. 2021). Biodegradable plastics are often promoted as environmentally preferable alternatives to conventional plastics, yet their impacts on root-associated soil microbiomes remain insufficiently understood. We investigated how microplastics prepared from conventional polyethylene (PE) and two starch-containing poly(butylene adipate-co-terephthalate) (PBAT) plastic bags designed for home composting (PBAT_HOME) or industrial composting (PBAT_IND) affect microbial communities associated with sunflower (*Helianthus annuus* L.) roots. Microplastics were mixed into agricultural soil at 0.5 and 1.0% (w/w dry soil), and sunflower plants were grown for eight weeks under controlled conditions. Root-associated soil was sampled at harvest, and bacterial and fungal communities, functional gene profiles and inferred microbial co-occurrence networks were assessed using shotgun metagenomics.

Microplastics altered the root-associated soil microbiome primarily in a polymer-dependent manner. PE-treated communities remained more similar to the control, whereas both PBAT treatments formed distinct communities, with fungal communities showing stronger treatment-specific responses than bacterial communities. PBAT treatments shifted fungal assemblages away from taxa associated with mutualistic or plant growth-promoting functions and toward saprotrophic fungi linked to decomposition of complex organic substrates. They also enriched genera containing potentially pathogenic or opportunistic members, including *Monosporascus*, *Ophiognomonia*, and *Pyrenophora*. In bacterial communities, PBAT_IND produced the strongest taxonomic signal and enriched taxa associated with anaerobic metabolism and carbon or sulfur transformations, such as *Geobacter*, *Desulfuromonas*, *Ruminiclostridium* and *Anaeromyxobacter*. Functional profiles further indicated polymer-specific changes in carbon- and nitrogen-cycling potential. PBAT treatments enriched genes related to carbon transformation, including methane metabolism, carbohydrate uptake and lipid or fatty-acid metabolism, while PE mainly affected genes linked to nitrogen scavenging and assimilation. PBAT_IND showed the clearest anaerobe-associated signature, with enrichment of genes involved in methane metabolism and carbohydrate transport. Co-occurrence network analysis suggested that microplastic exposure reduced inferred microbial network connectivity and robustness, with PBAT_IND showing the highest vulnerability in both bacterial and fungal networks.

Our findings show that biodegradable starch-containing PBAT microplastics can act as biologically reactive soil inputs rather than inert residues. Although marketed as compostable, these materials induced stronger shifts in the sunflower root-associated soil microbiome than conventional PE and altered microbial taxa, functional potential and network organization. Soil microbiome responses should therefore be included in environmental risk assessment, compost quality control and certification frameworks for biodegradable plastics.

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Intergenerational restructuring of the buckwheat grain microbiome following cold plasma seed treatment under field conditions

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With increasing pressure on agricultural production, cold plasma (CP) seed treatment is being explored as a non-chemical seed treatment with reported effects on germination, microbial contamination, and early plant performance (Mravlje et al., 2021a). However, its effects on seed-associated microbiomes under field conditions and their persistence into harvested progeny seeds remain poorly characterised; therefore, it is still unclear whether CP-induced microbiome changes persist beyond the initially treated seed lot or are reshaped by the environment. This study examined the impact of CP treatment on common (*Fagopyrum esculentum*) and Tartary (*F. tataricum*) buckwheat, integrating field performance measurements with shotgun metagenomic DNA profiling of grain-associated microbial communities in the initial grain lot and in harvested progeny.

Buckwheat grains were exposed for 60 s to low-pressure CP generated in a 50:50 O₂:N₂ atmosphere under previously optimised treatment conditions using a reactor system described previously (Mravlje et al., 2021b). Control and CP-treated grains were evaluated through laboratory germination assays and cultivation-based assessment of viable culturable fungal colonisation and were sown in the field the following day. During the growing season, plant establishment, physiological traits, biomass, and grain yield were measured. To investigate microbiome responses across generations, grain-associated bacterial and fungal communities from control and CP-treated grains were characterised using shotgun metagenomic sequencing and bioinformatic taxonomic profiling in the initial seed material (F0) and in grains harvested from the resulting plants (F1).

CP treatment delayed in vitro germination in both species and reduced final germination of Tartary buckwheat by approximately 20% relative to the control but had limited effects on field physiological traits and biomass. Despite reduced field seedling emergence, CP-treated buckwheat produced higher grain yield per established plant in both species (even up to 70% in common buckwheat). Cultivation-based assays showed up to 50% reduction in viable culturable fungal colonisation following CP treatment. Shotgun metagenomic DNA profiling revealed statistically supported CP-associated shifts in relative community composition of the F0-generation seed microbiomes of both species, including lower relative abundance of Actinobacteria and higher relative abundance of Proteobacteria, and an approximately 10% decrease in Pleosporaceae. In contrast, F1-generation grains were characterised by pronounced restructuring of fungal communities, with Pleosporaceae abundance increasing nearly 2-fold irrespective of CP pretreatment, suggesting that field environmental factors substantially overrode initial CP-associated microbiome signatures.

CP seed treatment induced detectable but transient changes in seed-associated microbiomes under field conditions. Bacterial community shifts showed limited persistence into harvested progeny grains, whereas fungal communities were largely reassembled in the next generation, consistent with a strong role of environmental acquisition and filtering. These findings position CP treatment as a short-term ecological disturbance to the seed microbiome and highlight the need to complement DNA-based profiling with viability assays and absolute-abundance measurements when evaluating microbiome responses to physical seed treatments.

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Understanding plant pathogens and pests

Small RNAs in plant immunity: Insights from plant–pathogen and plant–virus interactions

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Small RNAs (sRNAs) are key regulators of gene expression and play essential roles in plant development, stress responses, and immunity. During interactions with pathogens, plants employ diverse classes of sRNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs), to modulate defence pathways and restrict pathogen proliferation. Increasing evidence further indicates that sRNAs can function across species boundaries, mediating cross-kingdom communication between plants and their pathogens. Understanding the molecular mechanisms underlying sRNA-mediated regulation therefore represents an important step towards the development of innovative and sustainable crop protection strategies.

Our research focuses on the role of sRNAs in economically important perennial crops, particularly hop (*Humulus lupulus* L.) and grapevine (*Vitis vinifera* L.), and their interactions with fungal and viral pathogens. Using high-throughput sequencing, degradome analysis, target prediction, gene expression profiling and functional validation approaches, we investigated the contribution of sRNAs to plant defence responses and pathogen virulence.

In the hop–*Verticillium nonalfalfae* pathosystem, we identified conserved and novel miRNAs that respond differentially in susceptible and resistant cultivars following fungal infection. Their predicted and experimentally validated targets include transcription factors, auxin-response regulators, receptor-like kinases and other genes involved in defence signalling, root development and vascular tissue formation. Functional analyses suggest that miRNA-mediated regulation contributes to the establishment of resistance-associated responses and developmental reprogramming during infection. In parallel, we identified fungal microRNA-like RNAs (miRNAs) in *V. nonalfalfae* and demonstrated that their targets are enriched in pathways related to pathogenicity and stress responses, supporting a potential role in host colonization and cross-kingdom RNA interference.

In grapevine, small RNA sequencing revealed complex host responses to mixed viral infections and enabled the characterization of virus-derived siRNAs (vsiRNAs). Ongoing analyses indicate that vsiRNAs may target not only viral genomes but also host transcripts involved in defence signalling and RNA silencing pathways, highlighting an additional layer of regulation during plant–virus interactions.

Together, these studies demonstrate the central role of small RNAs in coordinating plant immune responses and shaping plant–pathogen interactions. Our findings provide novel insights into RNA-mediated regulatory networks and support the development of RNA-based approaches, including host-induced and spray-induced gene silencing, for sustainable disease management in agriculture.

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Association mapping of Verticillium wilt response in hop (*Humulus lupulus* L.) using a phased reference genome to support resistance breeding

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Verticillium wilt (VW), caused by the xylem-invading fungus *Verticillium nonalfalfae*, is a primary threat to European hop (*Humulus lupulus* L.) production (EPPO 2024). Development of the disease depends on a complex host–pathogen interaction; while susceptible cultivars often trigger vigorous but ineffective induced responses, resistant genotypes typically rely on more efficient, possibly constitutive, vascular defences (Luskare et al. 2022). Identifying the genomic loci governing these outcomes is essential for marker-assisted selection.

We analyzed a bi-parental F1 population from a cross between resistant ‘Wye Target’ and susceptible ‘BL2/1’. Disease response was quantified via a disease severity index (DSI; 0–5) following controlled inoculation. Progeny were genotyped by sequencing and mapped to a haplotype-resolved reference genome (Apollo; Kale et al. 2026), with both phases analyzed independently. Association mapping was conducted using five GWAS frameworks (MLM (Tassel 5) and MLM, MLM, FarmCPU, BLINK (GAPIT)) while controlling for kinship. To support breeding applications, association signals were integrated into a Random Forest prediction model.

While the quantitative nature of resistance and population size influenced the statistical stringency of genome-wide corrections, the analysis revealed several robust association signals. Cross-model comparison across both genome phases prioritized candidate loci with strong functional relevance to plant–microbe interactions. A prominent region on chromosome 1 was consistently detected and annotated with kinase-rich clusters, including wall-associated kinases (WAKs) implicated in perceiving fungal attack and activating downstream defence. Additional candidates included receptor-like kinases, supporting a model where variation in pathogen perception and vascular signalling determines resistance levels. The predictive model achieved strong rank correlation (~0.7–0.8), demonstrating its utility for early-stage screening.

Integrating haplotype-resolved GWAS with genomic prediction provides clear candidate loci and practical marker leads for VW resistance. The consistent identification of defence-signalling regions—specifically WAK/kinase clusters—offers new insights into the hop–Verticillium interaction and provides valuable resources for breeding programmes to combat this destructive vascular pathogen.

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Assessment of the possibility of mitigating the disease severity and reducing the incidence of grapevine infections with phytoplasma causing Flavescence dorée disease by treating vines with the endophytic bacterium *Paraburkholderia phytofirmans*

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Treatment of cultivated plants with microbial biostimulants can also affect plant pathogens and strengthen the plant's resistance mechanisms. Research has confirmed that among the microbes reported to inhibit the development of grapevine diseases are also endophytic symbiotic bacteria of the genus *Paraburkholderia* (e.g. the species *Paraburkholderia phytofirmans*). Previous studies have demonstrated that *P. phytofirmans* enhances grapevine resistance through multiple mechanisms, including the induction of plant defence responses, modulation of secondary metabolism, and improved tolerance to biotic and abiotic stresses (Miotto-Vilanova et al. 2019). In particular, *P. phytofirmans* has been reported to suppress the development of grey mould (*Botrytis cinerea*) in grapevine through both direct antagonistic activity and the stimulation of host defence mechanisms (Miotto-Vilanova et al. 2016, Vilanova et al. 2022). However, despite its well-documented biocontrol potential against fungal pathogens, its effects on phytoplasma-associated diseases, including golden vine yellowing (Flavescence dorée) caused by '*Candidatus Phytoplasma vitis*', have not yet been investigated.

In a pot experiment and in a vineyard experiment, we obtained data on the extent of the inhibitory effect of the *P. phytofirmans* bacterium against the phytoplasma '*Candidatus Phytoplasma vitis*'. In the pot experiment, we repeatedly treated vines infected with the phytoplasma causing FD with *P. phytofirmans* and found that the treatment significantly reduced the intensity of disease symptom expression. In the vineyard, we also observed a reduction in the intensity of infection symptoms on FD-infected vines. In addition, we placed uninfected vines in pots in a vineyard infected with FD phytoplasma and analyzed the extent of infection in treated vines compared with untreated vines. The experiment showed that treating vines grown in pots with the bacterium PP significantly reduced the proportion of plants that showed signs of infection in the first growing season compared to untreated vines. This contribution evaluates the potential of *P. phytofirmans* to help limit the occurrence and development of Flavescence dorée in vineyards.

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LongPolyASE: A framework for allele-specific gene and isoform analysis in polyploid plants using long-read RNA-seq

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Allele-specific expression analysis can reveal *cis*-regulatory differences (e.g., promoter variants, epigenetic changes) that cause imbalanced gene expression between haplotypes. Haplotype-resolved reference genomes and long-read RNA sequencing enable allele-specific expression analysis at gene and isoform-levels. However, existing tools are largely restricted to short-read RNA sequencing data and diploid organisms.

We developed [LongPolyASE](#), an end-to-end computational framework for allele-specific gene and isoform expression analysis in diploid and polyploid organisms using long-read RNA sequencing, consisting of three components: Syntelogfinder, for identifying syntenic gene relationships and annotation inconsistencies; longrnaseq, for novel isoform discovery and haplotype-level quantification; and PolyASE, for statistical testing and visualization of allelic imbalance and isoform usage.

We applied LongPolyASE to diploid rice, autotetraploid potato, allotetraploid rapeseed, and allooctoploid strawberry using Oxford Nanopore and PacBio long-read RNA-seq. The framework enabled identification of *cis*-regulatory variation, tissue-specific *trans*-regulatory effects, differential isoform usage, and haplotype-specific splicing differences. In addition, it facilitated the discovery of novel transcripts and genes with potential functional relevance in plant development.

LongPolyASE addresses a key methodological gap by enabling allele-specific expression analysis in polyploid organisms using long-read RNA sequencing. By combining haplotype-aware quantification with isoform-level resolution in a reproducible workflow, the framework provides a practical tool for plant researchers working with complex genomes. Its application to crop species highlights its potential to support the identification of regulatory variation and candidate targets for plant breeding.

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Gene expression during potato leaf response to wounding is determined through interaction between jasmonic acid and salicylic acid signalling

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Recent research on plant stress responses emphasizes the importance of response dynamics, involving crosstalk between different signalling pathways, in plant's ability to withstand stresses. The spatiotemporal dynamics of plant immune response can be best followed with genetically encoded biosensors. We investigated the spatial dynamics of potato (*Solanum tuberosum* L.) responses to herbivory (Colorado potato beetle) and mechanical wounding (Levak et al., 2025). We first followed the spatiotemporal response of jasmonic acid (JA) signalling, known to be involved in the response. We generated two sensor potato lines, one detecting degradation of JAZ transcriptional repressors (harbouring AtJas9-Venus-N7 construct; Larrieu et al., 2015)) and one detecting JA-dependent gene expression, namely by following expression of cysteine protease inhibitor multicystatin (harbouring *StMC::EYFP* construct; Lukan, 2018). Both of these two steps of JA-dependent response are known to be involved in defence against insect pests. Sensors' fluorescence was followed with confocal microscopy in nondetached potato leaves in response to herbivore and mechanical wounding. Obtained results showed that JA-dependent gene expression was absent in cells immediately adjacent to the wound, whereas JAZ degradation spread continuously outward from the wound site. This pattern occurred after both, herbivore attack and mechanical injury. To investigate this dichotomy, we measured salicylic acid (SA, Huang et al., 2006; Defraia et al., 2008) with high spatial resolution, which showed that SA predominantly accumulates at the wound edge. The role of SA in suppressing JA-dependent gene expression was further confirmed by following JA-dependent gene expression in plants with compromised SA accumulation, in which the *StMC::EYFP* sensor was active confluent from the wound edge. These results indicate that JA-SA crosstalk is involved in the dynamics of the response to herbivore as well as to mechanical wounding. Interestingly, analysis of tissue sections around the wound showed that transcription of redox signalling-related genes TRX and RBOHD showed the same spatial regulation as multicystatin.

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Microbes as plant's friends

From emerging threats to biological solutions: Rethinking the role of fungi in plant health

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Fungi play diverse and often contrasting roles ranging from destructive pathogens to beneficial partners and asymptomatic plant associates, and this presentation explores how these interactions collectively influence plant health.

Fungal pathogens are among the most important constraints to agricultural and forest productivity, causing substantial yield losses, reducing crop quality, and threatening ecosystem stability worldwide. The emergence of new fungal diseases has become a growing concern, driven by intensified global trade, changing climatic conditions, and the increasing movement of plant material. Slovenia reflects these broader trends, with recent reports of previously unrecorded fungal pathogens affecting various plant hosts. Accurate detection and identification of fungal species is clearly essential for effective disease prevention and management. At the same time, decades of intensive fungicide use have selected for fungicide-tolerant or resistant pathogen populations, significantly reducing the effectiveness of chemical control measures. These challenges are further amplified by current European Union policies aimed at reducing pesticide use and promoting more sustainable agricultural practices, increasing the need for alternative disease-management strategies that rely on alternative solutions for disease control.

Biological control is a promising alternative to conventional disease management by exploiting naturally occurring antagonistic microorganisms that suppress plant pathogens and contribute to plant health. In this context, antagonistic and beneficial fungi have attracted considerable attention as effective and sustainable biocontrol agents, providing multiple mechanisms for disease suppression. The ubiquitous yeast-like fungus *Aureobasidium pullulans* serves as an important model organism for studying beneficial plant-associated fungi. However, many other species of fungi are being intensively studied for their efficacy against numerous plant pathogens and offer innovative fungicide-free management strategies.

Future plant pathology will increasingly move beyond a pathogen-centred perspective towards broader plant health framework. Integrating pathogen surveillance, fungal ecophysiology studies, plant microbiome analysis, and biological control strategies will enable more resilient and sustainable plant protection systems. Understanding fungi as both threats and allies is essential for developing next-generation approaches to plant health management in a rapidly changing world.

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Understanding harmful and beneficial plant interactions through the study of harmful Potato virus Y and beneficial endophytic *Fusarium oxysporum* isolate

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Sustainable agriculture seeks to develop environmentally friendly strategies that ensure effective and long-term plant protection. Achieving this goal requires a thorough understanding of the interactions between plants and microorganisms. While considerable attention has been devoted to plant–pathogen interactions, it is equally important to investigate beneficial microorganisms, which can promote plant growth, enhance stress tolerance, and contribute to natural disease suppression.

To better understand interactions that negatively affect plant health, we examined the movement dynamics of Potato virus Y (PVY), one of the world's most economically important plant viruses. PVY is responsible for substantial yield losses in potato and other members of the Solanaceae family. Understanding the mechanisms of Potato virus Y (PVY) spread is therefore essential for developing effective disease control strategies. A key factor is the N-terminal region of the coat protein (CP), which plays an important role in virus particle assembly as well as local and systemic movement within plants. To identify CP regions involved in PVY spread, we constructed and analyzed a series of N-terminal CP mutants in a GFP-tagged infectious clone of PVY N605(123). The mutants were introduced into *Nicotiana clevelandii* plants by microbombardment, and viral movement was monitored using confocal microscopy and whole-plant imaging. Deletions of more than 40 amino acids from the CP N-terminus completely abolished cell-to-cell movement, while quantitative PCR confirmed that viral replication remained restricted to individual bombarded cells. Deletion of 26 amino acids delayed intercellular movement and prevented systemic infection, whereas deletions of 19–23 amino acids resulted in delayed systemic spread. In addition, site-directed mutations within a critical region identified specific amino acids required for efficient viral movement.

On the other hand, to explore potentially beneficial plant interactions, we characterized our novel endophytic *Fusarium oxysporum* isolate that was isolated from our potato plants grown in sterile conditions of tissue culture. After sequencing and assembly of the genome, comparative genomic analyses were used to compare our isolate with other pathogenic and non-pathogenic (endophytic) *F. oxysporum* strains. Gene Ontology overrepresentation analysis revealed some uniquely enriched processes in the novel endophytic isolate. Since our isolate originates from potato, we first evaluated its abundance in potato tissue cultures. To further characterize our isolate, a protocol for efficient plant inoculation using defined concentrations of *F. oxysporum* microconidia was established. Inoculation assays demonstrated disease-suppressive activity of our *F. oxysporum* endophytic isolate in tomato and bean.

In our study, we adopted a holistic approach to investigate both detrimental and potentially beneficial interactions for the plant. The results of our study provide important insights into the regions of the Potato virus Y (PVY) coat protein (CP) that are involved in viral movement. Understanding the role of these regions is essential for developing new strategies to prevent and control viral infections. Furthermore, our findings provide an integrative genomic, experimental, and ecological perspective on endophytic *F. oxysporum*–plant interactions and highlight the potential of beneficial endophytic strains as tools for sustainable disease management.

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Visualizing fast calcium response during beneficial microbe interactions in potato

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Plants perceive microbial signals and activate appropriate immune responses through complex signalling network. Beneficial bacteria, such as *Bacillus subtilis* and endophytic *Paenibacillus sp.*, have been shown to enhance plant resistance to pathogens including potato virus Y (Kalatskaja et al. 2023), although the spatial and temporal dynamics of the underlying signalling processes remain poorly understood. Our main objective is to characterize calcium-based signalling patterns induced by beneficial microbes and local tissue damage in potato plants.

To address this, we used transgenic potato lines expressing genetically encoded fluorescent sensor for cytosolic calcium, GCaMP3. Immune responses are monitored in intact leaves under controlled biotic stress conditions. Bacteria are applied locally to the leaf tissues in a droplet or combined with mechanical wounding to simulate natural stress scenarios. Real-time imaging is performed using live confocal microscopy, including a long working distance objective that enables minimally invasive monitoring of intracellular calcium dynamics in intact plant tissues. Imaging protocols are optimized to improve signal stability during extended time-lapse acquisition. Spatial signal propagation and temporal response characteristics are analysed at the cellular level.

Preliminary results indicate that both beneficial bacterial application and local leaf wounding trigger rapid and spatially structured calcium transients in potato tissues. Wounding causes distinct two-way calcium signal which was already described in *Arabidopsis thaliana* (Zhang et al. 2025). We also observe enhanced signal amplitudes in bacteria treatment, suggesting bacteria-specific modulation of immune signalling. Initial experiments suggest that beneficial bacteria may influence the pattern of calcium responses associated with stress perception.

The distinct calcium transient patterns observed between bacterial treatment and mechanical wounding suggest that potato plants can discriminate between different stress stimuli at the level of calcium signalling. The differences in spatial propagation and response dynamics point to a bacteria-specific modulation of early immune signalling, consistent with the concept of a calcium signalling code involved in beneficial-mediated immune priming (Bhar et al. 2023). Although the results are still preliminary, the observed patterns support the idea that beneficial bacteria actively shape the plant's calcium-based stress perception, rather than simply triggering a generic wound response. Future work will focus on quantitative characterisation of these signal signatures and their correlation with downstream hormonal and defence gene responses, with the broader aim of contributing to sustainable crop protection strategies.

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Drivers of arbuscular mycorrhizal communities in plant roots: from community composition to specific taxa

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Fertile soil is a limited resource that takes thousands of years to form. Potentially toxic metals (heavy metals) pose significant health risks due to their persistent presence in the environment. Soil washing with EDTA (ethylenediaminetetraacetic acid) can remove metals such as Pb, Zn, and Cd, but it may also disrupt soil microbial diversity. In this study we used 18S rRNA sequencing to examine the development of arbuscular mycorrhizal (AM) fungal communities in the roots of *Lolium perenne* following EDTA remediation. Plants were grown in calcareous and acidic soils, both remediated and unremediated, with or without a local inoculant (grassland soil and roots). The inoculant increased the richness and evenness of AM fungal communities while reducing compositional dissimilarity between soil types. The remediation process and inoculation altered the dominant fungal taxa, indicating mechanisms such as priority effects and competitive exclusion. These results highlight the importance of nature-based solutions, including indigenous inoculants and AM fungi, for sustainable soil management, particularly in restoring biodiversity in areas affected by contamination and various disturbances, including conventional agriculture and urbanisation.

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Application of chitosan and cyanobacterial biomass in agriculture for improving cereal crop productivity

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The growing demand for sustainable agricultural practices has encouraged the integration of agronomic management practices with innovative approaches. Among these, chitosan-based materials, including chitosan nanoparticles, and cyanobacterial biomass have emerged as promising tools for enhancing nutrient use efficiency, improving soil fertility, and increasing crop productivity (Molnár et al. 2023). Chitosan (nano)particles are recognized for their ability to facilitate nutrient delivery and stimulate plant metabolic processes, while cyanobacteria contribute to agricultural sustainability through biological nitrogen fixation and the production of bioactive compounds that promote plant growth. Despite the documented benefits of these technologies when applied individually, their combined use in cereal production systems remains insufficiently investigated.

This study evaluated the effects of chitosan (nano)particles and cyanobacterial biomass, applied separately and in combination, under both recommended and reduced nitrogen fertilization regimes in field-grown cereal crops, including maize, winter wheat, and winter barley. The objective was to determine whether the integration of these bio-based inputs could enhance crop performance while reducing dependence on mineral nitrogen fertilizers.

Field experiments demonstrated that treatments involving chitosan (nano)particles and cyanobacterial biomass significantly improved crop productivity compared with conventional fertilization practices. The combined application resulted in notable increases in grain yield, chlorophyll concentration, and several key growth parameters associated with plant vigor and development. The observed improvements in physiological performance indicated improved nutrient utilization and overall plant health, contributing to more efficient crop growth under field conditions. Furthermore, positive responses were observed even under reduced nitrogen inputs, highlighting the potential of these biostimulant-based strategies to maintain productivity while lowering fertilizer requirements.

The findings suggest that integrating chitosan-based materials with cyanobacterial biomass offers a synergistic approach for improving cereal crop performance and promoting sustainable nutrient management. This strategy could extend the effectiveness of conventional fertilizers, mitigate some of their environmental limitations, and support resilient agricultural production. Overall, the study demonstrates the potential of multidisciplinary approaches to enhance crop productivity while advancing the development of environmentally sustainable and resource-efficient farming systems.

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New methods in studying plants

Unraveling potato immune signaling in response to potato virus Y using spatially resolved small RNA and transcriptomic profiling

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Hypersensitive response (HR) is an effector-triggered immune response that leads to pathogen restriction and localized cell death. Although HR was described more than a century ago, the mechanistic basis of viral arrest remains incompletely understood. While transcriptional regulation of HR has been studied to some extent, the contribution of post-transcriptional small RNA (sRNA)-mediated processes remains largely unexplored. Because bulk analyses may obscure spatially restricted regulatory events, we applied a spatially resolved sRNAomics approach to uncover changes at the sRNA level and the dynamics of sRNA-gene regulatory networks in potato HR to potato virus Y (PVY). We show that miR160 is repressed in cells adjacent to HR lesions in the resistant cultivar, but not in PVY-sensitive, salicylic acid (SA)-depleted plants. Beyond its canonical regulation of *StARF10* and *StARF17*, miR160 controls a broader regulatory network spanning auxin signaling, cell wall remodeling, redox homeostasis, and tuberization. Elevated miR160 levels promote susceptibility-associated traits, reduce thermotolerance, and suppress genes strongly activated during HR. Mechanistically, we identify a novel miR160-*StARF10/StARF17-StPRX28* module that links miR160 repression to the induction of a class III apoplastic peroxidase during HR, with SA acting upstream. Our work identifies miR160 as a signaling hub linking SA, auxin, heat-shock, and reactive oxygen species pathways, thereby modulating growth and responses to viral infection and heat stress. Its dual role as a negative regulator of HR-associated genes and thermotolerance positions miR160 as a promising target for precision modulation to enhance multi-stress tolerance with minimal yield loss.

To further resolve the cellular basis of potato HR to PVY, we have also performed single-cell and spatial transcriptomic analyses at multiple time points after infection. These complementary approaches enable analysis of transcriptional responses in individual cell populations in relation to their spatial position relative to cell-death zones and their temporal dynamics during HR. Recently obtained results from these analyses will also be presented.

Integrating chromatin accessibility and transcriptomics to dissect potato responses to climate stress

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Improving potato resilience and yield under climate change requires understanding not only which genes respond to stress, but also how those responses are regulated. Within the ADAPT project, we conducted a multi-stress experiment in two potato cultivars, Desiree and Lady Rosetta, exposed to heat, drought, and waterlogging individually and in combination. Plants were profiled across multiple omics layers, including phenomics, transcriptomics, chromatin accessibility, proteomics, and hormone measurements. Here, we focus on the regulatory mechanisms linking stress perception to gene expression.

We generated genome-wide maps of chromatin accessibility in potato leaves across all stress treatments and controls, identifying regulatory regions and testing for stress-induced changes in accessibility. By integrating these data with matched transcriptomes, transcription factor motif analysis, footprinting, and co-expression networks, we identified candidate regulators and their likely target genes.

Stress altered chromatin accessibility across the genome, revealing both shared and stress-specific regulatory responses. Combined stresses triggered distinct responses that could not be explained by individual stresses alone. Different stress conditions engaged different sets of transcription factors, which were linked to known stress-response pathways. While the two cultivars shared a core response, the largest cultivar-specific differences appeared under drought and combined heat and drought. We also identified accessible but transcriptionally inactive loci that may represent stress-priming or memory sites.

These results provide insight into stress-responsive gene regulation in potato and generate a prioritized set of candidate regulators, target genes, and putative priming loci for future functional studies and breeding of more climate-resilient cultivars.

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Subcellular localisation of SAP11-like and SAP54-like effector proteins from '*Candidatus* Phytoplasma solani'

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Phytoplasmas are obligate intracellular pathogenic bacteria that inhabit the phloem of their plant hosts. Their parasitic lifestyle led to the loss of numerous genes involved in various metabolic processes, resulting in their small genomes. Phytoplasma effector proteins are a group of small secreted proteins and are an exception to this rule, as they show remarkable diversity. This diversity arises because phytoplasmas use effector proteins to interact with plant hosts in ways that increase their own fitness while evading detection by the plant immune system. Currently, the most studied phytoplasma effectors are the SAP originally discovered in aster yellows phytoplasma ('*Candidatus* Phytoplasma asteris', AY). SAP11 from AY contains a nuclear localisation signal and destabilizes members of the class II transcription factor family TEOSINTE BRANCHED 1, CYCLOIDEA AND PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR 1 (TCP). This results in dysregulated plant development and various witches' broom symptoms characteristic of phytoplasma infections. It also disrupts plant immune signalling by interfering with jasmonic acid signalling. SAP54 proteins, on the other hand, degrade MADS box transcription factors by binding to them and translocating them to the proteasome. Homologous genes have since been detected in various other phytoplasma strains. While they exhibit a similar mode of action, they have been shown to interact with different members of the respective transcription factor families. These specific interactions depend on both the host plant and phytoplasma species, explaining the symptom diversity observed in phytoplasma infections. SAP11-like and SAP54-like genes have been found in the genome of the phytoplasma '*Ca. P. solani*', which is causing devastation in many crop species, including tomato, potato, sugar beet and grapevine. SAP11-like from '*Ca. P. solani*' has already been demonstrated to interact with two TCPs and cause distinct developmental changes in transgenic Arabidopsis. Here, we report the preliminary results of subcellular localisation of SAP11-like and SAP54-like identified in '*Ca. P. solani*'. The gene sequences were codon optimised for *Nicotiana tabacum* and cloned into a vector containing an N-terminal yellow fluorescent protein tag. They were transiently expressed in *Nicotiana benthamiana* leaves by agroinfiltration and observed under a confocal microscope. Both proteins were found in the cytoplasm and the nucleus.

How to look closer at plants?

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Plants are beautiful, and when you look at them up close under a microscope, they are truly fascinating. Fresh plant tissue sections usually provide enough contrast to be observed with bright field microscopy. For detection of specific compounds, we can use a vast collection of histological staining and molecular localization protocols. Microscopic images can be used as research data or as educational material. As such, images need to include at least a scaling bar, indicating the size of structures shown in the image. We have developed a Fiji plugin to apply an appropriately sized scaling bar onto a spatially calibrated image (<https://github.com/aleskl/imagej>). Samples are sometimes too large to fit within a single field of view of the camera. In this case we can stitch a grid of overlapping images into a large panorama overview. When stitching images, we need to ensure that we use the correction of shading, an artifact in light microscopy when image brightness is reduced towards the image edges. Plant samples can also be observed using an epi-fluorescence microscope. Plant cells, especially cell walls, have strong autofluorescence, without the addition of external fluorochromes. We have used the changes in autofluorescence spectra under alkaline pH to characterize the secondary metabolites in the cell walls of the transport region of the maize kernel. Autofluorescence can also be used for the 3D reconstruction of the sample surface using structure-illuminated microscopy (SIM). However, the autofluorescence can pose a challenge in detecting signal from fluorochrome labelled probes, so the type of the fluorochrome label should be carefully selected not to overlap with intrinsic fluorescence of the examined sample. Molecular localization methods that we used in our research are immunolocalization and *in situ* hybridization. To avoid the signal confusion in fluorescence we prefer to use enzymatic reactions to detect the sites of antibody or nucleic acid probes binding, mainly using alkaline peroxidase labelled secondary antibodies and NBT/BCIP substrate. Images can also be used to extract quantitative data from our samples. Objects in an image can be measured manually using measurement tools in an image analysis software or they can be detected and measured automatically using machine vision methods. We used YOLO object detection to automatically measure the dimensions of stomata in the leaves of buckwheat genotypes with different ploidy levels. However, automatic object detection methods should be accompanied by manual measurements in a subset of samples to validate the accuracy of automatic measurements. An important method in our laboratory is the measurement of genome size using image densitometry. The relative amount of nuclear DNA is measured as optical density of Feulgen-stained nuclei. Using this method, we can compare genome size in species differing in ploidy level. We can also measure the absolute genome size if we measure optical density also in a standard species with known genome size. The most exiting research topic regarding genome size is measurement of somatic endopolyploidy. During differentiation, plant cells often switch to an endoreplication cell cycle, during which the DNA doubles, but the cells and nuclei do not divide, resulting in highly polyploid cells. Cells with multiple genome copies can grow larger, which is beneficial for example in storage tissues. We have measured genome size and cell size in seed storage tissue-the endosperm- of maize and related species. Cell size was positively correlated with genome size, but interestingly in direct correlation with cell surface, not volume.

Mineral element atlas of six underutilised dicot seeds revealed by high-content ion microscopy

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Mineral element deficiencies persist globally due to nutrient-poor staple crops. Diversification and/or biofortification of staple crops are potential strategies to alleviate this hidden hunger. However, the lack of tissue-specific elemental data hinders the understanding of element allocation in seeds and their within-tissue interactions. Here, we present a high-content tissue-specific element atlas of seeds from six underutilised dicot crops (caraway, chia, flax, poppy, quinoa, rapeseed). In this workflow (Figure 1), by coupling autofluorescence microscopy for seed tissue identification with quantitative and multielement ion microscopy, we uncovered distinct element allocation patterns: zinc predominates in the flax embryo, calcium concentrates in the testa of poppy and chia, chlorine, iron and manganese allocate to testa of quinoa, phosphorus and sulphur accumulate in embryo of rapeseed, and calcium, potassium and iron in testa of caraway. Unlike cereal grains, where most elements are confined to the aleurone and scutellum, dicot seeds show predominant elemental allocation to the embryo and endosperm, except calcium, which predominates in the testa. We further demonstrate a consistent positive correlation between iron and zinc in all seed tissues, suggesting that biofortification of dicots could simultaneously address multiple deficiencies. Our findings provide the first stepping stone towards informed dietary diversification strategies and biofortification opportunities for dicot crops.

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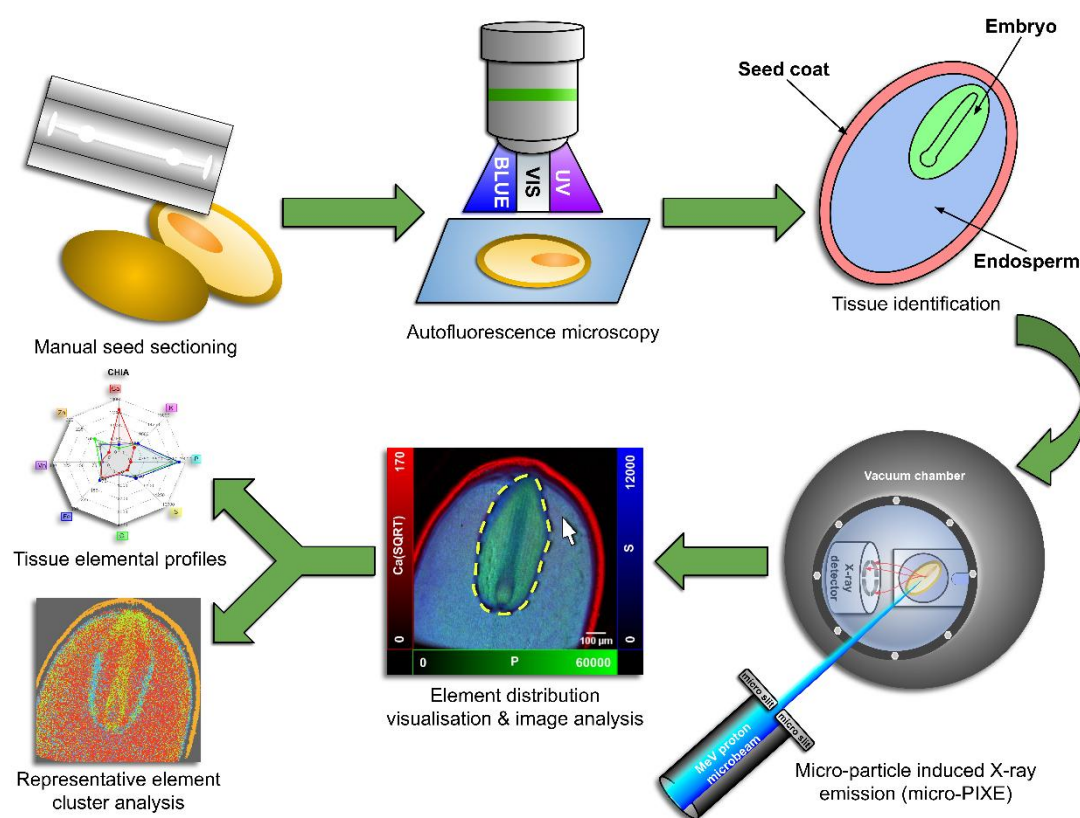


Figure 1: Workflow overview with key methods using chia seed as an example.

Plants are not alone: Ecology and conservation

Beyond the plant: rethinking plant conservation through ecological interactions

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For decades, plant conservation has focused on individual species, often overlooking the ecological interactions that determine their long-term persistence. Yet plants do not exist in isolation. Their survival depends on complex networks involving pollinators, seed dispersers, herbivores, microorganisms, neighbouring plants and people. Increasing evidence suggests that effective conservation requires moving beyond species-centred approaches towards understanding the ecological and social systems that sustain plant populations.

Plant-pollinator interactions provide one of the clearest examples of this complexity. Studies on *Salvia brachyodon*, a narrow endemic species from the Adriatic coast, demonstrated that limited pollination success may represent an important constraint on reproductive output, illustrating that conserving a threatened plant may also require conserving its ecological community. However, pollination represents only one component of a much broader conservation picture. Research on the endangered endemic *Moehringia tommasinii* revealed that multiple interacting pressures besides pollination, including human disturbance caused by recreational climbing or hybridisation with a closely related species, collectively influence the viability of a population. Such examples highlight that conservation challenges rarely arise from a single threat but rather from the cumulative effects of several interacting drivers. Addressing only one of these factors is therefore unlikely to ensure the long-term persistence of threatened species. Human-plant interactions, however, extend beyond threats. While harvesting can endanger vulnerable species, ethnobotanical research demonstrates that local ecological knowledge and sustainable use can also strengthen conservation. Increasingly, people are recognised not only as drivers of biodiversity loss but also as key partners in safeguarding plant diversity.

Recent scientific advances further expand this perspective by revealing previously overlooked biological interactions. It has, for example, been revealed that fungi and bacteria present in nectar (nectar microbiome) can shape plant-pollinator relationships by modifying nectar sugar concentrations, reducing its pH, reducing the concentration of toxic metabolites, etc. Such findings reinforce an important message: every newly discovered interaction adds another layer of complexity that was previously unknown.

Future conservation strategies should therefore aim not only to protect single threatened plant species but to preserve the ecological processes and interaction networks that sustain them. Conserving pollinators, maintaining habitat connectivity, understanding species interactions and engaging local communities should be viewed as complementary rather than separate conservation actions. Ultimately, successful plant conservation is no longer simply about protecting plants: it is about conserving the dynamic ecological and social networks of which they are an integral part.

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Evolutionary co-adaptation and functional architecture of the *Noccaea praecox* root-associated microbiome

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Cadmium and zinc hyperaccumulating *Noccaea* (formerly *Thlaspi*) *praecox* thrives in metalliferous and non-metalliferous soils in Slovenia (Likar et al. 2010). Like other plants, hyperaccumulators are complex holobionts: they do not function in isolation; rather, they actively recruit and influence their root-associated microbiome (Abedini et al. 2021). While the mechanisms of uptake, translocation and accumulation of metals in hyperaccumulating plants have been extensively studied, little is known about the composition and functionality of their microbiomes, especially when grown in the field.

Therefore, the entire *N. praecox* plants with the rhizosphere soil still attached were collected in a non-metalliferous site in Lokovec (Slovenia) and at the metalliferous site in Žerjav (Slovenia). Total genomic DNA was extracted from the rhizosphere using the DNeasy® PowerSoil® Pro Kit (Qiagen) and from roots using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma Aldrich). Shotgun metagenomic sequencing was conducted with the Illumina HiSeqX platform (2 × 150 pair-ends), using TruSeq DNA kit (Illumina).

The abundance of bacterial phyla was dominated by Actinomycetota (formerly Actinobacteria) and Pseudomonadota (formerly Proteobacteria), regardless of the sampling site and root-associated compartment. Interestingly, bacterial phyla were more homogeneous than fungal classes across both root-associated compartments. While most fungal and bacterial taxa were found on both sampling sites, the metalliferous site comprised more unique taxa than the non-metalliferous site. The co-occurrence network revealed that most bacterial phyla form positive interactions within the bacterial community, except for *Firmicutes*, *Gemmatimonadetes* and *Bacteroidetes*. The compartment was the primary driver of the overall microbial functional profile, whereas microbial functions related to metal tolerance and resistance (i.e., the resistome) were driven by both soil metal concentrations and the root-associated compartment. Additionally, in the resistome, we found several microbial functions enriched at the metalliferous compared to the non-metalliferous site.

Together, our results demonstrate that both the environment and plant host play interactive roles in shaping the root-associated microbiome. The observed findings provide new insight into the evolutionary co-adaptation between the plant host and its microbiome, revealing how specific selective pressures shape the taxonomical and functional architecture of the root-associated microbiome to actively mitigate extreme metal toxicity.

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Water extracts from cereal weed corn cockle (*Agrostemma githago*) showed plant growth regulator efficiency

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Corn cockle (*Agrostemma githago* L. (Lychnis Githago (L.) Scop.)) was once a common weed in cereal fields in the hinterland and southern Europe, but today it is less prevalent due to the use of herbicides and mechanised agriculture. Corn cockle is the main ingredient in some plant preparations for biostimulation in agriculture and elicits many positive responses (Ambrožič-Dolinšek et al., 2021). The logical next step was to investigate whether the plant source itself also exhibits plant growth regulator, cytokinin- or/and auxin-like activity at different growth stages.

The Cucumis and Triticum bioassays were used to determine cytokinin-like activity, and the Cucumis and Mung bean bioassays were used to determine auxin-like plant growth regulators (PGRs) activities. Water extracts of seeds, as well as fresh and dry plant material at different developmental stages, showed auxin-like activity in the Cucumis cotyledon root and Mung bean bioassays (Ambrožič-Dolinšek et al., 2025). We have shown that fresh and dry plant material of *A. githago* also contains auxin- and cytokinin-like PGRs. The activity in the Cucumis bioassay corresponded to 0.5 to 2 mg L⁻¹ IBA and in the Mung bean bioassay corresponded to 0.5 to 4 mg L⁻¹ IBA. While the Cucumis cotyledon expansion bioassay showed weak or no cytokinin-like activity, the Triticum bioassay showed activity of about 0.5 to 1 mg L⁻¹ BAP, which is comparable to synthetic analogues. LC-MS analysis confirmed its presence.

By confirming that *Agrostemma githago* dry and fresh plant material is a source of natural auxins, it can potentially be used in organic and sustainable agriculture for root induction as an innovative strategy to improve plant growth, development, and other quality traits of various plant species, with the potential to enhance productivity and sustainability.

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Mulberry germplasm diversity as a foundation for european sericulture, biodiversity conservation and cultural landscapes

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Mulberry (*Morus* sp.) trees have played a central role in the history of European sericulture, while also shaping rural landscapes, local biodiversity, and cultural heritage. However, many old mulberry trees are now threatened by land-use change, abandonment of sericulture, and insufficient documentation of genetic resources. Within the ARACNE project (<https://aracneproject.eu/>), a comprehensive study was conducted to identify, characterise, and preserve mulberry trees across European sericultural regions and germplasm collections. The study included accessions maintained by CREA (Padua, Italy), the Scientific Centre of Sericulture in Vratsa (Bulgaria), IMIDA in Murcia (Spain), and the University of Maribor (Slovenia), together with field-recorded old local mulberry trees from historically important sericultural regions. From 2023 to 2025, mulberry accessions were systematically described using morphological, phenological, and reproductive descriptors. This three-year systematic documentation underpins the Catalogue of Mulberry varieties (<https://aracneproject.eu/catalogue-of-the-mulberry-varieties/>) and helps maximise the use of these resources by supporting the identification of high-yielding morphotypes.

In parallel, a large-scale inventory of historical trees was carried out using MorusAPP (<https://morusapp.aracneproject.eu/>). The application enabled researchers, schools, and citizens to record tree locations and morphological traits. This approach contributed to the development of a comprehensive database and the Mulberry Story Map (<https://aracneproject.eu/aracne-mulberry-storymap/>), which presents old mulberry trees as living monuments of European cultural landscapes. To strengthen the reliability of cultivar identification, genetic analyses based on single nucleotide polymorphism markers, were performed that helped to clarify relationships among accessions, reduce redundancy within germplasm collections, and distinguish major genetic groups. The results provided insights into the historical dissemination of mulberry from Asia to Western Europe and support the reconstruction of cultural pathways linked to sericulture.

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Posters

Microbiome of liverworts: a case study of the complex thalloid liverwort *Mannia triandra* (Aytoniaceae) using shotgun metagenomics

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Microbial associations with liverworts present important avenues for studying ecological aspects of land plant evolution and their interactions with microorganisms. In this study, we investigated the taxonomic and functional structure of microbial communities associated with the protected liverwort *Mannia triandra* (Scop.) Grolle and its edaphic microhabitat across four geographically distinct sites. Using shotgun metagenomic sequencing, we compared plant-associated and bulk soil microbiomes to assess community composition, functional potential, and drivers of community assembly.

Both read- and assembly-based approaches revealed highly diverse microbial communities dominated by *Actinomycetota* and *Pseudomonadota*, with several lineages also classified as *Acidobacteriota*, *Chloroflexota*, *Cyanobacteriota*, *Myxococcota*, *Verrucomicrobiota* and other soil-dominant taxa. Across sites, microbial communities were generally similar in diversity but exhibited clear geographic structuring, indicating a strong influence of local environmental conditions. In contrast, differences between plant-associated and bulk soil compartments were less pronounced. Nevertheless, differential abundance analyses identified a subset of taxa significantly enriched in the *M. triandra*-associated microbiome, including several plant growth-promoting rhizobacteria in the *Alpha*- and *Gammaproteobacteria* classes. Consequently, despite overall functional stability across metagenomes, plant-associated accessions showed enrichment of specific genes related to nutrient acquisition, carbon utilisation, nitrogen and sulfur metabolism. These patterns suggest enhanced microbial adaptation to plant-derived resources within the rhizosphere-like environment. With the use of scanning electron microscopy, we provide visual details of microbial assemblages on the rhizoid surfaces.

Altogether, our results provide evidence that *M. triandra* supports a functionally distinct rhizosphere-like microbial compartment, whereas the overall microbial community structure is primarily shaped by site-specific environmental factors. This study contributes to a growing understanding of bryophyte microbiomes and gives suggestions for further work.

Uncovering metabolic signatures of plant–pathogen interactions

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Secondary metabolites are important components of plant defense, but their responses to different pathogen groups remain difficult to compare across studies because of substantial biological and methodological heterogeneity. This study used association rule mining (ARM) to identify interpretable co-occurrence patterns linking metabolite class and the direction or magnitude of concentration change with pathogen group.

The analysis included 241 observations from 25 peer-reviewed studies, representing 18 plant species and 20 pathogen species. Pathogen group, metabolite class, metabolite percentage-change, and sampled tissue were converted into binary variables. Association rules were generated using the Apriori algorithm, with each rule predicting one pathogen group. Other pathogen variables, absence indicators, and tissue information were excluded to avoid obvious associations caused by how the data were organized. Rules were filtered for minimum co-occurrence, pathogen-specific support and confidence, and statistical redundancy. Strength of association was evaluated using confidence, representing the probability of a pathogen group given a metabolite pattern, and lift, representing enrichment relative to the pathogen's baseline prevalence. Fisher's exact test and odds ratios were calculated, with Benjamini-Hochberg correction.

Phenolic compounds showed the clearest pathogen-specific patterns. Phenolic increases of at least 100% were strongly associated with nematodes (confidence = 0.857; lift = 3.501; $q = 3.2 \times 10^{-12}$), whereas phenolic decreases of at least 20% were associated with oomycetes (confidence = 0.714; lift = 3.310; $q = 1.16 \times 10^{-6}$). Terpenes were linked to bacterial infections in leaves (confidence = 0.727; lift = 3.273) and roots (confidence = 1.000; lift = 11.000), although the root result was based on only six observations. Nitrogen-containing compounds were strongly associated with fungal infections in leaves (confidence = 1.000; lift = 3.375). Virus and fungi data were too limited for reliable conclusions.

ARM revealed some distinct, interpretable metabolite-pathogen co-occurrence patterns within a heterogeneous literature dataset. The direction of phenolic change differentiated nematode from oomycete-associated observations, whereas terpene and nitrogen-containing compound patterns were tissue dependent. These results provide testable hypotheses for pathogen-associated metabolic biomarkers. However, the rules describe statistical associations rather than causal mechanisms and should be validated using larger, more standardised datasets.

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Antagonism as a powerful tool of a less virulent pathogen in apple fruit colonization

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Apple fruit are susceptible to numerous fungal pathogens that differ markedly in virulence and ecological strategies. Species of *Monilinia* are economically important and widespread causal agents of brown rot, whereas *Lambertella corni-maris* has been reported as the causal agent of yellow rot only in certain regions (Amiri et al., 2017). Both are present in Serbia, and previous studies have shown that they differ in virulence on fruit of different apple cultivars, with *L. corni-maris* being less virulent than *Monilinia* spp. (Vasić et al., 2018, 2022). This raised the question of whether the lower virulence of *L. corni-maris* is compensated by strong antagonistic activity that facilitates colonization of apple fruit previously occupied by *Monilinia* spp. Interactions between two *L. corni-maris* isolates (L1 and L2) and four *Monilinia* species (*M. fructigena*, *M. polystroma*, *M. laxa*, and *M. fructicola*) were evaluated using dual-culture assays on potato dextrose agar (PDA), scanning electron microscopy (SEM), and co-inoculation experiments on apple fruit slices (cv. Idared). Colony growth inhibition was determined after seven days. After three weeks, pigmentation responses in *Monilinia* colonies were assessed, while SEM was used to examine hyphal interactions. Colonization success on apple slices was evaluated by re-isolation of fungi after four weeks. Both *L. corni-maris* isolates significantly inhibited the growth of all four *Monilinia* species *in vitro*. Growth inhibition ranged from 37.3–52.5%, with the strongest inhibition observed against *M. fructicola*. No significant differences in overall antagonistic activity were detected between isolates after seven days. However, after three weeks, isolate L1 induced broader darkly pigmented zones in *Monilinia* colonies than isolate L2, indicating stronger long-term antagonistic effects. SEM observations revealed severe hyphal damage of *M. polystroma*, including cell wall lysis and hyphal collapse, confirming antibiosis-related interactions. Interestingly, *M. fructigena* and *M. laxa* exhibited co-antagonism only against isolate L2. Despite these differences *in vitro*, both *L. corni-maris* isolates completely replaced all four *Monilinia* species on apple fruit slices. After four weeks, only *L. corni-maris* was successfully re-isolated from co-inoculated fruit, whereas *Monilinia* species were recovered only from control treatments. This study demonstrates that *L. corni-maris*, despite its relatively low virulence, possesses remarkable antagonistic capacity against *Monilinia* spp. Complete replacement of *Monilinia* from apple tissues indicates that antagonism is an effective ecological strategy enabling successful host colonization. These findings highlight that ecological fitness among fruit pathogens depends not only on virulence but also on the ability to suppress competing microorganisms. Such competitive interactions may play an important role in pathogen succession and disease development in apple fruit.

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Influence of hydrogen peroxide and peroxyacetic acid application on total phenolics, anthocyanin content, and antioxidants in strawberries and blueberries

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Berries, such as strawberries and blueberries, are an important part of a healthy human diet due to their high levels of bioactive compounds, particularly antioxidants (phenolic compounds, flavones, flavonoids, anthocyanins), tannins, and vitamins. These compounds contribute to antioxidant and anti-inflammatory effects, helping to reduce oxidative stress and potentially lower the risk of chronic diseases in humans (Soltaniband et al. 2022). Climate change, environmental stress, and soil degradation are increasingly threatening agricultural production systems, creating a need to apply more sustainable plant-supporting compounds. alternatives to conventional agrochemicals. In this context, various biostimulants, which are a heterogeneous group of biologically derived substances, beneficial microorganisms, or inorganic compounds with physiological activity, have some or several of the listed effects; they increase plant growth and productivity, improve stress tolerance, and provide a more environmentally friendly alternative to conventional inputs for fertilization or plant protection (Khurshid et al. 2026). In this study, hydrogen peroxide and peroxyacetic acid were applied to strawberries and blueberries, using different application methods and concentrations. Yield and fruit quality parameters, including fruit firmness, color component parameters (using Minolta colorimeter and L, a, b color definition space), and sugar (total soluble solids) content, were evaluated. Total phenolic content (Ainsworth et al. 2007), DPPH radical scavenging activity (Senekovič et al. 2025), and anthocyanin content (Lee et al. 2005) were subsequently determined using spectrophotometric methods. Some differences were observed in the chromatic colour parameters a* and b*, whereas no differences were detected in fruit lightness (L*) in either species. In strawberries, the highest values of redness (a) and yellowness (b) were recorded in hydrogen peroxide foliar treatments, indicating enhanced red and yellow coloration compared to the control. Total soluble solids (TSS) content remained unaffected by biostimulant application in both strawberries and blueberries, indicating the stability of this primary quality trait despite the treatment-induced increase in yield. Significantly higher anthocyanin and total phenolic contents, together with greater antioxidant activity, were observed in hydrogen peroxide-treated strawberries and blueberries. The effects of peroxyacetic acid treatments on these parameters were less pronounced. These findings suggest that the applied treatments may influence the phytochemical composition and antioxidant profile of the fruits.

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Phased genome assembly of potato cultivar Désirée

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Potato (*Solanum tuberosum*) is a globally important crop and a valuable model for plant responses to environmental and microbial challenges. Genomic analyses of cultivated potato have long relied on the double-monoploid (DM) *S. tuberosum* Phureja reference (Pham et al., 2020), which does not capture the heterozygous, autotetraploid gene repertoire of cultivated varieties. This limitation is particularly relevant for studies of host genotype effects on pathogen responses, beneficial microbial associations, and stress adaptation, where genes absent from the DM reference can greatly influence the responses. We present a phased (i.e. haplotype-resolved), chromosome-level genome assembly and annotation of potato cultivar Désirée (Godec et al., 2025), a cultivar that has been widely used in research. The assembly was generated using PacBio HiFi reads and Illumina Hi-C scaffolding, with Illumina paired-end data for quality assessment and Oxford Nanopore Technologies (ONT) reads for DNA methylation profiling. Gene annotation was performed independently for each haplotype using an integrated pipeline combining *de novo* prediction, transcriptome-guided annotation, deep-learning-based prediction, and reference-based transfer from the UniTato DM annotation (Zagorščak et al., 2024). The final phased assembly spans 3.3 Gbp and shows high completeness, supported by a BUSCO score of 99.4% for Solanaceae-specific orthologs. We demonstrate that a substantial part of the genome is absent in the DM potato reference genome, since 17.24% of genes annotated in Désirée are absent in the DM reference. We provide a public genome browser (<https://desiree.nib.si/>) that visualizes the phased genome sequence, gene models with functional annotations, DNA methylation, and repeat regions. We are working on the Désirée v2 assembly that will incorporate in-house produced ONT ultra-long reads to improve assembly structure and long-read transcriptomics for better prediction of gene models. We will also prepare the genome for more streamlined integration into pangenomes.

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From weed to plant fortifier. Silicon from *Equisetum arvense* improves drought tolerance during germination of Styrian oil pumpkins.

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The Styrian oil pumpkin plays an important role for Styria as a cultural asset, and all of its products - especially Styrian pumpkin seed oil - are of significant economic importance. In 2025, approximately 35,000 ha (Statistik Austria, 2026) were cultivated in Austria. As spring drought conditions intensify, the shell-less seed (Teppner, 2000) is subjected to drought stress as early as the germination stage, leading to reduced plant growth and yield losses.

According to Thakral et al. (2024), silicon can play a crucial role in alleviating both biotic and abiotic stressors in plants. The “weed” field horsetail can serve as a natural source of silicon; as a silicon hyperaccumulator, it consists of approximately 20% silicon (Greger & Landberg, 2024). A study by Salim et al. (2021) indicates that silicon applied as a foliar fertilizer has positive effects on growth, flowering, and yield in garden squash (*Cucurbita pepo* L.) under irrigated conditions. To support the plant even during the germination phase, priming the seeds with *Equisetum arvense* extract could be beneficial.

To investigate a possible effect, two germination experiments were conducted. In these experiments, the seeds of the Styrian oil pumpkin were pretreated in different ways. Two concentrations of horsetail extract were used to strengthen the seeds through priming; hydropriming and no seed pretreatment served as controls. The seeds were germinated in Petri dishes for one week; a 5% PEG 6000 solution was used as stressor, and deionized water was used for normal conditions. During germination, the seeds were monitored for growth, and at the end of the experiment, hypocotyl length, root volume, and dry matter content were measured.

Treatment with PEG resulted in a significant reduction in growth, hypocotyl length, root volume, and dry matter content. Pre-treatment of the seeds via priming resulted in improved growth. Both hydropriming and horsetail extract priming demonstrated improved growth capacity under drought stress. This trend was also observed in the measurements of hypocotyl length, root volume, and dry matter content.

In summary, silicon can play an important role in alleviating biotic and abiotic stressors, and field horsetail can serve as a natural source of it. This property can help support the Styrian oil pumpkin even under adverse conditions, such as drought stress during germination.

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Are SWEETs the stress nexus in grapevine–phytoplasma interactions?

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Bois Noir (BN), caused by *Candidatus Phytoplasma solani*, is one of the most important phytoplasma diseases affecting grapevine (*Vitis vinifera* L.), characterized by leaf yellowing in white cultivars, or reddening in red cultivars, leaf curling, irregular ripening, and berry withering, ultimately resulting in substantial reductions in vine vigor, fruit quality, and yield (Casarin et al., 2023; Pierro et al., 2024). Despite extensive research on grapevine–phytoplasma interactions, no grapevine cultivar exhibits complete resistance or tolerance to BN, with existing cultivars showing only varying levels of sensitivity. The phytoplasma–grapevine interaction involves extensive cellular, metabolic, and transcriptional reprogramming, including alterations in hormone signalling, secondary metabolism, oxidative stress responses, and carbohydrate allocation. These coordinated changes are driven by phytoplasma effector proteins that manipulate host susceptibility pathways to facilitate pathogen colonization. Among these processes, increasing evidence indicates that phytoplasmas actively reprogram host carbon metabolism through the modulation of sugar transport pathways. SWEET (Sugars Will Eventually be Exported Transporters) proteins have emerged as key susceptibility factors in numerous plant–pathogen interactions by facilitating pathogen access to host-derived sugars. They regulate carbon allocation between source and sink tissues and occupy a central position at the interface between plant metabolism and pathogen nutrient acquisition (Dermastia, 2019; Dermastia et al., 2023; Musetti, 2010). In grapevine, transcriptomic studies have reported differential expression of SWEET genes during BN infection; however, their functional role in grapevine susceptibility and disease development remains unknown (Dermastia et al., 2021). Moreover, differential gene expression alone cannot establish whether SWEET transporters actively drive disease susceptibility or simply reflect downstream physiological responses to infection.

Determining the functional role of SWEET transporters is therefore fundamental to understanding how *Ca. P. solani* exploits host sugar transport during infection and whether these transporters represent the molecular nexus linking pathogen-induced metabolic reprogramming with host susceptibility. Resolving this question could reshape current perspectives on BN pathogenesis, identify new host-directed targets for disease management, and provide the biological foundation for future functional studies and precision breeding or targeted genome-editing strategies aimed at developing grapevine cultivars with enhanced and durable tolerance to BN.

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Silicon supplementation had greater effects on barley (*Hordeum vulgare* L.) than on cucumber (*Cucumis sativus* L.) exposed to water deficit stress in hydroponics

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Silicon (Si) is a beneficial element that can help plants tolerate drought stress (Coskun et al., 2016). However, plant species differ in their ability to accumulate Si. Barley is considered a high Si accumulator, whereas cucumber accumulates lower amounts of Si (Ma and Takahashi, 2002). The aim of this study was to compare the effects of Si supplementation on physiological performance, biomass production, and mineral composition of barley and cucumber grown under simulated water-deficit conditions.

Hydroponic experiments involved cucumber (*Cucumis sativus* L.) and barley (*Hordeum vulgare* L.). Barley was exposed to four treatments (Si+, Si0, Si+PEG, and Si0PEG), while cucumber received the same treatments plus two additional controls (HR and PEG). Seedlings were grown in a nutrient solution supplemented with either 0 or 1 mM Si supplied as sodium silicate. NaCl was added to treatments without Si (Si0 and Si0PEG) to balance Na concentrations in nutrient solution of treatments Si+ and Si+PEG. Water deficit stress was induced by adding 10% polyethylene glycol (PEG-6000). At the end of the experiments, dry biomass, relative water content (RWC), potential photochemical activity of photosystem II (Fv/Fm), and concentrations of Si, K, and Mn in leaves and roots were determined. The effect of Si under water-deficit conditions in both species was evaluated by comparing Si+PEG and Si0PEG treatments.

Silicon supplementation significantly increased Si concentration in leaves of both species, while root Si concentration increased significantly only in barley. Si-treated barley had significantly higher leaf dry mass and Fv/Fm than plants without Si, which indicated improved photosynthetic performance and stress tolerance. In cucumber, leaf and root biomass, and RWC tended to increase with Si supplementation, but the differences were not statistically significant. Silicon significantly decreased K concentrations in both barley leaves and roots. In cucumber, leaf K concentration was slightly lower, while root K concentration was significantly higher in Si-treated plants. Manganese concentrations tended to decrease with Si supplementation in both leaves and roots of barley and cucumber. However, the effect was statistically significant only in cucumber roots.

The results showed different responses of barley and cucumber to Si under water deficit conditions. Barley responded to Si supplementation with higher photosynthetic efficiency, greater leaf biomass and increased Si accumulation. In cucumber, the effects of Si were mostly similar to those observed in barley, but the differences were not statistically significant. The stronger response of barley indicates that Si may be more effective in species that accumulate larger amounts of Si.

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Effects of precursor feeding and elicitation on flavonoid metabolism in ginkgo (*Ginkgo biloba* L.) callus cultures

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Biflavonoids are a relatively understudied group of plant specialized metabolites, and ginkgo (*Ginkgo biloba* L.) is among the few plant species known to accumulate them. Despite their pharmacological importance, little is known about the regulation of their biosynthesis. To investigate biflavonoid biosynthesis, a ginkgo callus culture was successfully established and maintained for more than two years. Different elicitation approaches were applied, including precursor feeding with naringenin and apigenin, exposure to high salinity and UV radiation, and assessment of biflavonoid composition under biotic stress caused by natural mold/bacterial infection. The contents of amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin were analyzed by HPLC-DAD. The results did not reveal significant changes in the composition of the analyzed biflavonoids following any of the applied treatments. However, in cultures supplemented with naringenin and apigenin, an additional chromatographic peak appeared at a retention time corresponding to biflavonoids. Since its identity could not be confirmed based solely on chromatographic and spectral characteristics, further characterization using mass spectrometry is required. Although the elicitation treatments did not result in substantial alterations in biflavonoid profiles, clear changes were observed in the composition of monomeric flavonoids, indicating that precursor feeding and stress treatments influenced flavonoid metabolism. These findings suggest that the biosynthesis of biflavonoids in ginkgo callus cultures is relatively stable and less responsive to the elicitation strategies tested, while other branches of the flavonoid biosynthetic pathway remain more susceptible to metabolic modulation.

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Effects of proline and L-Dopa seed priming on germination of wheat and radish under salt stress

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Salt stress is a major agricultural problem affecting around 20 % of irrigated land and limiting crop productivity (Kopittke et al., 2019). Salt stress induces osmotic, ion and oxidative stress resulting in reduced germination and growth. Seed priming, a simple and cost-efficient pre-sowing treatment, aims to activate metabolic and protective pathways to improve germination and stress tolerance (Rhaman, 2025). Proline, a naturally occurring amino acid, well known as an efficient osmolyte and an important part of the antioxidative system was already used as priming agent (Renzetti et al., 2025). L-dopa is an allelochemical molecule found in leaves of faba bean (*Vicia faba*) resulting in a high antioxidative capacity of these leaves (Duan et al., 2022). In this study proline and L-dopa were used to prime seeds of radish (*Raphanus sativus*) and wheat (*Triticum aestivum*) to study germination and various stress markers under salt stress conditions.

Germination experiments were performed in Petri dishes with primed (water; 15 mM proline; 15 mM L-dopa) and non-primed (control) seeds exposed to NaCl (0 mM, 100 mM, 150 mM). Germination was recorded daily and after eight days plants (leaves, roots) were sampled for analysis of proline, antioxidative capacity (DPPH) and plant pigments.

Increasing salt concentrations reduced germination and growth in both species, with radish generally being more sensitive than wheat. Salt stress typically reduces chlorophyll and carotenoid levels and increases oxidative stress markers such as proline and antioxidant activity (Munns and Tester, 2008). In both species, these expected stress responses were visible, although the magnitude differed between wheat and radish. Root and shoot length, leaf size and fresh weight decreased with increasing salinity in both species, except for a slight increase in radish shoot fresh weight at 100 mM NaCl. Priming (hydropriming, proline, L-Dopa) consistently improved early germination speed compared to the control, especially within the first two days. However, this effect diminished on the further days, and only slight and non-significant effects could be observed at the end of the experiment (day 8). Analysis of stress and vitality markers (proline, antioxidative capacity, leaf pigments) showed only modest priming effects.

Seed priming enhanced early germination under salt stress, particularly in wheat, but benefits on later growth and on stress markers were limited under the tested conditions. Proline and hydropriming tended to be more effective than L-Dopa under the tested conditions. Further studies varying concentrations and stress intensity are needed to clarify the potential of L-dopa and to optimize priming strategies for different species.

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Mulberry as a versatile tree: Transforming sericulture by-products into sericin nanoparticles for delivery of bioactive phenolics

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ARACNE project safeguarded Europe's silk heritage, preserving traditional skills while weaving them into a shared cultural identity. It focused on exploring innovative applications of mulberry (*Morus* sp.), the exclusive feed of silkworms, that is rich in phenolics with proven pharmacological properties (Urbanek Krajnc et al. 2022; Urbanek Krajnc et al. 2023). The study evaluates the incorporation of mulberry phenolic extracts into sericin-based nanoparticles, with the aim of developing functional coatings with enhanced antioxidant capacity and UV-shielding properties. Previous research has shown that sericin nanoparticles loaded with polyphenolics exhibit high encapsulation efficiency, controlled release, and strong antioxidant activity (Orlandi et al., 2020). Methanolic extracts of mulberry leaves, bark, and soroses were analysed for total and individual phenolics and DPPH antioxidant activity. Extracts were encapsulated in sericin nanoparticles by spray-drying technique and their inclusion was evaluated through phenolic profiling (HPLC-PDA-MS), while scanning electron microscopy (SEM) was employed for morphological analyses. Mulberry parts exhibited distinct phenolic profiles. Leaves were rich in caffeoylquinic acids (19.54 mg g⁻¹ DW; 52% of total phenolics), bark in stilbenoids (5.58 mg g⁻¹ DW; 47%), and soroses in anthocyanins (2.82 mg/g⁻¹ FW, 58%). Spray-drying retained up to ~83% of caffeoylquinic acid, quercetin, and kaempferol derivatives, while *p*-coumaric and caffeic acid derivatives showed lower retention. Sericin nanoparticles loaded with leaf extract achieved the highest phenolic content and antioxidant activity. Particles exhibited spherical shape. Their size is mostly governed by the concentration of encapsulating material and spray-dryer settings. In conclusion, incorporation of mulberry extracts into sericin nanoparticles resulted in high phenolic contents and antioxidant activity with greatest efficiency for leaves extracts.

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The role of antioxidant systems and photosynthetic pigments in cabbage defence against slug herbivory

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Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the most commonly produced vegetables because of its affordability, availability, and numerous health benefits, making it an important component of diets worldwide (Moreb et al. 2020). However, its cultivation is frequently limited by pests, including slugs, which are among the most destructive herbivores in vegetable production (Rowson et al. 2014). The Spanish slug (*Arion vulgaris*) and the gray field slug (*Deroceras reticulatum*) can cause substantial damage to cabbage crops, particularly in moist and temperate climates (Rowson et al. 2014). While plant responses to insect herbivory are relatively well understood, the physiological effects of molluscan feeding remain poorly studied. Slug feeding is characterized by extensive tissue abrasion, mucus deposition, and prolonged contact with plant surfaces, which may trigger distinct oxidative and signalling responses. This study investigated the effects of slug herbivory on the ascorbate–glutathione (ASC–GSH) system and photosynthetic pigments in cabbage seedlings. The ASC–GSH cycle plays a central role in maintaining cellular redox balance and detoxifying reactive oxygen species (ROS) (Foyer & Noctor 2011), while changes in photosynthetic pigments contribute to photoprotection under oxidative stress (Massacci et al. 2008). The pot experiment was established with three treatments: cabbage exposed to two *A. vulgaris* individuals, cabbage exposed to two *D. reticulatum* individuals and an untreated control. Ten days after exposure, leaves and roots were sampled for biochemical analyses. The contents of ASC, GSH, cysteine, and their oxidized forms and xanthophylls were determined by HPLC, while chlorophylls and carotenoids contents were analysed spectrophotometrically. Slug feeding induced pronounced systemic oxidative stress in both leaves and roots, reflected by reduced antioxidant pools and increased proportions of their oxidized forms. Photosynthetic pigments were also significantly affected, with decreased chlorophyll and carotenoid contents and activation of the xanthophyll cycle toward increased zeaxanthin accumulation. In most measured parameters, seedlings exposed to *D. reticulatum* showed stronger responses than those attacked by *A. vulgaris*. These findings indicate that slug herbivory triggers systemic defence-related metabolic adjustments and that the gray field slug caused more severe physiological stress on cabbage plants.

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Assessing H₂O₂ accumulation and antioxidant enzyme activities in miR160-mediated thermotolerance in potato

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Small non-coding RNAs are important regulators of plant stress responses. MicroRNA160 (miR160) was recently identified by our group as a key regulator of development and immune responses in potato (*Solanum tuberosum* L.) in response to potato virus Y infection and heat stress. Here, we investigated whether miR160 modulates thermotolerance in potato by affecting reactive oxygen species (ROS) dynamics and antioxidant enzyme activities.

We first optimized protein extraction, H₂O₂ quantification, and activity assays for major ROS-homeostasis enzymes, including peroxidases (PRX), ascorbate peroxidases (APX), catalase (CAT), and superoxide dismutase (SOD), in potato leaf tissue. We then applied these protocols to non-transgenic plants (NT; cv. Rywal), miR160-overexpressing plants, and miR160-knockdown plants (mir160b) exposed to elevated temperatures (30 °C day/28 °C night) for 10 days.

Under control conditions, mir160b plants showed slightly higher PRX activity and greater H₂O₂ accumulation than NT plants. Under heat stress, PRX activity did not differ between mir160b and NT plants, whereas H₂O₂ accumulation was reduced in mir160b plants. This reduction may be associated with higher APX activity and lower SOD activity in mir160b plants under heat stress.

In summary, even though we observed changes in H₂O₂ levels and antioxidant enzyme activities, these changes alone cannot explain miR160-mediated heat-stress tolerance. However, our optimized protocols provide a useful basis for future studies in potato.

Effect of proline seed priming on early growth of pepper seedlings under salt stress

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Seed priming is a pre-sowing treatment that partially activates seed metabolism before germination and may improve seedling establishment under abiotic stress conditions. The aim of this study was to evaluate the effect of proline seed priming on the early growth of pepper (*Capsicum annuum* L.) seedlings exposed to salt stress. Pepper seeds were subjected to proline seed priming, hydropriming or left untreated. Following priming, seeds were germinated under either control conditions or salt stress. Seedling biomass together with root and shoot length were evaluated during the early stages of seedling development. Proline seed priming showed the most pronounced positive effect under salt stress, where seedlings exhibited greater biomass and enhanced root development compared with both untreated and hydroprimed seeds. In contrast, shoot growth was only slightly influenced by the applied treatments. Under non-stress conditions, proline priming also stimulated root elongation, whereas no clear improvement in shoot growth or overall seedling biomass was observed. Overall, the results indicate that proline seed priming primarily promotes root development during early seedling establishment, particularly under saline conditions. Enhanced root growth may improve water and nutrient acquisition, thereby increasing seedling resilience to salinity stress. These preliminary findings support the potential of proline seed priming as a simple and sustainable strategy for improving the early performance of pepper seedlings under adverse environmental conditions.

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The allelopathic potential of a spreading invasive species: *Senecio inaequidens*

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South African ragwort (*Senecio inaequidens*) is among the most invasive plant species in Europe, especially in mountainous and Mediterranean regions (Bazzato et al., 2024). *S. inaequidens* was first recorded in Slovenia 35 years ago and is now present in the Primorska region, where it colonizes disturbed anthropogenic habitats (Glasnović and Fišer Pečnikar, 2010). Invasive plants can gain ecological dominance by releasing secondary compounds into the soil. These compounds may negatively affect neighboring plants, a process known as allelopathy (Hierro and Callaway 2003). This study investigated the allelopathic potential of aqueous extracts from *S. inaequidens* leaves. Radish (*Raphanus sativus*) seeds were sown on filter paper in Petri dishes and treated with 0.5%, 1%, and 2% leaf extracts; the control group received distilled water.

After 3 days, seed germination was inhibited by up to 40%, showing a clear concentration-dependent effect (Fig. 1A). Radish seedling root length was significantly reduced only by the 2% extract, whereas lower extract concentrations had stimulatory effect (Fig. 1B). Oxidative stress-related parameters, including lipid peroxidation and total antioxidant capacity, remained unchanged in treated roots. When radish plantlets were exposed to ground *S. inaequidens* material mixed with vermiculite for 36 days, their growth and physiological status were unaffected, suggesting that seedlings are more sensitive to allelochemicals than mature plants (Šoln, 2026).

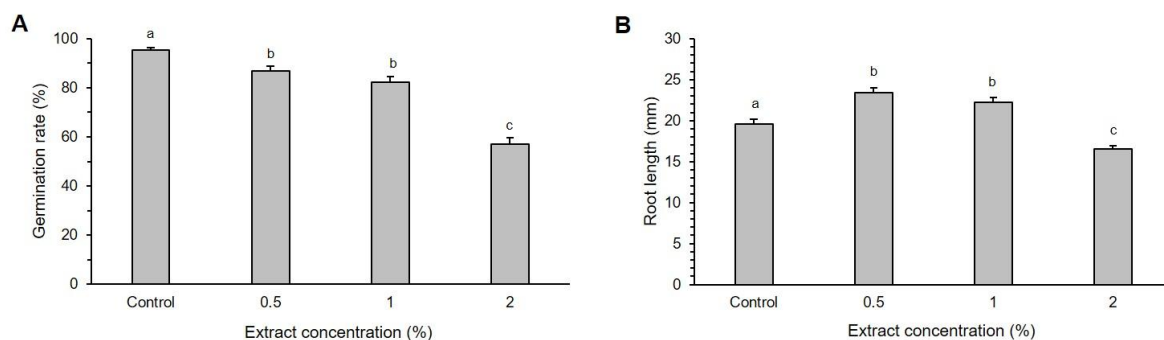


Fig. 1. Germination rate of radish seeds (A) and the length of radish primary root (B) after 3-day exposure to aqueous extracts of *Senecio inaequidens*. Data are means $\pm$ SE ($n = 10$; each mean represents one Petri dish with 50 seedlings). Different letters indicate statistically significant differences between treatments (ANOVA and Bonferroni-Holm test, $p < 0.05$).

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Unmasking mulberry bacterial blight: Characterizing mulberry-associated *Pseudomonas* phytopathogens in samples collected across Slovenia

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White mulberry (*Morus alba* L.) is an economically and ecologically valuable tree. Its leaves are the primary food source of the silkworm *Bombyx mori*. Mulberry trees also contribute to sustainable land use, erosion control, carbon sequestration, and the phytoremediation of degraded soils. Mulberry bacterial blight (MBB) is a disease that causes leaf lesions, necrosis, defoliation, and shoot dieback in white mulberry trees. MBB-associated pathogens include Gram-negative bacteria of the genus *Pseudomonas* (Gomila *et al.*, 2017; Xin *et al.*, 2018).

This study aimed to isolate, identify and characterize bacterial causative agents of MBB symptoms on old local white mulberry trees (*Morus alba* L.), growing in various regions of Slovenia. Samples from symptomatic mulberry trees from the UM mulberry collection, as well as samples from old trees from the Gorizia and Posavje regions, were selectively cultured on bacterial media supplemented with boric acid, ampicillin, and crystal violet. Bacterial isolates were identified by 16S rDNA, *hrpZ*, and whole-genome sequencing on an Illumina NextSeq 2000 platform, generating 2 × 150-bp paired-end reads. Biochemical characterization included LOPAT tests, gelatin hydrolysis, xylose, adonitol, and mannitol utilization, and growth on King's B agar. The Polish MBB reference isolate Ma001, originally identified as *Pseudomonas syringae* pv. *mori* (Krawczyk & Łochyńska, 2020) and subsequently reclassified as *Pseudomonas amygdali* pv. *mori*, was included for comparison.

All three Slovenian bacterial isolates included in the study – ZMUM_B001_MB01 (from the UM collection, isolate collected from an old tree from Goričko), ZMUM_B002_KP01 (from the Gorizia region), and ZMUM_B003_KK01 (from the Posavje region) – were identified as *Pseudomonas* at the genus level based on 16S rDNA and *hrpZ* sequences. Whole-genome analysis confirmed that isolate ZMUM_B001_MB01 is *P. amygdali* pv. *mori*, and that isolates ZMUM_B002_KP01 and ZMUM_B003_KK01 are *P. caspiana*. The Slovenian isolates exhibited the same LOPAT profiles and inability to degrade gelatin as the Polish reference isolate Ma001. However, differences were observed in carbohydrate utilization. ZMUM_B001_MB01 did not utilize xylose or mannitol, whereas both *P. caspiana* isolates and the Polish reference isolate utilized these substrates. These differences reveal phenotypic diversity among mulberry-associated *Pseudomonas* and may indicate adaptation to local environmental conditions.

In conclusion, integrating classical microbiological isolation methods with genetic markers, whole-genome sequencing, and phenotypic characterization is crucial for identifying MBB-associated bacteria from the genus *Pseudomonas*.

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