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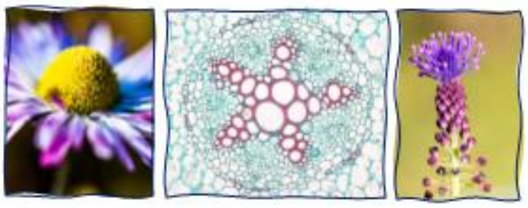

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Understanding Chloroplast Gene Expression for Rational Engineering of Plastid Genomes and Biotechnological Applications

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Chloroplasts are essential organelles that enable the photoautotrophic growth of plants and algae through their sophisticated photosynthetic machinery. Most key components of the thylakoid complexes are encoded by the chloroplast genome, a relic of their cyanobacterial ancestor. To produce this limited set of proteins, chloroplasts also possess their own gene expression machinery, including a multisubunit RNA polymerase, ribosomal proteins and RNAs, and a complete set of tRNAs.

Plastid genomes are readily transformable in several model species and crop plants. Numerous studies have demonstrated that the high expression capacity of plastids enables both the enhancement of endogenous gene expression to regulate photosynthetic capacity and the production of foreign gene products for biotechnological applications.

In the first part of my talk, I will discuss two case studies that demonstrate the successful manipulation of plastid genomes for the production of biotechnological products (antimicrobial peptides) and for insect pest control (via the expression of dsRNA). Each of these studies illustrates that, while we can technically modify chloroplast genomes with precision, we still lack a deep understanding of the fine-tuned regulation and mode of action of the gene expression machinery.

In the second part, I will provide a brief overview of my recent work aimed at uncovering fundamental aspects of transcriptional regulation and the mode of action of plastid RNA polymerases, with a focus on transcription termination and the evolutionary significance of this mechanism for plastid genome shaping and gene organization.

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Inducible Expression of Trichome-Associated Transcription Factors in *Cannabis sativa* Suspension Cultures

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Cannabis sativa represents a significant repository of diverse phytochemicals, attracting increasing commercial interest owing to their inherent bioactivity. Secondary metabolites produced by this species include cannabinoids, terpenes, and phenolic compounds. This intricate array of biomolecules is primarily synthesized within the glandular trichomes of female inflorescences and demonstrates considerable variability contingent upon cultivar and environmental growth parameters. Modern molecular methodologies and genome editing technologies present avenues for the manipulation of cannabis biosynthetic pathways, thereby potentially augmenting the yield of specific metabolites. However, cannabis exhibits recalcitrance to genetic transformation, and robust protocols for in vitro regeneration remain a challenge. In this context, cell cultures constitute a compelling alternative for the industrial-scale production of specialized metabolites, facilitated by their cultivation under controlled conditions and the potential for direct genetic modification, bypassing the requirement for whole plant regeneration. To explore the possibility of using Cannabis cell suspension cultures to produce secondary metabolites, we generated stably transformed cell lines using the biolistic transformation. Transcription factors implicated in trichome development, belonging to the R2R3 MYB MIXTA and HD-ZIP IV families, were selected for the generation of distinct transformed cell lines, with the objective of inducing cellular differentiation and the subsequent activation of the cannabinoid biosynthetic pathway. The expression of these factors was regulated via a dexamethasone-inducible expression system. The impact of varying dexamethasone concentrations on transcriptional activation was assessed through the utilization of reporter genes, facilitating the identification of optimal induction treatments. Gene expression analyses conducted on transformed cell lines revealed that the heterologous expression of an HD-ZIP IV factor from *Solanum lycopersicum* and a MIXTA factor from Prunus persica can transactivate endogenous cannabis transcription factors associated with trichome development and structural genes of the cannabinoid biosynthetic pathway, particularly when transgene induction was combined with methyl jasmonate elicitation. The genetic transformation of cell cultures has proven to be a valuable method for studying the interaction between developmental and secondary metabolism genes, enabling transgenesis studies in a plant where it would otherwise not be possible. Furthermore, a more detailed characterization of the obtained cell lines could help to understand whether they could constitute a potential biotechnological approach for the production of cannabinoids.

Auxin Response Factors in Reproductive Development: A Comparative Study of *Os*ARF1 and its Orthologs *At*ARF11/*At*ARF18

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Auxin is a key phytohormone regulating essential plant processes, including cell differentiation and reproduction [1]. The AUXIN RESPONSE FACTOR (ARF) family mediates auxin signalling [2,3], with OsARF1 playing a crucial role in Oryza sativa growth and fertility [4,5]. However, OsARF1 function in reproductive development remains unknown, making it a promising target for enhancing rice productivity and adaptation to environmental challenges.

Firstly, we generated Osarf1 mutants using a CRISPR-Cas9 multiplex approach. Phenotypic analysis of Osarf1 T1 mutant lines revealed an increased number of unfertilised flowers and aborted seeds compared to the wild-type. These findings strongly suggest that OsARF1 plays a critical role in reproductive development.

Given the conservation of auxin signalling, we have planned to study OsARF1 orthologs in Arabidopsis thaliana. Consequently, by phylogenetic studies we have identified AtARF11 and AtARF18 as OsARF1 orthologs. We have generated Atarf11 and Atarf18 single and double mutants by CRISPR-Cas9 technology with the aim to uncover conserved and species-specific ARF-mediated reproductive mechanisms between rice and Arabidopsis.

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Pollen-Mediated Control of Ovule Development in *Ginkgo biloba*: Insights into Auxin Regulation and Epigenetic Mechanisms

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In angiosperms, pollination and fertilization occur in close temporal succession. In contrast, gymnosperms such as *Ginkgo biloba* display a substantial gap of 4–5 months between these two events. Notably, pollen reception plays a pivotal role in guiding female gametophyte differentiation and seed coat development [1]. Our previous work revealed that pollen capture modulates auxin distribution, represses programmed cell death (PCD)-associated genes, and upregulates those involved in DNA replication and cell cycle progression, while the inverse pattern characterizes unpollinated ovules [2]. The present study aims to further dissect the mechanisms by which pollen controls ovule development. To this end, ovules were manually pollinated using non-viable pollen or treated with exogenous auxin, and were monitored over time for growth dynamics, histological analysis, and gene expression profiles. Interestingly, our findings show that, unlike auxin application, the mere physical arrival of pollen is sufficient to trigger an initial increase in ovule size. However, ovules subsequently aborted after approximately ten days, suggesting that pollen germination - typically occurring one week post-pollination - acts as a secondary developmental checkpoint.

ChIP-seq analysis of pollinated ovules, conducted before and after pollen germination, revealed shifts in the genomic distribution of H3K27me3-marked regions. These results suggest that pollen perception reduces PRC2 (Polycomb Repressive Complex 2) activity, specifically affecting genes involved in auxin biosynthesis whose epigenetic regulation post-fertilization has been previously described in Arabidopsis. Taken together, our data uncover a conserved regulatory framework and provide novel insights into the molecular and epigenetic control of ovule development in non-model plant systems.

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Unravelling Reproductive Mechanisms in *Ginkgo biloba*: From Male Cone Development to Pollen-Ovule Cross-talk

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Understanding reproductive mechanisms across seed plants is crucial in order to better understand their evolutionary history. While angiosperm reproduction has been extensively studied, the diversity within gymnosperms, comprising four of the five major seed plant lineages, remains largely unexplored. *Ginkgo biloba* is the only extant species of Ginkgophytes and occupies a critical phylogenetic position, thus offering unique insights into early spermatophyte evolution.

In Ginkgo pollination and fertilization are separated by several months, during which the male gametophyte grows and develops inside the ovule. Pollination is crucial for triggering further ovule development, including seed coat differentiation, with unpollinated ovules typically aborting. However, the molecular underpinnings of pollen-ovule interactions in Ginkgo remain largely unknown.

To unravel these mechanisms, our research integrates the study of Ginkgo pollen formation, ultrastructure, and its germination dynamics within the ovule. Ginkgo pollen, composed of four cells encased in a double wall, undergoes significant hydration-dependent morphological changes when captured by the pollination drop. Upon entering the pollen chamber, it germinates and forms a highly branched haustorial pollen tube, penetrating between the cells of the nucellus and extracting nutrients for several months.

We hypothesize a multi-step communication system for effective pollen-ovule recognition, potentially involving signals on the pollen exine, exposed intine components (e.g., arabinogalactan sugar moieties), and *de novo* synthesized molecules (e.g., small peptides, small RNAs). Comparative chemical analyses of Ginkgo pollen with other gymnosperms will be conducted to test these hypotheses and broaden our understanding of seed plant reproductive strategies.

In parallel, our research encompasses male cone development. We have identified three key developmental stages for molecular analysis: dormant winter buds with hibernating cone primordia, opening buds with actively dividing microspores, and mature cones prior to dehiscence. Initial phytohormone immunolocalization suggests a significant role for auxin in male cone development. Furthermore, we are analysing RNA sequencing data to decipher the gene regulatory networks governing Ginkgo male cone development and their interplay with hormonal signaling.

By dissecting male cone morphogenesis and pollen production, together with *in vivo* and *in vitro* analysis on pollen germination and further male gametophyte development, we aim to unravel the reciprocal recognition and cross-talk between pollen and ovule in *Ginkgo*. Our ultimate goal is to identify the signalling molecules involved in this communication throughout ovule development, from pollination to fertilization, and to challenge the prevailing notion of autonomous female gametophyte development in gymnosperms by demonstrating its potential interdependence on signals from the male gametophyte.

Plant Responses to Vibrational and Acoustic Stimuli: Biochemical and Molecular Responses in Arabidopsis

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The PRIN DAMATIRA project investigates how plants perceive and respond to acoustic and vibrational stimuli by integrating tools and methodologies from multiple scientific disciplines. Using Arabidopsis thaliana as a model organism, we employed laser Doppler vibrometry to record the vibrational signals generated by Eurydema oleracea during feeding. These recordings were used to determine an average leaf resonance frequency that maximizes mechanical deformation.

Based on this analysis, we developed two artificial vibrational stimuli: one replicating the natural feeding vibrations of E. oleracea on the abaxial leaf surface, and another designed to target the resonance frequency of A. thaliana leaves, thereby inducing maximal mechanical deformation. Finite Element Analysis (FEA) simulations were used to predict the biomechanical effects of these stimuli on leaf structures. The vibrations were then applied in vivo using a custom vibrational platform to assess the plants' biological, biochemical, and molecular responses.

A key technological advancement in this study was the development of high-resolution, three-dimensional models of A. thaliana using X-ray micro-computed tomography (micro-CT). This technique, not previously successfully applied to living non-woody plants, enabled the generation of realistic 3D reconstructions under vital conditions. Optimization of scanning parameters suggests promising applications in clinical botany.

Plant responses to both types of vibrational stimuli were evaluated through morphometric analysis (germination rate, fresh biomass, leaf number, and primary root length), biochemical profiling (total phenolic content and antioxidant capacity), and gene expression studies. The molecular analysis focused on genes involved in cell wall remodelling (XTH11, XTH19, XTH29, PME12, PME18, PME34) and mechanosensation (PIEZO1, MSL2, MSL6, MSL9). Additionally, in plants exposed to feeding-derived vibrations, we assessed carotenoid content and volatile organic compound (VOC) profiles, which are also being analysed under artificial stimuli.

This integrative, multi-level approach offers novel insights into plant mechanosensitivity and expands our understanding of how plants respond to mechanical cues at structural, physiological, and molecular levels.

New Insights into Tomato P4H3 Function: A Key Regulator of Plant Architecture, Fruit Development, and Seed Resilience

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The plant cell wall is a fundamental structure in plants, serving multiple roles in vegetative growth and managing responses to environmental stresses, including water deficit, salinity, and heat. The cell wall is dynamic and complex, containing a fibrillar component (largely cellulose) distributed within a matrix of pectins, hemicelluloses, glycoproteins, and secondary metabolites. Among the glycoproteins, hydroxyproline-rich glycoproteins (HRGPs) - such as extensins and arabinogalactan proteins - play significant roles in cell wall structure and signaling, structural support, and defense against biotic and abiotic stresses. However, the precise functions and structural interactions among these components are not yet well established.In plants, HRGPs have been reported to play crucial roles in cell wall organization, growth regulation, stress responses, and fruit development. Recently, the HRGP Prolyl 4-Hydroxylase 3 (P4H3) has been identified as a potential regulatory factor in these processes.

In this work, we elucidate the developmental role of P4H3 in *Solanum lycopersicum* by analyzing both RNA interference (RNAi) knockdown mutants and transgenic lines overexpressing P4H3 (OEX), using a combination of molecular and cell biology approaches. Comparative analyses with wild-type plants revealed significant alterations in plant morphology, fruit development, and seed performance. Anatomical and cellular differences among the genotypes suggest that P4H3 plays a role in both vegetative and reproductive development.

Furthermore, gene expression profiling and *In-situ* hybridization analysis have been employed to investigate the molecular functions of P4H3 in regulating seed and fruit maturation. Mutant lines exhibited aberrant seed development and impaired germination patterns, supporting a role for P4H3 in seed resilience. We also explored the contribution this molecule to stress response pathways during seed germination.

Moving forward, we aim to better define the mechanistic role of P4H3 in conferring developmental plasticity, particularly under environmental stress conditions associated with climate change. Ultimately, these findings may inform new strategies for improving fruit quality and productivity, supporting more resilient and sustainable agricultural systems.

Crat-Seq: A Novel Reduced Representation Sequencing Technique Targeting Protein-Coding Regions In Plant Genomes

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In modern breeding programs, molecular genotyping techniques have become increasingly important, being able to characterize entire genomes of plant crops at a relatively low price through second- and third-generation sequencing. Examples include techniques such as Whole Genome re-Sequencing and exome sequencing. Even if these techniques are able to detect large amounts of variants without the need of any previous knowledge, their cost and complexity increases the size of the genome and the number of genotypes analyzed arise. As a result, these techniques are rarely applied for routine or large-scale applications, especially on non-model species. In such cases, simpler and cheaper techniques such as those based on on-chip hybridization (SNP-arrays) or probe-based techniques (e.g. SPET) have been developed and widely applied. However, these methods require a deep knowledge of the study model and are often based on panels of variants previously identified, reducing their applicability to non-model or poorly characterized species. Also, reduced representation sequencing (RRS) techniques, based on restriction enzymes such as RAD-seq or GBS, have been applied in population genetics but they randomly select regions across the genome, thus valuable or unique markers are scant, and require extensive bioinformatic expertise for data analysis.

To overcome these drawbacks, we developed Coding region Amplicons Targeted sequencing (CrAT-seq), a universal, low-cost, non-restriction-based RRS method which enables variant discovery in protein-coding regions of plant genomes. Our methodology, partly inspired by SCoT markers technique and based on common PCR reactions and short read sequencing, does not require prior optimization on the species of interest. This approach is being developed and tested on rye (*S. cereale*), a traditional crop species cultivated in marginal lands across the Alps. Preliminary results on a panel of selected accessions highlighted that this methodology allows the identification of a significantly higher number of SNPs and INDELs compared to RAD-seq. The platform was also tested on tomato and rice plants to test its applicability to other model species while future implementations will include the development of primers optimized across different plant taxa or targeting specific large gene families or domains.

The development of this methodology will include tools for reproducible data analysis and accessibility leveraging cutting-edge digital technologies. Finally, CrAT-seq will improve efficiency of breeding programs in agriculture, paving the way for new opportunities in agricultural crop improvement as well as in plant conservation.

The Endoplasmic Reticulum Quality Control of Glycoproteins is Involved in Cadmium Stress Perception and Response in *Arabidopsis thaliana* through the Brassinosteroid Signaling Pathway

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The Unfolded Protein Response (UPR) pathway is widely conserved in all eukaryotic cells and plays an important role in the response to several abiotic and biotic stresses impacting on ER homeostasis. Defects in the UPR pathway cause root growth inhibition and increase tolerance to several abiotic stresses, including cadmium (Cd) treatment [1,2,3]. The UPR pathway is closely related to the Endoplasmic Reticulum Quality Control (ERQC) machinery, which surveils the folding process of glycoproteins trafficking along the secretory pathway. However, less attention has been paid to investigate the role of ERQC in: (I) plant development and (II) response to abiotic stresses. In this contest, we have characterised at a phenotypic and biochemical level a mutant of the ERQC pathway in the experimental model A. thaliana subjected or not to Cd stress. Under standard growth conditions, a short root phenotype was observed in the mutant line compared to Wt plants, implying that some glycoproteins folded under the ERCQ machinery play a role in root development. Interestingly, Cd treatment seems not to be perceived by the mutant line, which shows a higher tolerance to different Cd concentrations in comparison with the Wt genotype.
Among the many N-glycosylated proteins proceeding into the secretory pathway, we followed the biochemical pattern of the brassinosteroid receptor BRI1. The data here presented highlight that increased brassinosteroids signalling and the BRI1 protein level may be one of the reasons for the higher tolerance to Cd stress displayed by mutant plants.

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Selection of Plant Species with Anti-Inflammatory and/or Antimicrobial Properties and *in vitro* Callus Production

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Plants are a primary source of molecules with various biological activities beneficial to humans, making them valuable resources for pharmaceutical and cosmetic applications. This ongoing study aims at the isolation and characterization of phytocomplexes with anti-inflammatory and/or antimicrobial properties. A dataset was created after deep bibliographic research with 85 articles. Four key words (anti-inflammatory, anti-microbial, Europe and plants) were used to select several plant species (102 different species) with the features that were needed in this project. An article about this dataset, with all the processes of selection, is currently being written. Four selected species (*Perilla frutescens* L., *Lythrum salicaria* L., *Tribulus terrestris* L., *Filipendula ulmaria* L.) were chosen to start developing *in vitro* cultures and *in vivo* plants. The number of previous studies was a key aspect, that helped the selection to focus the research mainly on plants species that could be define "innovative".

Different MS media were tested on different plants organs (leaves, roots, stem). The samples were obtained from both *in vivo* plants (all organs from *in vivo* were sterilized) and shoots germinated *in vitro* (MS medium). The main aims are to find the highest levels of callus production based on three different hormones: BAP, 2-4D and NAA. BAP seems to have the highest yield, but more tests are currently carried out.

This PhD project is at its initial stage. The isolation and characterization of the selected phytocomplexes will happen when callus will be developed and when the most efficient extraction method will be found. The findings of this studies will have the potential to contribute to the development of novel plant-derived bioactive compounds with healthy and industrial applications, developing also sustainable practices. By developing *in vitro* cultures from species having a low amount of literature studies, it will be possible to create new protocols, based on the type of target metabolites. The creation of a dataset about plants species with anti-inflammatory and anti-microbial properties could be helpful for future researchers.

This PhD study received funding from the European Union - NextGenerationEU (Piano Nazionale Di Ripresa e Resilienza (PNRR) - ex D.M. 630/2024.

Marine Diatom Extract as Sustainable Tools for Enhancing Plant Resistance Against Fungal Pathogens

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The growing concern over the environmental and health impacts of synthetic pesticides is driving the search for sustainable, bio-based alternatives in crop protection. In this study, we investigated the potential of an aqueous cellular extract derived from the marine diatom *Cylindrotheca closterium* VRUC 291 to enhance plant resistance against the fungal pathogen *Botrytis cinerea*. The diatom was mass-cultivated in indoor photobioreactors under controlled conditions of temperature, light, and photoperiod (25 °C, 80 μmol photons m^{-2} s^{-1}, 12:12 h light/dark cycle).

Using a green extraction protocol, we obtained a water-soluble extract that was applied as a foliar spray to four-week-old *Arabidopsis thaliana* plants prior to inoculation with *Botrytis cinerea*. Treated plants showed a significant reduction in lesion size and incidence compared to untreated controls. The protective effects were also confirmed in crop species, including *Solanum lycopersicum*, *Solanum melongena*, and *Capsicum annuum*, with approximately 80% reduction in disease symptoms across all tested species. This protective effect was mainly associated to a mechanical mechanism involving the reduction of fungal spore adhesion, linked to dose-dependent changes in leaf surface hydrophobicity. In fact, contact angle measurements revealed that the extract altered the physicochemical properties of the leaf surface, reducing hydrophobicity in a dose-dependent manner and preventing fungal spore adhesion.

Moreover, this effect could not be attributed to any specific molecular weight fraction of the extract ($x<3$kDa; $3<x<30$ kDa; or $x>30$ kDa) but was significantly greater when plants were treated with the whole extract rather than with individual fractions. Finally, the chemical composition of the extract was analyzed by FTIR and NMR spectroscopy. These results support the potential of *C. closterium*-derived extracts as promising, eco-friendly tools for enhancing plant resistance to fungal diseases.

Optimized Extraction of Bioactive Compounds from Brazilian Green *Coffea arabica* L. Beans Using Conventional Methods and Natural Deep Eutectic Solvents

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Coffee is one of the most consumed beverages and a major global trade product. *Coffea arabica* L. is the most appreciated species for its flavor, aroma, and organoleptic qualities, which are influenced by its complex chemical properties. This study investigates the efficiency of different extraction methods on the composition of five commercial Brazilian green coffee bean samples, focusing on total polyphenols, chlorogenic acids, and caffeine, and their antioxidant activity. A further objective is the optimization of extraction processes using Natural Deep Eutectic Solvents (NaDES), an environmentally friendly alternative to conventional solvents.

Three extraction methods were firstly applied: two using organic solvents (95% methanol and 70% ethanol) and one using hot water (100°C) to simulate the traditional beverage preparation. Hot water treatment proved to be the most efficient for extracting total polyphenols and chlorogenic acids, yielding up to 74.3 mg GA eq/g FW (total polyphenols), 44.0 mg/g FW (chlorogenic acid), 9.9 mg/g FW (cryptochlorogenic acid), and 5.23 mg/g FW. Conversely, caffeine was better extracted with ethanol treatment, yielding up to 10.8 mg/g FW. Antioxidant activity was highest in the hot water extracts, ranging from 22.3 to 37.3 mg AA eq/g FW.

Additionally, eight different NaDES formulations were tested under four incubation times and temperatures, to optimize polyphenols and caffeine extractions. NaDES, have numerous advantages compared to other solvents, such as lower toxicity, reduced environmental impact and cost-effectiveness. Choline chloride-lactic acid showed the highest efficiency for polyphenol extractions (up to 38.9 mg GA eq/g FW), while betaine-glycerol yielded the highest levels of chlorogenic acid (61.5 mg/g FW) and caffeine (13.3 mg/g FW) among the tested formulations. Extracts obtained with NaDES also demonstrated superior antioxidant activity compared to organic solvents extracts.

This study provides valuable insights into the identification of the most effective extraction strategies for selected bioactive compounds in coffee bean samples and highlights the potential of NaDES as sustainable alternatives to conventional solvents in food and natural product applications.

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Exploring the Function of Rice Florigenic Proteins During Inflorescence Development

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Rice (*Oryza sativa*) is an herbaceous annual plant of Asian origin whose transition to flowering is accelerated under short-day (SD) conditions. Among the key regulators of this process are florigens, small proteins encoded in rice by the *Hd3a* and *RFT1* genes. After being synthesized in the phloem companion cells of the leaves, florigens are transported to the Shoot Apical Meristem (SAM), where they induce the switch from vegetative to reproductive development. Among the genes directly activated by florigens at the apex, *FT-L1* is one of the most strongly induced. Mutations in *FT-L1* lead to delayed flowering, increased inflorescence branching, and a substantial decrease in fertility. These phenotypes are more severe when *ft-l1* mutants are combined with either *hd3a* or *rft1*, supporting a model in which FT-L1 functions together with Hd3a and RFT1 to promote the floral transition and the acquisition of determinate spikelet meristem identity, ensuring proper reproductive development.

Inflorescence analysis of *rft1* mutants revealed an increase in the number of branches, a phenotype that is further exacerbated in *ft-l1 rft1* double mutants, suggesting an additive role of the two florigenic proteins in regulating panicle architecture. Ongoing work includes evaluating the effect of combined absence of Hd3a, RFT1, and FT-L1, adopting an RNAi approach.

FT-L1 also appears to impact the expression of other regulators: in *ft-l1* backgrounds, expression levels of *OsMADS34* and *OsMADS5*, two transcription factors involved in floral meristem identity determination, are significantly reduced. These factors are known to repress the antiflorigenic gene *RCN4*, which counteracts florigen activity and maintains indeterminate branch meristem fate. We have generated mutants for *RCN1* and *RCN4* in both wild-type and *ft-l1* backgrounds. In *rcn1 rcn4* double mutants, the number of secondary branches is reduced compared to the control, as expected from literature; interestingly, the *ft-l1* mutation, combined with *rcn1 rcn4*, partially compensates this phenotype, suggesting an antagonistic relationship between FT-L1 and RCN proteins. In parallel, we are exploring whether the targeted mutation of a key aminoacid in FT-L1, using Prime Editing approach, can convert it into an antiflorigenic-like protein, allowing us to understand the basis of its function.

To understand the causes of sterility in *ft-l1* lines, we performed *in situ* hybridization and localization studies with FT-L1:eYFP marker lines on rice flowers. FT-L1 is predominantly expressed and localized in the pistil, although the transcript is also detected in vasculatures and male reproductive organs. Reciprocal crosses revealed significantly reduced seed number when *ft-l1* plants serve as the seed bearer, suggesting that sterility may be mainly linked to defects in the female organ. Analysing flower morphology, while single *ft-l1* mutants show only mild floral abnormalities, combining *ft-l1* with florigen mutations results in severe floral defects, including homeotic conversions from anthers to pistil-like structures, or irregular organ numbers. These phenotypes are supported by the downregulation of C, D, and E-type floral identity genes in mutant backgrounds. These findings suggest a cooperative role for FT-L1 and florigens in ensuring the proper development of spikelets and flowers in rice.

Sorghum bicolor subsp. *bicolor* as a Source of Bioactive Compounds with a Cholesterol Lowering Effect: First Data on its Lipid-Derived Extracts

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The use of phytochemicals in medicine and nutraceutical sciences has become increasingly popular for treating various diseases and pathologies. Specifically, policosanols and phytosterols are currently considered for their enhanced lipid-lowering efficacy. These compounds, particularly abundant in several cereals, seem to act mainly on dietary cholesterol, reducing its absorption in the intestine. However, their mechanism of action and their eventual interaction with the protein responsible for cholesterol transport from the intestinal lumen into the enterocytes (Neiman-Pick C1 Like1- NPC1L1) are still not completely clear. Moreover, the information on the specific policosanol and phytosterol composition of several quite commonly cereals, among which *Sorghum bicolor* (L.) Moench subsp. *bicolor* (here-after *S. bicolor*), is still quite scarce and no data on their specific efficacy in the treatment of hypercholesterolemia are available in the literature yet.

In this context, the objectives of this study are: 1. to obtain a comprehensive and detailed characterization of the composition of phytosterols and policosanols in *S. bicolor*; 2. to evaluate the impact of this mixture on reducing cholesterol transport in enterocytes through the modulation of NPC1L1 expression.

To this purpose, policosanol and phytosterol extracts were obtained from *S. bicolor* grains; an initial crude lipid extraction followed by saponification and purification by column chromatography was conducted to obtain different fractions of the extracts, enriched in each of the considered groups of compounds. These fractions were then characterized using gas chromatography coupled with mass spectrometry (GC-MS). Furthermore, their biocompatibility was evaluated with a MTT-based in vitro cell viability assay, carried out on an intestinal barrier model consisting of Caco-2 and HT-29 cells. After assessing the monolayer integrity measuring the trans-epithelial electrical resistance, cells were treated for 24 hours with increasing doses of the extracts, assaying NPC1L1 protein levels and its transcription factor STAT-3 by Western Blot techniques.

The findings confirmed a high content of both policosanols and phytosterols in *S. bicolor* grains highlighting the presence of several cholesterol-lowering compounds including 1-hexacosanol, 1-octacosanol, campesterol and stigmasterol. Notably, some of these compound, such as 1-tricosanol and fucosterol, have not been previously reported for this sorghum variety. Furthermore, no adverse effects of the tested extracts fractions were observed on cell viability and trans-epithelial electrical resistance. Lastly, the phytosterol fraction appears to play a relevant role on reducing significantly NPC1L1 protein levels by about 40% compared to controls.

Acknowledgements

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Evaluation of Phytotoxicity of Essential Oils Derived from By-Products of Aromatic and Medicinal Plants

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The cultivation of Medicinal and Aromatic Plants (MAPs) is widely practiced in Western Liguria, with a notable impact on the local economy, thanks to the continuous demand from the Northern European market. This cultivation involves the production of significant quantities of plant by-products, as the plants grown in pots must be maintained in the size and shape required by the horticultural market. In the present study, we have analysed by-products from *Lavandula angustifolia* Mill. 'Essence Purple' (aerial parts) and *Helichrysum italicum* (Roth) G.Don (leaves) cultivated in Albenga (Savona). Micromorphological and anatomical investigations confirmed the typical secretory structures of both these species. Analysing the fragmented aerial parts of *L. angustifolia* we detected non-glandular dendritic trichomes, peltate glandular trichomes and different types of capitate glandular ones, including those with a multicellular stalk, a short neck cell and a unicellular head, present only on the inner surface of corolla. In the fragmented leaves of *H. italicum* a woolly-tomentose indument composed of very long and flexible multicellular uniseriate non-glandular trichomes was observed. Mixed among these, biseriate glandular trichomes were also identified, which show from 4 to 5 parallel pairs of cells, culminating in two cephalic cells covered by a subcuticular space where the secretion accumulates.

The essential oil (EO) from *L. angustifolia* showed high amounts of oxygenated monoterpenes (40.71%), followed by oxygenated sesquiterpenes (28.47%); the main components were τ-cadinol (23. 09%), linalyl acetate (14.07%) and γ-cadinene (12.07%). The EO from *H. italicum* showed high amounts of sesquiterpene hydrocarbons (41.49%) followed by oxygenated sesquitepenes (39.89%), being the main components γ-curcumene (15.47%) and eudesm-4(14)-en-11-ol (10.71%). The two EOs were tested for a possible phytotoxic activity on the seeds of two weeds (*Lolium multiflorum* Lam. and *Sinapis alba* L.) and two crops (*Raphanus sativus* L. and *Hordeum vulgare* L.). Both EOs showed inhibitory activity on germination and radical elongation of both weed seeds, especially on *S. alba*, and a milder phytotoxicity on the two crops. The results could lay the foundation for the use of EOs obtained from by-products of MAPs as alternatives to traditional industrial pesticides, in a green and eco-sustainable perspective.

Engineering Biocompatible Nanosystems to Improve Delivery of Plant Biostimulants

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The increasing global demand for food, coupled with growing environmental challenges, fosters the quest for new and sustainable agricultural practices. Plant biostimulants - substances or microorganisms that enhance plant nutrition and growth independently of their nutrient content - have been shown to improve plant nutrition and stress tolerance, acting at very low doses. Therefore, they hold great promise for reducing reliance on traditional chemical fertilizers. However, their application in the field can be limited by their chemical or physical status, namely their high environmental degradability or volatility. Nanotechnology offers a novel solution to these limitations, specifically through the nanoencapsulation of the bioactive compounds, which may enhance not only the biostimulant stability and resistance to environmental degradation, but also the efficiency of their delivery to plants.

The present work aims to develop, in collaboration with the company ILSA S.p.A., novel nanoencapsulated eco-friendly biostimulants and biopesticide fertilizers, collectively called NanoBios. Two classes of nanosystems have been selected for the nanoencapsulation: Metal Organic Frameworks (MOFs) and Hydrogen-bonded Organic Frameworks (HOFs), 3D-ordered porous materials characterized by high porosity, tunability and biocompatibility. Preliminary synthesis trials were conducted using MOFs nanosystems. The successful encapsulation of the bioactive molecules was confirmed by chemical and structural characterization of the NanoBios, carried out by transmission and scanning electron microscopy, as well as X-ray diffraction and mass spectrometry. Experiments to exclude cytotoxicity and check potential internalization of NanoBios are ongoing in *Arabidopsis thaliana* cell suspension cultures. Viability assays showed the absence of cytotoxic effects of empty HOFs; concerning MOFs, since they degrade at low pH, suspension-cultured cells are currently being adapted to culture media at neutral pH, before viability tests can be conducted. To check the potential calcium-mediated perception of NanoBios, Arabidopsis seedlings and cell suspension cultures expressing the genetically-encoded calcium reporter aequorin will be used in luminometer-based Ca^{2+} measurement assays. The potential size-dependent uptake of NanoBios into plant cells will be assessed via laser scanning fluorescence microscopy, after fluorophore loading. Subsequently, investigations on NanoBios growth promoting activity and/or priming of plant defence responses will be conducted on plants *in toto*, using different plant experimental systems. This work will allow the evaluation of the NanoBios effectiveness, by unravelling their effects on plant physiology, and generate an analytical platform to test additional future formulations.

The Chemical Composition and Biological Activity of *Nigella sativa*

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The use of *Nigella* species seeds in the culinary field, or of the aerial parts infusions in the pharmaceutical and cosmetic fields, is widely reported. The biological activity of these plants, demonstrated over the years, are closely related to its phytochemical content. This investigation focused on the study of the fixed oil isolated from seeds of *Nigella sativa* collected in India. The fixed oil was chemically analysed by GC-MS.

Studies were conducted on the possible antiproliferative activity and DNA damage on Caco-2 tumor cell lines and antioxidant activity on HaCat cells. The extract showed a strong antiproliferative and antioxidant power.

In Depth Metabolomic Characterization of Autochthonous Legume Landraces from Apennine Italian Regions

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Autochthonous landraces or local varieties are plant genetic resources characterized by high genetic variability, specific adaptation to the environmental conditions of the cultivation area (i.e., tolerance to biotic and abiotic stresses), and the presence of secondary metabolites (terpenoids, flavonoids, alkaloids) and health-promoting compounds. However, local varieties are gravely threatened by extinction mainly for their replacement by modern genetically uniform varieties. Untargeted metabolomics is a strong approach that allows the investigation of a wide range of metabolite classes and gives crucial data for the identification of distinct local varieties, identifying their metabolic fingerprints, and promoting their valorization and conservation. The current study aimed to provide a global view of the metabolite diversity of three autochthonous lentil landraces from different villages of Molise region (Italy) - Capracotta, CA; Rionero Sannitico, RS; and Agnone, A - in comparison to one landrace from Umbria region (Italy) - Castelluccio di Norcia, CS. Untargeted metabolomics, performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) allowed the detection of 662 differential accumulated metabolic features (DAMfs). The annotation of the DAMfs, by consulting homemade spectral and MS libraries and the molecular network approach, allowed 50.3% of them to be assigned to 15 metabolic categories (6 pathway families and 9 chemical families). Amino acids and peptides (85 DAMfs), flavonoids (55 DAMfs), and lipids and fatty acids (35 DAMfs) were the most represented metabolic categories. Significant variations in the metabolite composition of lentil landraces were also observed through the combination of univariate and multivariate statistical analyses. According to the PCA score plot, the metabolite compositions of lentil landraces differed significantly from one another. In detail, A, CA, and RS landraces were separated from CS by PC1, which explain 56 % of variance. The hierarchical cluster analysis (HCA), based on the DAMfs intensities, confirmed these results. Furthermore, the HCA coupled with the enrichment analysis allowed the identification of specific landrace metabolic features, which include flavan-3-ols, proanthocyanidins, and benzoic acids (for CS); amino acids and peptides (for CA and RS); carbohydrates and polyketides (for RS and CS); terpenoids, and lipids and fatty acids (for A). Finally, the functional enrichment analysis and Pearson correlation coupled with the molecular network analysis identified enriched metabolic pathways and putative landrace biochemical markers, respectively. In the next period, the antimicrobial and antioxidant activities of lentil seed extracts will be evaluated and analyzed in the light of the distinct metabolic profiles of each landrace in order to explore their putative beneficial effects.

Sustainable Production of Betanin-Rich Extracts from *Beta vulgaris* Leaves in SETIS® Bioreactor

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Beta vulgaris (beetroot) is a valuable source of phytochemicals, in particular betanin, which are increasingly studied for their potential health and industrial applications. In this study, *B. vulgaris* shoot explants were successfully cultivated in vitro using SETIS temporary immersion bioreactor, producing biomass and an efficient metabolite accumulation. The biomass showed vigorous and rapid growth in this system, confirming the suitability of the SETIS bioreactor for *B. vulgaris* cultivation. Unlike most studies that focus on the root juice, this work employs an extract obtained from leaves, which are a less explored but faster-to-grow source of bioactive compounds. The extracts obtained from the leaves showed a high content of betanin, as confirmed by preliminary phytochemical analyses. Furthermore, antioxidant activity assessed through the ABTS assay revealed a remarkable radical scavenging capacity. Notably, both betanin content and antioxidant activity were similar or higher to values reported in the literature. Furthermore, the SETIS bioreactor system offers a scalable, rapid, and reproducible platform for the sustainable production of *B. vulgaris* biomass rich in high-value compounds, supporting its application in the development of natural antioxidant ingredients for the food, cosmetic, and pharmaceutical industries.

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Functional Characterization of the *AUXIN RESPONSE FACTOR 11/MONOPTEROS (OsARF11/MP)* In *Oryza Sativa* During Reproductive Phase

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Auxin is one of the most studied plant hormones. The cellular concentration of auxin is translated into a cellular response by the AUXIN RESPONSE FACTORS (ARFs). These transcription factors activity is modulated at post translational level in an auxin-dependent manner. In rice have been described 29 ARFs that have been subdivided in 3 classes depending on their capability to regulate targets as transcriptional activator or repressor(Shen et al., 2010).

AUXIN RESPONSE FACTOR 11/MONOPTEROS (*OsARF11/MP*) plays a pivotal role in plant development since the loss of function mutant *osarf11*$^{TOS\text{-}17}$ is completely sterile (Sims et al., 2021).

In our laboratory, we generated a mutant line named *osmp1*, using CRISPR-Cas9 technology, carrying a single-base insertion located 25 bp downstream of the ATG start codon. This mutation appears to compromise both fertility and panicle architecture in the plants. Interestingly, our observations differ from previously published data on *osarf11*$^{TOS\text{-}17}$ loss of function mutants.

I will discuss the phenotypical characterization of *osmp1* mutant focussing on reproductive development.

Peptide Hormones in Fruit Development: Insights from Tomato

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Fruit development and ripening are genetically and environmentally regulated processes that are crucial for plant reproduction and food production. These processes rely on complex signaling networks involving hormones, transcription factors, and other regulatory molecules. Among them, peptide hormones (PHs) have emerged as key players in mediating both local and systemic signaling events[1]. Despite increasing evidence of their involvement in various aspects of plant development, their specific roles in fruit formation and ripening remain poorly understood.

To address this gap, we explored the function of PHs in fruit development by targeting common steps in their biosynthetic pathways[2]. Specifically, we interfered with key post-translational modifications responsible for PHs biological activity, focusing on enzymes such as subtilase proteases and Tyrosyl Protein Sulfotransferase (TPST)[3]. We used two strategies: overexpression of microbial protease inhibitors[4] and gene silencing via artificial microRNAs (amiRNAs). These manipulations were driven in a fruit-specific manner in tomato, used as a model system.

Our results highlight the importance of secreted PHs in the regulatory networks governing fruit development, offering new insights into the molecular mechanisms underlying this crucial stage of the plant life cycle.

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New Insights into the Role of CLE Peptides in Fruit Ripening

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CLE (CLAVATA3/EMBRYO SURROUNDING REGION RELATED) peptides are small, secreted signaling molecules that regulate diverse aspects of plant development, yet their roles in fleshy fruit biology remain largely unexplored. Recent annotation of the *Solanum lycopersicum* CLE family has uncovered many peptides with no assigned function, and by means of transcriptomic analyses we revealed that a specific subset — *SlCLE5*, *SlCLE11*, *SlCLE38*, and *SlCLE51* — is upregulated during fruit development and ripening. Intriguingly, the expression of these genes is closely linked to ethylene, the key hormone that acts as the primary regulator of climacteric fruit ripening. This ethylene dependence strongly suggests a regulatory role for these CLE peptides in the ripening process.

To further investigate this, we studied CLE expression in tomato fruits though promoter analyses. By driving the expression of a *eGFP-GUS* reporter construct under the control of CLE promoters, we observed that CLE expression varies across different fruit tissues. This spatial analysis revealed that certain CLEs are preferentially expressed in specific morphological regions, suggesting that their signaling may play localized roles in coordinating region-specific developmental programs during fruit growth and ripening. It will be particularly interesting to assess how these spatial patterns are altered following hormone (e.g. ethylene, ABA) treatment of promCLE::eGFP-GUS lines.

Moreover, we employed a genome-editing strategy to generate *S. lycopersicum* lines carrying CRISPR/Cas9-mediated *knockouts* of multiple *CLE* genes. By targeting these peptide-encoding genes, we aim to perturb key nodes within the signaling network that governs fruit growth, development, and ripening. To date, we have successfully generated *knockout* lines for *CLE11*, which will serve as a valuable line to investigate the role of this peptide within the complex regulation of ripening.

Altogether, albeit still preliminary, these analyses suggest that fruit-expressed CLEs may contribute directly to the ripening process by participating in hormonal cross-talks. Elucidating how ethylene regulates these peptides, identifying the receptors that mediate their signaling, and understanding how their distinct signals co-participate in ripening regulation could uncover previously unknown regulatory layers. These insights could pave the way for novel strategies to improve fruit quality and post-harvest performance in tomato and other climacteric crops.

Plant Sensitivity to Inorganic Arsenic Exposure: A Comparison Between Two Rice (*Oryza sativa* L.) Genotypes, Carnaroli and Zhonghua 11

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The presence of arsenic (As) in the environment is still a critical issue worldwide. The highly toxic inorganic forms, arsenite (As^{III}) and arsenate (As^{V}), negatively affect plant development and human health (IARC 2012) (1). Arsenic has been classified as one of the major public health concerns by the World Health Organization (WHO) (2) and many others authoritative organizations have established strict guidelines to mitigate human exposure to As. Particularly, the European Commission has recently set new limits (EU 2023/465) to reduce the presence of inorganic As (iAs) in rice-based foods, as a safeguard of public health. Indeed, rice (*Oryza sativa* L.) is among the main exposure sources of As for humans. Flooded rice cultivation facilitates iAs entrance through the roots, wherein the largest accumulation occurs, altering their development and hormonal homeostasis (3).

Italy is the leading rice producer in Europe, whose production covers more than 50% of the total amount, thanks to the regions of Piedmont and Lombardy. In particular, Vercelli is the largest producer of Carnaroli variety (*O. sativa* subsp. japonica), which was obtained by crossing Vialone and Lencino varieties 80 years ago in Lombardy (4). Nowadays, this variety is still the most cultivated and exported all over the world thanks to its organoleptic qualities and good yield. However, Carnaroli is still poorly studied concerning the plant sensitivity to As. To date, due to the steady presence of As in paddy fields, it is essential to manage to select As-tolerant rice varieties capable of excluding or limiting this harmful semimetal in the grains.

This research aims to investigate the iAs effects on the development of Carnaroli variety. Rice seedlings were grown *in vitro* in presence, or not, of As^{III} and As^{V}, added at sublethal concentrations to the culture media, and analyzed at the morphological, cytohistological, and chemical levels. The responses of this variety were compared with those of the As-sensitive variety Zhonghua 11 (*O. sativa* subsp. japonica) (5).

The first results show a lower sensitivity to iAs of Carnaroli rice plantlets, despite a higher root uptake compared to Zhonghua 11. The lateral root (LR) production was significantly reduced only with As^{III} treatment compared to the Control in Carnaroli, whereas this decrease was more pronounced with both iAs forms in Zhonghua 11. The histological analysis of adventitious roots (AR), which are the main part of the rice root system, confirmed the results of morphological analysis and revealed anomalies in the development of LRs. Our results suggest deepening the knowledge of the mechanisms that allow different rice genotypes to cope with the presence of As^{III} and As^{V} in the substrate and evaluating the involvement of the shoot. Hence, further analyses are currently underway.

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Sorghum bicolor Salt-Sensitive Genotype Grown in Saline Soil Benefitted the eBL Seed-Priming Treatment

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Soil salinization is a phenomenon increasing and spreading worldwide and continuously damaging ecosystems and reducing yield of crop, mainly in the major cereals. Thus, it is crucial to identify salt-tolerant crops and/or technologies able to improve salt tolerance in sensitive ones, without harming the ecosystem. This is necessary to ensure food availability for the increasing world population in the changing environment. *Sorghum bicolor*, being a salt-tolerant crop, represents a staple food in many arid and semi-arid regions of the world, thus it is a good candidate for this study, although differences among genotypes were found (2). Brassinosteroids (BRs) are a class of steroidal hormones well known for their role in plant development and tolerance to different stresses, including salt-stress. Brassinosteroids could be exogenously applied, also as seed-priming, to improve salt resistance in crops with minimum impact to the environment (1). In this study the effects of salt stress and of the seed priming treatment with the 24-epibrassinolide, a BR active precursor, were evaluated on two sorghum genotypes, Bianca, a salt-tolerant genotype, and Tonkawa, a salt-sensitive one. The aim was to evaluate if the seed-priming could mitigate the negative effect of the salt-stress in plant cultured in pots. The results obtained from 40-days-old plants in vegetative phase evidenced the efficiency of eBL treatment in enhancing the photosynthetic activity and in mitigating the negative salt effects on the leaf anatomy in the salt-sensitive genotype. Gas exchange analyses showed a significant increase in the net photosynthetic rate and a decrease in the intercellular CO_2 content, leading to a higher carboxylation efficiency in plants grown in saline soil with the pre-treatment with eBL in comparison to the salt treatment alone. Shoot morphological analyses showed that the priming treatment was beneficial for Tonkawa's parameters under saline soil conditions. Histological analyses highlighted more differences among treatments in Tonkawa, with respect to Bianca, especially between plants treated only with salt and those pre-treated with the hormone. Altogether, the results show that seed priming, with a specific eBL concentration, can increase salt-tolerance in the salt-sensitive genotype.

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GERAS a Regulator of Arabidopsis Silique Senescence

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The senescence process is a highly organized and finely regulated mechanism through which tissue aging and death occur, allowing for nutrient recycling. Senescence has been extensively characterized in leaves, as it is considered a crucial trait in agronomy, particularly for crop production. However, senescence is also critical in fruits, and its elucidation paves the way for future fruit manipulation strategies.

Several transcription factors have been identified as key regulators of senescence. Among them, the NAC family represents a plant-specific group of transcription factors found not only in *Arabidopsis thaliana* but also in other crops such as tomato, rice, and grapevine. NAC proteins play a pivotal role in regulating senescence and they participate in various plant developmental processes, ranging from vegetative growth to floral development, as well as responses to biotic and abiotic stresses.Mutations in NAC genes have been shown to affect fruit senescence. For instance, the *atnap* mutant, corresponding to the first NAC transcription factor discovered to regulate senescence in both leaves (Guo and Gan, 2006) and siliques (Kou et al., 2012), exhibits delayed fruit senescence.

In our laboratory, we isolated and studied a NAC transcription protein, GERAS, that modulates silique senescence. Our findings indicate that GERAS acts as a negative regulator of senescence, since its disruption accelerates silique yellowing. A *geras* knockout mutant, generated through genome editing, was analysed to better understand the molecular mechanisms underlying the senescence process and our results will be discussed.

Regulatory Roles of NAC Genes in Fruit Elongation, Senescence, and Xylem Formation in *Arabidopsis thaliana*

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A fruit is the mature ovary of a flowering plant, typically containing seeds, and develops from a fertilized ovule following pollination. Its primary functions include protecting, nourishing, and dispersing seeds, thereby playing a crucial role in the plant's reproductive cycle. While fruits exhibit a wide range of structures, compositions, and functions, they are generally recognized for their contribution to seed dispersal and their nutritional value to both humans and animals.

Given their essential roles in both biological processes and human nutrition, fruits are of significant importance to the global agricultural economy. As a result, there is strong interest in understanding the molecular mechanisms that regulate fruit development and maturation, with the aim of improving crop yield and quality, and ultimately advancing agricultural productivity.

To investigate the molecular networks involved in fruit development and ripening in *Arabidopsis thaliana*, we conducted a transcriptome analysis using RNA deep sequencing of siliques collected at various developmental stages. Among thousands of differentially expressed genes, we focused on the NAC family of transcription factors. Our analysis revealed that mutations in individual NAC genes can significantly influence fruit elongation and senescence. Three NAC genes - *NAC37*, *NAC76*, and *NAC105* - contribute to secondary cell wall construction and their disruption affects xylem formation in the replum and mainly in the funiculus.

Maternal Regulation of Nutrient Availability Plays a Pivotal Role During Ovule and Seed Development in Arabidopsis

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In Angiosperms, the communication between the maternal sporophytic tissues and the embryo sac is essential for the proper development of the ovule and, after the double fertilization, the seed. In Arabidopsis, the sporophytically expressed ARABIDOPSIS Bsister (ABS) and SEEDSTICK (STK) genes encode for MADS-domain transcription factors (TFs), that act in concert to control ovule differentiation and seed development. In the *abs stk* double mutant, seed set is severely compromised, resulting in a few viable seeds. Moreover, the *abs stk* ovules are characterized by defective integuments, together with increased starch accumulation within the embryo sac. Although most *abs stk* ovules are properly fertilized, as we have shown by analysing the presence of zygotes, the embryo is delayed and arrested a few days after fertilization in most of the developing seeds. To unravel the role of ABS and STK during ovule and early seed development, we used transcriptomic and metabolomic approaches followed by a detailed analysis of the double and single mutant phenotypes before and after fertilization. These approaches revealed that the sugar metabolism is widely targeted by these two TFs, and is affected in the double mutant. By modulating the starch accumulation in the *abs stk* ovules we were able to partially rescue the defects in embryo and seed development, highlighting the importance of the sugar balance in these processes. Finally, by assessing callose deposition in the maternal tissues, we postulated a model that involves the appropriate modulation of nutrient flux during the ovule-to-seed phase transition. Overall, our data emphasize the importance of the interplay between metabolic and developmental processes, regulated by the maternal tissues, for the growth of the fertilization products.

Unveiling Transcriptional Networks Governing Seed Coat Control of Endosperm Development in *Arabidopsis thaliana*

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In most angiosperms, the seed comprises maternal tissue-derived seed coat, triploid endosperm, and diploid embryo. Coordinated development of these three tissues is critical for optimal seed viability and germination. The rate of seed lethality is sensitive to parental genome dosage balance. In the model species *Arabidopsis thaliana*, as in other flowering plants, deviations from the canonical 2 maternal and 1 paternal (2m:1p) genomes in the endosperm, lead to altered seed development that affects seed size, endosperm proliferation and cellularisation, and seed viability. We have focused on the role of *TRANSPARENT TESTA 8* (*TT8*), an evolutionary conserved bHLH transcription factor encoding gene, which null mutation causes viable seeds to develop from paternal excess crosses (Zumajo-Cardona et al., 2023). We examined seeds resulting from intraploid and interploid crosses in *tt8* mutant, as well as in the wild-type background, by morphological and transcriptomic characterization.

Our investigations point out that TT8 protein is localized in the endothelium, the cell layer surrounding the endosperm. Analyses on mutants indicated that, in tt8 seeds, the endosperm cellularises earlier while the embryo development is sensibly delayed; the resulting seed is significantly smaller. Interestingly, these phenotypes may highlight possible non-autonomous effects of *TT8* gene on endosperm development. To identify which processes are mainly affected at transcriptional level when *TT8* is not functional or parental genome contribution is unbalanced, a bulk RNA-seq was performed on early developing seeds; the resulting high-throughput data were used to infer causal relations between expressed genes by taking advantage of a network-based approach using RTP-STAR pipeline, which implements GENIE 3 regression tree algorithm (Spurney et al., 2019). Lastly, through the observation of marker lines with confocal microscopy, we focused on the effect that paternal excess and *tt8* mutation have on the timing of expression of some Auxin Response Factors (ARFs) which are known to initiate endosperm cellularization. Our study may lead to deeper understanding of transgenerational communication mechanisms in the seed and shed light on the mechanisms of action of TT8 as an important regulator of post-zygotic hybridization barriers.

HEATSTOP: Understanding The Impact of Heat Stress on Pollen-Pistil Communication in Crops

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Background: The steady rise in average temperatures predicted for the near future will have a significant impact on several ecological areas. This phenomenon will lead to a drastic reduction in crop productivity and yields, especially for agronomically important plants with such as *Brassica napus* L., commonly known as canola or rapeseed. One of the worst consequences of this scenario is the partial or complete male sterility of the flower (Lohani et al 2020). Therefore, the HEATSTOP project aims to propose a new approach by studying the molecular mechanisms underlying pollen-pistil interactions and how they are affected by high daytime temperatures.

Method: Two rapeseed cultivars, *i.e.* Phoenix Cl. and Dariot, were harvested under optimal (22°C) and heat-stressed (35°C) conditions. The heat stress response was monitored by measuring the photosynthetic efficiency in stressed and control groups throughout the experiment using a chlorophyll fluorometer (HANDY PEA – HANSATECH). The effect of heat stress on pollen development and performance was assessed using a combination of imaging and molecular techniques, comparing dimensions, viability, and germinability between stressed and control plants, but also evaluating the presence or potential differences of nanovesicles released during the germination, analyzing different enzymatic activities *i.e.* ROS, and assessing the lipophilic and protein profile of the granules (Suanno et al. 2023).

Results: It was observed that there were no significant differences in photosynthetic efficiency between cultivars and groups, nor in the pollen grain size measured by optical and ESEM microscopy. In contrast, the viability and germination rates of the stressed group were significantly lower than those of the control group, 90% and 20% ±SD (n=1) and 99% - 30 % ±SD (n=1), respectively. Furthermore, the Dariot cultivar exhibited a pronounced thermal sensitivity and a reduced production of pollen grains. Nanovesicle trafficking showed no significant differences, as the nanovesicles were extracted, analysed and their concentrations were determined.

Conclusions: Our *in vitro* experiments indicate that the selected cultivar Phoenix Cl. does not show thermo-sensitivity to heat stress, whereas Dariot does. The vegetative parts of the plants were unaffected by the stress, therefore, more emphasis should be placed on understanding the molecular mechanisms involved in pollen development and germination and pollen-pistil communication, as pollen production and performance were affected by the stress. Future studies in the HEATSTOP project will focus on the proteomic profile and enzymatic activity of the pollen grains.

Fig.1 Canola field in Bologna interland

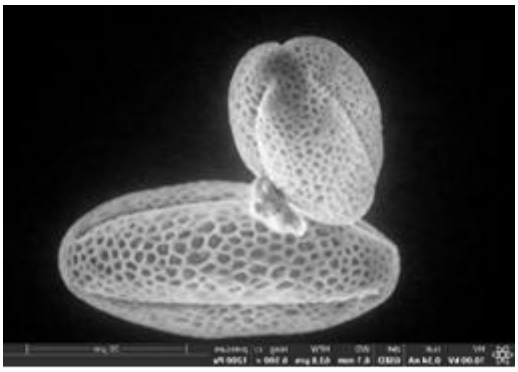

Fig.2 Fresh and hydrated pollen grain
of Phoenix Cl. – ESEM

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Plant Responses to Micro- and Nanoplastic Contamination Under Short- and Long-Term Exposure: A Case Study on *Ocimum basilicum* L.

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Although plastic pollution poses a major threat to terrestrial ecosystems, the effects of interactions between micro- and nanoplastics and plants, as well as their potential transfer into the food chain, remain poorly understood.

This study aimed to investigate, through an integrated approach, the effects of polyethylene micro- and nanospheres (MNPs) on *Ocimum basilicum* L. cultivated hydroponically. We analyzed biometric traits, cytological and physiological responses, and oxidative stress-related parameters. The experimental design consisted of two phases: a short-term and a long-term treatment. The applied MNPs concentration (0.1% w/v) and broad diameter range (200–9900 nm) were selected to simulate realistic environmental contamination, characterized by the coexistence of particles of varying sizes, as reported in the scientific literature.

To track their presence in plant tissues, MNPs were stained with Nile Red. In the short-term treatment, seeds were germinated for five days in an MNPs suspension. In the long-term treatment, seeds were initially germinated in the absence of MNPs; seedlings were then grown hydroponically for 20 days before being exposed to MNPs for an additional 15 days.

In both treatments, fluorescence microscopy revealed MNP adhesion to root tegmental tissues, their presence in the cortex, and the possible translocation of smaller particles into the vascular tissues. MNP adsorption onto root hairs likely reduced water uptake, thereby affecting plant physiology. Short-term exposure impaired root growth and meristem activity, with clear signs of cyto-genotoxicity and oxidative damage. Additionally, oxidative stress was observed in the shoots of treated plants, as indicated by elevated hydrogen peroxide levels.

Long-term exposure compromised physiological parameters, notably reducing CO_2 assimilation due to stomatal limitations, with consequent effects on photosynthetic efficiency. It also impacted biometric traits, including root length and biomass, leaf area, and dry matter content.

Oxidative stress and related damage increased in leaves, boosting phenolic compound production. Despite exhibiting lower hydrogen peroxide levels, roots experienced the most significant oxidative damage. The antioxidant response involved elevated levels of reduced ascorbate and glutathione, while proline content decreased in both leaves and roots.

These findings underscore the detrimental impact of MNPs on *O. basilicum* growth and development. Further research is essential to elucidate the mechanisms of MNP uptake and translocation, and to assess the potential risks posed by MNP contamination to plant health, food safety, and ecosystem integrity.

Populus alba (white poplar) Thiol-Peptide Compounds Allow Intracellular and Extracellular Cadmium Detoxification

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Cadmium (Cd) is a highly toxic, non-essential heavy metal, widely distributed in the environment due to natural and anthropogenic activities. Its high mobility and bioavailability make it easily taken up by plants. To cope with Cd toxicity, plants have evolved a range of detoxification mechanisms, including the chelation and compartmentalization of Cd by thiol-containing peptides such as glutathione (GSH) and phytochelatins (PCn). Previous work on the liverwort *Marchantia polymorpha* revealed a dual function of GSH and PCn in Cd detoxification, involving both intracellular complexation and extracellular release. Building on this evidence, we explored whether a similar strategy is adopted by *Populus alba*, a woody species known for its high Cd resistance and good potential for phytoremediation techniques. Briefly, we developed an HPLC-ESI-MS/MS method to quantify thiol-peptides in *P. alba* under both short- and long-term Cd exposure. Plants were assessed for biometric parameters, photosynthetic performance, and thiol-peptide profiles under control and stress conditions (0–50–100 µM $CdSO_4$ in solid medium over two months and 0, 5, 10, 20 and 36 µM $CdSO_4$ in liquid medium over 24–48–72 h). Our results show that *P. alba* can detoxify high Cd concentrations (50–100 µM) without significant effects on growth or photosynthetic efficiency. Under both long- and short-term Cd stress, PCn levels increased significantly, particularly in roots. GSH levels also increased, especially during early exposure times before declining at later timepoints, likely due to oxidation or conversion into PCn. Noteworthy, both GSH and PCn were detected extracellularly, with GSH levels comparable to intracellular concentrations, and PCn detected mainly as PC2. The extracellular presence of thiol-peptides supports the hypothesis of an active efflux mechanism. To explore this, we conducted inhibition experiments using sodium orthovanadate, a known plasma membrane transporter inhibitor. The treatment strongly suppressed GSH efflux, while only partially reducing PCn release, suggesting that these peptides may rely on distinct transport pathways. These findings demonstrate that *P. alba* performs a dual detoxification strategy, through intracellular chelation and vacuolar sequestration, as well as through extracellular extrusion of thiol-Cd complexes. This mechanism, previously described in bryophytes, now appears conserved in tracheophytes, highlighting the evolutionary significance of thiol-peptides in plant responses to metal stress.

Response to Heat Stress of Different Microalgae

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Human activities and economic development, with the use of fossil fuels, have had and continue to have a significant impact on the environment and the global climate. According to the United Nations Intergovernmental Panel on Climate Change (IPCC), global temperatures could rise between 1.8° C and 5.8° C, with more marked increments in high latitudes regions. Climate change affects significatively the health of both terrestrial and aquatic ecosystems. Among the various aquatic ecosystems, those most affected by climate change are lakes, especially the less deep ones that, because of their high surface/volume ratio, have higher hydrological sensitivity. When an organism perceives a stressful situation, a series of biochemical and molecular signals are activated and transmitted to the nucleus, triggering an appropriate response at cellular level. The present study focuses on the variations in morphological and biochemical characteristics associated with tolerance to heat stress in four freshwater microalgal species: two green microalgae, *Desmodesmus abundans* and *Chlorella vulgaris*, and two cyanobacteria, *Nostoc sp.* and *Anabaena sp.* The aim of these study is the safeguard of ecosystems and conservation of biodiversity, but it could also have potential biotechnological applications. The cultures were divided into three groups, those defined as control were maintained in a growth chamber at 23°C, while those defined as stressed were grown at two different temperatures, 28°C and 33°C. All cultures, both control and stressed, were grown under a light intensity of 2000 lux and a photoperiod of 16 hours in the light and 8 hours in the dark. Cell growth was analysed by counting cells in a Bürker chamber under an optical microscope. All experiments were conducted during stationary phase. Both the control and stressed cultures were observed under the confocal microscope using also colorant such as Fluorescein Diacetate (cell viability marker) and Nile Red (neutral lipid marker). After freeze drying, cultures were also subjected to biochemical analyses. Following heat stress, a reduction in cell density was recorded in all microalgae, particularly at 33°C, but with lower values for *Chlorella vulgaris*. From the morphological analyses, an increase in cell diameter was observed in *Desmodesmus abundans* and *Nostoc sp.* under heat stress conditions and a decrease in the number of cells per filament was recorded in both cyanobacterial species. Biochemical analyses of chlorophylls and carotenoids showed differences in all microalgae but with different trends and values. The results obtained so far suggest that, among all the species analysed, *Chlorella vulgaris* is the most resistant to heat stress. This work also shows how different species of microalgae respond differently to heat stress suggesting that climate change will affect species with different modalities over time.

Targeting Gene Expression and Culture Conditions to Enhance Fucoxanthin Production in *Thalassiosira rotula*

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Fucoxanthin (Fx), a high-value carotenoid derived from marine organisms, has attracted considerable interest due to its potential applications in the pharmaceutical, nutraceutical, and cosmetic fields. Attention in Fx biosynthesis is largely driven by its unique molecular structure—which cannot be replicated through chemical synthesis—making it a sustainable and environmentally friendly bio-compound, as well as by its essential biological functions. This pigment allows diatoms to efficiently absorb light across a broad spectrum—particularly within the blue to green/yellow wavelengths (460–570 nm)—and plays a crucial role in supporting cellular growth as well as adaptation to changing light conditions [1, 2]. In this study, the effects of different culture conditions were examined in the marine diatom *Thalassiosira rotula*, a species with high potential for biotechnological applications. Two treatments, specifically low light intensity and high nitrate availability, on biomass production and fucoxanthin accumulation were examined. Given the recognized health benefits and economic significance of Fx, its biosynthetic pathway has been the focus of intensive research, particularly in diatoms. Nevertheless, this pathway remains only partially understood, with several key enzymes still unidentified and uncharacterized [3, 4]. This study highlights the expression pattern in *T. rotula* of key genes in control condition as well as after the treatments, focusing more attention on the genes associated with the final steps of the Fx pathway, not yet completely characterized in diatoms. Furthermore, to investigate the potential for achieving high growth rates while promoting fucoxanthin accumulation, we have tested a two-phase cultivation approach in a short time, spanning just five days [5]. The dry weight, biovolume, and the Fx content were estimated and also the other pigments were analysed by HPLC. Globally, our results suggest that the culture condition and/or method established herein could represent a promising strategy to boost fucoxanthin production in *T. rotula*, which might prove to be a valuable natural source of commercial fucoxanthin.

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The Microalga *Neochloris oleoabundans* for the Re-Use of Fruit By-Products

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Emilia-Romagna is one of the leading fruit production regions in Italy, especially for pears, apples, apricots and peaches [1]. Closely linked to fruit production, the industrial fruit processing sector generates large quantities of by-products (fruit processing matrices – FPMs) every year, mainly composed of peels, seeds, and fruit pulp. These residues still contain nutrients, such as sugars, fibers, polyphenols and other bioactive compounds, but also possible traces of toxins or pesticides, so that they are generally treated as wastes with consequent environmental, economic, and logistical challenges, especially during seasonal production peaks. Within this context, microalgae are photosynthetic microorganisms well-known for their versatile metabolism, also linked to abilities in bioremediation practices [2,3], and can represent an attractive strategy for a sustainable re-use of FPMs as low-cost culture feedstocks, avoiding disposal. Indeed, depending on microalgae species, the sugars and nutrients contained in FPMs can be metabolized. Among microalgae, the chlorophyte *Neochloris oleoabundans* is known to be a mixotrophic alga able to exploit apple residues after vinegar production [2]. The present study aims to better explore its cultivation using fruit pulp wastes after the processing by a food industry. Pre-trials were set up cultivating the microalga in BG11 synthetic medium added with different doses (low, medium, high) of aqueous extracts of apple, apricot, peach and pear pulp wastes aiming at selecting the optimal type and concentration of the substrate for algal biomass production. Growth results showed that algae cultivated with pear extract at the medium dose reached a higher biomass production compared to both autotrophic control cultures and the other mixotrophic ones. Growth, morpho-physiological aspects, and biochemical composition of microalgae were investigated during cultivation. Significant differences in biomass production, and content in polysaccharides, photosynthetic pigments, total proteins, polyphenols and mycosporine-like amino acids between autotrophic and mixotrophic cells were revealed during cultivation. NMR analysis gave a first characterization of the spent culture media highlighting the specific sugars consumption, especially glucose. Investigation to assess the bioactivity of both algae and the spent medium is ongoing to find new effective biotechnological application of microalgae as bio-based solutions for the valorization of wastes.

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Enhancing *Emiliania huxleyi* Growth With Recycled Calcium From Oyster Shells

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Coccolithophores are a group of microalgae belonging to the Haptophyte that have the ability to produce scales of calcium carbonate, called coccolith, that play a crucial role in the ocean carbon cycle as a carbon sink. Moreover, coccoliths are currently being studied for a wide range of different applications, such as enzyme carriers, for drug delivery, in paper manufacturing and more. Despite their potential, coccolithophores are not currently exploited, probably because of low cells production and coccolith formation, since carbon is required for both photosynthesis and coccolith formation, and limitation can easily occur. However, Jakob and colleagues [1] proved that using $CaCO_3$ instead of $CaCl_2$ was enough to increase cell production and coccolith formation in *Emiliania huxleyi*, a model coccolithophore. Our aim is to further explore *E. huxleyi* potential applications, also using sustainable resources, such as bivalve shells deriving from food waste as a source of $CaCO_3$.

In this work, we studied the growth and coccolith production of *Gephyrocapsa huxleyi* CCAP920/8, formerly known as *Emiliania huxleyi*, using the pulverised shells of *Magallana gigas* (also known as Pacific oyster) as the source of $CaCO_3$ in ESAW medium enriched with f/2 nutrients. Culture media was modified by replacing $CaCl_2 \times 2H_2O$ with (a) pure $CaCO_3$, and (b) pulverised shells of *M. gigas* (composed of about 90% $CaCO_3$), maintaining the final Ca^{2+} concentration of 366 mg L^{-1}. Cell growth was monitored using Thoma counting chamber for 14 days. Morphological changes in cells and the presence of coccoliths were recorded by scanning electron microscopy. The concentrations of pigments, proteins and polyphenols were also evaluated. As expected, cultures grown in the presence of pure $CaCO_3$ reached a higher cellular density compared to the control cultures grown with $CaCl_2$. Remarkably, the cultures grown in the presence of $CaCO_3$ from pulverised shells of *M. gigas* reached the highest cellular concentrations, about 20-fold the concentrations of the control cultures and 4 times the concentrations of the cultures grown with pure $CaCO_3$. Moreover, cells cultured with the powder from shells of *M. gigas* were smaller (radius = 2.03 ± 0.37 μm) compared both to the control cultures (radius = 2.87 ± 0.34 μm) and the cultures grown in presence of pure $CaCO_3$ (radius = 2.62 ± 0.42 μm), but the pigment concentrations per cell did not differ. These results highlight the potential of food waste valorisation in promoting coccolithophore growth, paving the way for sustainable and cost-effective biotechnological exploitation of these algae.

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Root Meristem Organization is Associated with an Auxin-Related Determination of Redox Status

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Previous work has shown that the *Zea mays* quiescent center (QC), a population of slowly dividing cells in the root meristem just proximal to the root cap, contains relatively high levels of the oxidized forms of both ascorbic acid and glutathione, leading us to conclude that the QC must have a relatively oxidized status. Subsequent work, however, using a redox-reporting GFP (*roGFP*) to measure *overall* redox status (the redox potential) in *Arabidopsis thaliana* roots, showed that the QC maintained a relatively reduced redox potential compared to surrounding root cell types. Redox potentials involve electron transfers and are one of the most commonly occurring and important biochemical reactions. Based on the observation that the endogenous auxin maximum is highest in the QC, and thus coincident with the region of most reduced redox status, we hypothesized that there may be an association between endogenous auxin levels, redox potential, cell proliferation and organization of the root meristem. Here we show that perturbing auxin levels in the root tip results in marked changes in the redox patterns. Using the QC marker pWOX5H2BYFP, and several mutants (*Axr3/IAA17* [a gain of function mutant], plus *aux1-7* and *eir1* [auxin transport mutants]), we show that applied auxin/auxin analogues, depending on concentration, alter both the root redox potential and meristem patterning and organization. We conclude that the mechanism of auxin-regulated root development likely involves auxin influencing the root's redox potential profile.

Some Responses of the Maize Root to Mechanical Stress

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In field conditions, roots penetrate and explore the soil to grow and acquire water and nutrients. Growing roots sense mechanical force exerted by soil particles and obstacles, affecting their structure and functioning. Studies on the effect of mechanical stress on the *Zea mays* root concern different levels of organization and different root characteristics, like morphology, anatomy, and histochemistry, but also the mechanical properties of the root tissues. Here, the results of some of these studies are presented.

In the experiment examining the response of the maize root apical meristem (RAM) to mechanical impedance distributed circumferentially or applied from opposite sides, apex deformation and a change in RAM organization from closed to open were observed. The cessation of mechanical stress resulted in the restoration of both cellular organization and apex morphology to their original state.

Like in other grasses, the apical part of the maize root is covered by a two-layered deposit of extracellular material, named the pellicle, which, together with the outer periclinal wall of protodermal cells, forms the three-layered epidermal surface. Root apices subjected to mechanical impedance showed microdamage in all the layers of the epidermal surface, however, protodermal cells in the meristematic zone remained viable. This leads to the conclusion that the pellicle may play a protective role against the negative effects of mechanical stress.

Maize roots growing in media of different physical density and structure showed differences in measurable morphological traits, cell arrangement, and mechanical properties. The growth medium also affected the rate of maturation of exodermis and endodermis. Of the media used in the experiment, the one of high density and fine movable particles (soil) appeared to be most favorable for the maize root growth and development.

Based on the results of this fairly wide range of studies, it can be concluded that the root is an easily adapting plant organ, responding to changes in the environment at different levels of organization.

Vascular Cambium: The Key to Lifelong Adaptability in Woody Plant Roots

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Regarding the growth and development of the root system, literature from the past few decades has revealed the existence of very sophisticated regulatory mechanisms based on the movement of large molecules (such as small peptides, RNA, transcription factors, and plant hormones) from synthesis cells to target cells that are often very distant from each other. The availability of new techniques and *Arabidopsis* mutants has clarified that from the first division of the zygote until the completion of the primary and secondary structure of the root system, growth and development are regulated by two main mechanisms: 1) the homeostasis of a limited group of stem cells distributed in various positions of the body; 2) the ability of meristematic cells (or those that have reverted to such) to divide asymmetrically (in a periclinal or anticlinal transverse/radial direction). These mechanisms appear to be well conserved (albeit with some variations) in all representative organisms of different evolutionary lines and are "inherited" from the vascular cambium. In the overall development of woody perennial plants, the dogma dominates that the root architecture is determined by events (the emission of lateral roots) occurring only during the construction of the primary body due to the activity of some pericycle cells. Hence, the loss of the pericycle following the appearance of the vascular cambium makes alterations in root architecture possible only as a result of wounds and subsequent callus-induced rhizogenesis. This type of root system development in woody perennial plants openly contrasts with the development scheme of the aerial part, which can be modified throughout the plant's life due to the presence of latent vegetative buds generated during the development of the primary body. Our studies surpass the dogma and establish that architectural modifications can also occur when the root system is composed solely of secondary tissues. In this case, the production of new lateral roots seems to be entrusted to the vascular cambium, which would designate some initial cells to abandon their role as wood and phloem builders to become mother cells of a new root primordium. This event seems to be controlled by the same factors (transcription factors, hormones, small peptides) that were active in defining the root architecture of the primary structure. The cytological, histological, and anatomical differences found in the vascular cambium of roots of different plants suggest the possible existence of different types of vascular cambium, which could make the results obtained from studies conducted with model plants only indicative.

The Role of the *DEEPER ROOTING 1* (*DRO1*) Gene Family in Tomato (*Solanum lycopersicum* L.) Organ Development and Response to Drought Stress

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Drought stress is a major abiotic stress that significantly limits plant growth and development. Although plants activate various defense mechanisms, severe drought often exceeds their capacity to adapt [1]. The root system is the primary organ to perceive the water stress and, consequently, to modify its architecture in order to ensure the survival of the plant. In particular, the root growth angle has been shown to play a critical role in drought avoidance, influencing the plant's capacity for water uptake. The ability of roots to shape their growth angle is regulated by the *DRO1* gene family, which belongs to the IGT protein family [2]. Recent studies on *Oryza sativa* have shown that increased expression of the *DRO1* gene enhances root growth angle, improving the plant's ability to tolerate water stress [3]. In addition, overexpression of *DRO1* gene in *Arabidopsis* and in *Prunus domestica* led to deeper-rooting phenotypes [4]. In this context, the aim of this work was to functionally characterize the *DRO1* gene family in *Solanum lycopersicum* (L.), a globally important crop, and understand their involvement under water stress conditions. For this porpouse, a gene expression analysis was first performed in all tomato organs during different stage of plant development, and *in situ* hybridization was conducted on root and stem. Subsequently, the *SlDRO1* gene expression profile was analyzed in plants exposed to three different water stress condition for 10 days. The obtained results showed a distinct spatial-temporal expression profile of the *SlDRO1* gene in all tomato organs. In addition, the *SlDRO1* cyto-histological domains were confined throughout the meristematic zone, in the rhizodermis and in the vascular tissues within the root differentiation zone. In the same way, the *SlDRO1* transcripts were detected in the vascular tissue of the stem. Concerning *SlDRO1* gene expression under drought stress, our findings revealed an overall upregulation of *SlDRO1* across all plant organs, with a pronounced increase observed under conditions of extreme water stress. In conclusion, our findings reveal a distinct expression profile and spatial distribution pattern of the *SlDRO1* gene, supporting the hypothesis of its site-specific involvement in plant development and functional regulation. In addition, the upregulation of the *SlDRO1* gene in plants subjected to water stress suggests a possible involvement of this gene in the response *pathways* to drought stress.

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SlDRO1 as a Candidate Regulator of Root Adaptation Under Drought Stress in Tomato

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Drought stress poses a critical constraint on crop productivity, emphasizing the need for improved water-use efficiency without yield compromise. Modifying root system architecture through genes such as Deeper Rooting 1 (*DRO1*) offers a promising strategy for enhancing drought resilience. This study evaluates the role of *SlDRO1* in shaping root architecture and conferring drought tolerance in *Solanum lycopersicum* (cv. Micro-Tom).

Morphological and high-throughput phenotyping under drought revealed reduced total root length and fine root fraction, coupled with increased root mass ratio, indicating a shift in resource allocation. Despite prolonged drought (30 days), root surface area and fineness remained stable, as assessed by WinRHIZO and scanning imaging. Near-infrared (NIR) imaging via LemnaTec 3D Scanalyzer confirmed reduced water content, evidenced by elevated reflectance, underscoring the utility of spectral indices for drought assessment.

qPCR analyses showed that *SlDRO1* expression in roots remained stable early but was significantly upregulated at 30 days of drought, while stem expression declined. This tissue-specific expression pattern suggests differential regulation under drought, with potential functional relevance in adaptive responses. In situ hybridization confirmed *SlDRO1* localization in both tissues.

Ongoing development of knockout and overexpression lines aims to further elucidate *SlDRO1* function in tomato, marking the first such effort in this species. These findings provide novel insights into genetic determinants of root plasticity and lay the groundwork for breeding drought-resilient cultivars.

A Symbiotic MLO Gene Regulates Root Development via RALF-Triggered Ca^{2+} Signalling in *Lotus japonicus*

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Mildew Locus O (*MLO*) genes belong to a plant-specific gene family with pleiotropic functions in plant development, reproduction and interaction with filamentous microbes. They encode transmembrane proteins whose long-sought biochemical function has recently been unravelled as Ca^{2+}-permeable channels, activated by Rapid Alkalinization Factors (RALFs) in plant reproductive cells. Nevertheless, their involvement in Ca^{2+} signalling during plant-microbe interactions and root growth remains unexplored.

In this work, we investigated the function of a clade IV *MLO* (*LjMLO4*) in arbuscular mycorrhizal (AM) symbiosis and root development in the model legume *Lotus japonicus*. Although GUS staining showed a strong activation of the *LjMLO4* promoter in colonized cortical cells of mycorrhizal roots, no significant differences could be observed in *mlo4* mutants in comparison with wild-type plants regarding mycorrhizal colonization with the AM fungus *Rhizophagus irregularis*. On the other hand, the *LjMLO4* promoter was found to be active at the root tips and lateral root primordia, which aligns well with a striking root phenotype of the *mlo4* mutant. Indeed, *mlo4* plants exhibited shorter roots and reduced lateral root density, both in the presence and absence of AM fungi. Consistently with this phenotype, roots of *mlo4* plants showed decreased sensitivity to RALF treatment, as indicated by Ca^{2+} signalling and primary root growth. Furthermore, by heterologous expression of *LjMLO4* in aequorin-expressing *E. coli* cells, we validated the functioning of the encoded protein in Ca^{2+} mobilization across the plasma membrane. Overall, we provide novel insights into the role of a symbiotic *L. japonicus* MLO protein in regulating plant root development via RALF-mediated Ca^{2+} signalling.

New Insight into the Role of microRNAs on Wheat (*Triticum aestivum* L.) Seminal Root Development

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Winter wheat (*Triticum aestivum* L.) is one of the most widely cultivated crops in the world, but the current grain yield rate will result insufficient in the next decade. Thus, the aim of the present contribution is to investigate the regulative role of some miRNAs on the cellular and molecular processes which participate to winter wheat (Altamira variety) seminal root growth and function. Indeed, root represents a fundamental organ, and it is strongly related to crop yield and health status. Hence, it is important to reach a better comprehension of the phenomena linked to the developmental biology of seedlings from this species and to favour the design of novel biotechnological approaches able to promote their resistance, productivity and quality. An initial transcriptomic approach on roots from wheat seedlings grown for 4 and 8 days after seeding (henceforth DAS) revealed a modulation of some molecular pathways already known in the literature to be associated with root system architecture and function. To have a better overview on the expression of miRNAs during wheat root growth, a miRNome differential expression analysis was carried out at the same DAS. The results revealed the potential modulation of several pathways mediated by microRNAs and in line with the evidence obtained by transcriptomics. Our attention was focused on two specific miRNAs, miR398 and miR397, whose targets are Cu/Zn SOD (Cu/Zn Superoxide Dismutase, involved in the detoxification of superoxide anion) and Laccase 2 (Multi-domain cupredoxin oxidoreductase, involved in the lignification process and secondary cell wall biosynthesis) respectively. We found a down-regulation of both miRNAs during the root development (i.e., 4, 6, 8, 10 and 12 DAS), while the level of transcript for Laccase 2 remained stable and that of Cu/Zn SOD accumulated. These data suggested a different regulatory mechanism for the two miRNAs on their respective targets (i.e., in that order, inhibition of translation and degradation of the mRNA). These hypotheses were confirmed by evaluating the protein level of Cu/Zn SOD and Laccase 2 through Western Blotting analysis and the translational level of their transcripts by polysome profile analysis. Providing a more general overview of this kind of regulation, the activity of both these enzymes and the transcript level of other Laccases and SODs were also monitored, confirming the role of the two investigated miRNAs on their targets and not on other isoforms. Concerning the lignin biosynthetic process regulated by Laccase 2, the total level of lignin and the activity of the main enzymes involved in its production was measured by specific kits, confirming the involvement of miR397 in increasing the plasticity of root tissue during the development. On the other side, a decrease of the total antioxidant status, a reduction of the expression and activity of other antioxidant enzymes, and a specific distribution of radical oxygen species was registered. All these results evidenced how miRNA-linked changes in lignification and redox equilibrium are crucial to allow the correct cell division and differentiation of wheat seminal root.

A Novel Dual-Compartment Hydroponic System for Investigating Inter-Plant Root Signalling Responses to Water Stress

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Conventional experimental methodologies for investigating inter-plant communication often involve the compartmentalisation of root systems, altering physiological interactions. To overcome these limitations, an innovative hydroponic system based on a dual-compartment spatial configuration (Confined Sector - CS; Free Sector - FS) was developed. The 3D printed architecture allows for the unidirectional growth of roots from FS towards CS through non-linear holes across the separating septum, designed to enable root extension from FS while preventing intrusion from CS. The implementation of a slow and controlled drip irrigation system, combined with the use of a high-percolation substrate, enabled the selective application of water stress (20% polyethene glycol, ψ_w= -0.5MPa) to individual plants, while maintaining the continuity of root communication with co-cultivated conspecific or heterospecific plants. Two species were selected, *Solanum lycopersicum* L. and *Tagetes patula* L. Preliminary analyses of root exudates, volatile organic compounds obtained from the substrate, morphological plant traits (e.g. root and shoot biomass and length, total leaf area, root system morphology) are providing insights into the molecular mechanisms involved in inter- and intraspecific communication in response to water stress.

Effect of an Urbanisation Gradient on the Root Microbiota of *Celtis australis*

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According to recent estimates, nearly 70% of the world's population will live in urban environments by 2050. Urban green areas represent crucial components of modern cities as they can provide many ecosystem services. Urban trees can act as reservoirs for microbial species adapted to disturbed environments. Our research, carried out within the Italian National Biodiversity Future Center (https://www.nbfc.it), aims to study the microbiota of the European nettle tree (*Celtis australis* L.), a resilient Mediterranean endemic tree, commonly used in urban planning and forestry, by combining culture-dependent and -independent (DNA meta-barcoding) approaches across an urban gradient. In autumn 2024, the superficial young roots of seventy adult plants of *C. australis* were collected across an urbanisation gradient in Turin (Northern Italy) considering two distinct environments, urban parks and tree-lined avenues. The rhizosphere and endosphere compartments were separated and DNA was extracted to perform 16S/ITS amplicon sequencing to profile bacterial and fungal communities. In addition, bacteria were isolated from the rhizosphere and the endosphere of eight trees along the urbanisation gradient. This resulted in 250 strains that were taxonomically identified using MALDI-TOF. In parallel soil physico-chemical parameters, microbial respiration and enzymatic activities have also been assessed. Preliminary results show that beta-glucosidase activity is higher in tree soils along streets than in urban parks while other enzymatic activities and microbial respiration did not show any significant difference. This suggests that the urbanisation gradient does not have a strong impact on these general soil microbial parameters. Bacterial strains will be further characterized *in vitro* for assessing plant growth-promoting activities (e.g. phosphate solubilisation, siderophore production). Synthetic microbial communities will be created and tested on *C. australis* seedlings under controlled conditions. Abiotic stress factors present in cities, such as high temperature or presence of pollutants, will also be considered, in order to select the microbial consortia with the greatest positive impact on plant growth and health.

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Brassinosteroids and Jasmonates Counteract Arsenic Stress in *Arabidopsis thaliana* Differently Modulating Root System Plasticity

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Arsenic (As) is a widespread environmental contaminant. Its highly toxic inorganic forms, arsenate (As^{V}) and arsenite (As^{III}), are frequently present in soil and water and easily absorbed by plant roots, causing negative effects on plant growth and development. As^{III} reacts with sulfhydryl groups of enzymes and proteins, inhibiting cellular functions and causing plant death. As^{V} is an analogue of phosphate and competes with it in essential metabolic processes [1]. After root uptake, As^{V} is widely reduced to As^{III}, which becomes the prevalent form in the plant. Plant responses to different abiotic stresses involve complex interactions among phytohormones, including brassinosteroids (BRs) and jasmonates (JAs). However, to date there is a limited knowledge about the effects of BRs or JAs application to plants exposed to As pollution and no clear information concerning their interaction in plant responses to As$^{III/V}$ stress. The BR signaling is involved in controlling different aspects of Arabidopsis root development, i.e., stem cell activity in the apical meristem, cell elongation rate and termination of cell elongation [2]. Moreover, it is known that BR-induced stress tolerance is associated with increased antioxidant functions and enhanced response to heavy metal toxicity. Jasmonic acid methyl ester (JAMe) is one of the JA-related compounds involved in plant growth regulation and response to many stresses [2]. The role of JAMe in mitigating As-toxicity is well known for *Oryza sativa* [3], however, recent results reveal differences between the two inorganic As forms in the interaction with JAs, suggesting that similar variations might exist in Arabidopsis.

The aim of this research was to determine synergistic or antagonistic roles of JAMe (0.01 µM) and BRs, provided as epibrassinolide (eBL, 0.01 µM), in mitigating the damages induced by As (As^{III}, 25 µM $NaAsO^2$; or As^{V}, 100 µM $Na_2HAsO_4 \cdot 7H_2O$) in *Arabidopsis thaliana* root system development. Results showed that As^{III}, differently from As^{V}, strongly reduced the elongation of the primary root (PR), and increased the formation of lateral and adventitious roots (LRs and ARs), mainly when combined with eBL. The quiescent center in PR, LR and AR apices was irregular under As^{III} more than As^{V}, and eBL enhanced the formation of regular LRs and ARs to counteract As^{III} toxicity. The pericycle periclinal proliferation leading to xylogenesis was positively mediated by JAMe, as alternative defense-strategy against As^{V}. Altogether, data show that BRs and JAs act on different aspects of the changes in root architecture caused by As, enriching or fortifying the root system, respectively.

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An Emerging Approach on Plant-Microbe Interaction under Abiotic Stress Conditions

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The rhizosphere is a dynamic terrestrial ecosystem where plant-soil-microbe interactions have an essential impact on global nutrient cycles. In general, plants are able to attract or repel microorganisms through the production of enzymes or exudates, as well as altering their surroundings. At the same time, microbes have characteristics that help plants to manage stress and survive. However, the persistent presence of these soil organic and inorganic contaminants in the environment endangers diverse plant and microorganism populations, disrupting biocenoses and compromising ecosystem stability. The impact of heavy metal concentrations on rhizosphere interactions has recently been studied in part by combining spatial mapping of enzyme activity (zymography) with analyses of specific root features and structural/functional diversity of microbial communities. However, obtaining a full understanding of plant-microbe interactions and dynamics under organic contamination has proven to be a substantial issue. With this in mind, the Rhizo_Tongue project [funded by the European Union - Next-GenerationEU - National Recovery and Resilience Plan (NRRP) - MISSION 4 COMPONENT 2, INVESTIMENT N. 1.1, CUP: H53D23005300006] aims to observe the rhizosphere environment as a single dynamic entity, analyzing to what extent the interactions among different organisms (plant-bacteria-fungi, in a three-way model) diverge under soil contamination with organochlorine pesticides (OCPs). The Rhizo_Tongue research intends to investigate these intricate connections using *Arabidopsis* plants grown on degraded soil, with and without the addition of organic contaminants and specific microbial communities. Part of the research will examine how the substances released by the plants affect the surrounding soil environment by using a 2-D zymography technique to find and measure the activity of three specific enzymes (β-glucosidase, leucine aminopeptidase, and acid phosphatases). Understanding variations in the intricate spatial gradients of enzyme activity within the *Arabidopsis* rhizosphere will be crucial for discovering mechanisms governing root-microbe interactions and ensuring long-term plant ecosystem services.

Bio-activated Biochar for Recovering Degraded Soils: Response of Forest and Agri-food Species

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The restoration of degraded soils is a critical ecological challenge with significant implications for environmental conservation and agri-food production. In this context, biochar - a material obtained through the pyrolysis of plant biomass - emerges as a promising solution for soil restoration due to its porous structure, chemical stability, and potential as a microbiome carrier in the bio-activation process. This study investigates the effectiveness of bio-activated biochar in enhancing the physical, chemical, and biological properties of soils, particularly by promoting microbial biodiversity and plant growth.

Aleppo pine (*Pinus halepensis* Mill.) and tomato (*Solanum lycopersicum* L. cv Roma) were selected as model species representing the forestry and agri-food sectors, respectively, to assess the impact of bio-activated biochar on degraded agricultural and urban soils. The experimental design included the inoculation of raw biochar into healthy soils (an healthy well-managed agricultural soil and a natural forest soil) for several months to enable bio-activation through the enrichment of native microorganisms and nutrients. Subsequently, a pot experiment was conducted in a controlled growth chamber for both species. Degraded soils were amended with either non-activated or bio-activated biochar to evaluate effects on soil quality and plant development. After 85 days of growth for *Pinus halepensis* and 30 days for *Solanum lycopersicum*, seedlings were harvested. Analyses included biomass measurements (leaves, stems, roots), root morphological traits (e.g., total length, diameter distribution), aerial plant characteristics (e.g., leaf surface area, plant height), and assessments of soil and rhizosphere microbiomes.

Preliminary results suggest that both types of biochar can variably influence soil properties and plant growth, depending on soil type, with important implications for the ecological restoration of degraded soils.

Aquatic Moss as New Multipurpose Live Biomass: From Biofiltration to Bioproduction

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Due to their extraordinary surface extension, aquatic mosses *Taxiphyllum barbieri* and *Leptodictyum riparium* perform very well in N metabolization without preliminary microbiological activation and in capturing HMs and metalloids as well as trapping NPs. Therefore, aquatic moss biomasses are gaining attention to be considered as valuable and efficient biofilter media applicable in many different scenarios.

At the same time cultivation of the biomass may be a limiting factor since growth is relatively slow. In our work we evidence how the moss cultivation may also be part of a productive chain where biomass synthesize valuable products while gaining mass.

First, we identified plant growth regulators with significant biostimulatory effects. Secondly, we are exploring the possibility to genetically engineer these organisms to create innovative and efficient molecular farms.

Monitoring Microbial Communities in Urban Soils: Insights into Biodiversity and Antibiotic Resistance Between Bulk Soil and Plant Rhizosphere

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Urban soil pollution is an increasingly pressing issue due to its adverse effects on both environmental health and human being. Anthropogenic pressures are leading to the accumulation in soils of both well-known pollutants and emerging contaminants, such as antibiotics, which can reduce soil biodiversity and cause the spread of antibiotic resistance (AR) phenomenon, posing a serious threat to public health. In this context, Nature-Based Solutions (NBS), involving the synergistic use of plants and their associated microbial communities, offer a sustainable remediation strategy.

In this preliminary study, we investigated microbial communities in an urban brownfield located near a disused railway yard in Milan, Italy. Bulk soil and local plant rhizosphere samples were collected from fifteen representative points across the site. Culturable bacteria were quantified using the plate count method and the cultivated communities were investigated molecularly through next generation sequencing (NGS) of the hypervariable regions of the 16S rRNA gene. Approximately 300 bacterial strains were isolated and characterized at both phenotypic and molecular levels. Gram staining revealed a predominance of Gram-negative bacteria (54%) over Gram-positive (46%), with morphological diversity including bacilli (71.4%), cocci (4.8%), coccobacilli (16.2%), and filamentous forms (7.6%). Microbial abundance was markedly higher in plant rhizosphere samples compared to bulk soil. In contrast, the Shannon and Simpson biodiversity indices, calculated from both NGS data and culturable isolates, indicated greater microbial diversity in bulk soil than in the rhizosphere, reflecting the selective pressure exerted by root exudates that may favour specific microbial taxa. At the same time, to evaluate the presence of antibiotic-resistant bacteria, all isolates were tested against five antibiotics (Ampicillin, Tetracycline, Rifampicin, Gentamicin and Vancomycin) across a concentration gradient (0.2 $\mu g\ mL^{-1}$ to 10,000 $\mu g\ mL^{-1}$). Survival rates, monitored at 28°C after 24/48 hours, confirmed the widespread presence of antibiotic-resistant bacteria, with 56.8% of isolates exhibiting high levels of resistance. In fact, the complete bacterial mortality was observed only for Vancomycin at the high concentration of 3,000 $\mu g\ mL^{-1}$.

These highly resistant strains were significantly less frequent at sampling points with greater plant abundance, suggesting that some environmental factors, such as root exudate and microbial interactions, may influence AR distribution and persistence. Moreover, it's noteworthy that Sanger sequencing of some of these multidrug-resistant bacteria isolated from both bulk soil and plant rhizosphere revealed functional traits involved in plant growth promotion and pathogen suppression (*e.g.*, *Pseudomonas brassicacearum*, *Peribacillus frigoritolerans*), with no evidence of infectivity toward humans. Our research provides preliminary insights into microbial dynamics and antibiotic resistance in urban soils. It emphasizes the ecological importance of rhizosphere processes and supports the development of Nature-Based strategies for urban soil restoration, aimed at enhancing microbial and plant biodiversity, mitigating contamination, and promoting overall soil health.

Adaptive Responses of *Triticum dicoccoides* and *Triticum durum* to Natural CO_2 Enrichment: A leaf anatomical and Physiological perspective

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How plants regulate gas exchange and photosynthesis is crucial, especially given the current rise in atmospheric carbon dioxide ($[CO_2]$) levels beyond pre-industrial times. Responses to CO_2 ($e[CO_2]$) enrichment include the regulation of stomatal conductance (g_s) and changes in leaf morphology, particularly regarding stomatal density (SD) and size (SS). An active stomatal control is fundamental to successful plant stress responses. Stomatal conductance (g_s) can be regulated by modifying stomatal morphology, such as size and density in newly developing leaves, or by physiologically adjusting the openings of stomatal pores. Despite ongoing research, the relationship between anatomical structure and physiological function remains complex and not yet fully understood, especially under long-term conditions and in natural environments. In this study, we investigate the coordination between leaf anatomical traits, such as stomatal density and size, and physiological responses, including stomatal conductance and photosynthesis in *Triticum dicoccoides* and *Triticum durum landraces*. These two varieties were grown near Rapolano Terme (Siena), where there are natural CO_2 outgassing sources. This environment offers a stable and natural gradient of $e[CO_2]$ (up to 3000 $\mu mol\ mol^{-1}$), enabling the observation of physiological and anatomical adaptations under realistic yet controlled conditions. The objective of this study is to: (i) evaluate changes in stomatal density and size in response to varying levels of CO_2; (ii) examine the relationship between leaf morphological traits and gas exchange performance under natural CO_2 enrichment; (iii) compare the responses of *Triticum dicoccoides* and *Triticum durum landraces* to identify potential adaptive differences; and (iv) investigate the relationship between leaf morphological traits and photosynthetic performance under natural CO_2 enrichment. This study's results will offer insights into the relationship between morphology and physiological adjustments. Grasping this connection is essential for understanding how crops may adapt to future climate scenarios. Ultimately, this knowledge could contribute to the development of more resilient plants in the face of global change.

Modifications of Traits of the Ni hyperaccumulator *Odontarrhena chalcidica* in Relation to Different Plant Provenience and Cultivation Regimes

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Nickel (Ni) plays a crucial role in the current economy, mainly serving as a key component in producing stainless steel and lithium-ion batteries. However, the traditional extraction of nickel from ore can be energy-intensive and significantly contributes to greenhouse gas emissions. A promising alternative currently being developed is agromining. This innovative process leverages hyperaccumulator plants to extract and recover metals directly from the soil, offering a more sustainable and eco-friendly method for metal production. Hyperaccumulators can remarkably uptake elevated concentrations of metals, such as Ni, from the soil. These metals are subsequently translocated to the plant's aerial parts, frequently exceeding the hyperaccumulation threshold, defined for nickel as concentrations higher than 1,000 $\mu g\ g^{-1}$ in shoots dry weight. Notably, species from the genus *Odontarrhena*, especially *Odontarrhena chalcidica*, have proven exceptionally efficient at Ni uptake. This species is particularly significant in Europe, where its prevalence and high hyperaccumulation capacity position it as a candidate for agromining. The aims of this study are: 1) to compare plant traits of four genotypes of *O. chalcidica* belonging to four different accession sites scattered across West Macedonia and Epirus (Greece); 2) to assess the consistency or modifications of traits in different cultivation regimes: aeroponics, hydroponics and pot.

This comparative approach allowed the assessment of plant morphological and physiological traits while quantifying their Ni accumulation capabilities in roots, stems and leaves.

Morphological traits (plant height, projected ground area, stomatal and trichome density, specific and total leaf area, leaf fresh/dry weight, biomass of roots/stems/leaves, number of secondary branches, and main stem length) and physiological traits (chlorophyll content, photosynthetic parameters: assimilation rate, transpiration rate, and stomatal conductance) were evaluated. Ni content was determined through monochromatic X-ray fluorescence (XRF).

The results showed great phenotypic diversity among the four genotypes and demonstrated consistency in almost all plant traits across different cultivation systems. Hydroponics and aeroponics emerged as reliable methods for phenotyping this species. The only exceptions were biomass yield and Ni accumulation, where hydroponics consistently yielded the best performance for these plants. The exceptional Ni accumulation capabilities make *O. chalcidica* a valuable candidate for future genetic research into hyperaccumulation traits, paving the way for developing optimized genotypes that excel in biomass productivity and metal extraction efficiency to be used for agromining.

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Agrivoltaics as a Sustainable Strategy to Enhance Food Security under Water Scarcity

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In recent years, European policy has intensified competition for land between agriculture and renewable energy to promote decarbonisation. Encouraged by energy policies, many farmers have switched from food production to photovoltaic energy for economic gain. This creates a paradox: policies designed to support sustainable energy may inadvertently threaten global food security. Agrivoltaic systems offer a promising solution to reconcile the growing demand for renewable energy with the need to ensure food security in the context of climate change. By integrating photovoltaic (PV) panels into agricultural landscapes, agrivoltaics enables the simultaneous production of electricity and crops, thereby mitigating the trade-offs typically associated with land-use change. Using a transdisciplinary approach, this research investigates the effects of panel-generated shade on crop yield, food quality, water use and ecosystem services, focusing on chicory and tomato crops under different irrigation regimes. In the case of chicory, the study was conducted over two different growing seasons - spring-summer and winter-spring - and showed consistent results in terms of increased biomass production under agrivoltaic shade in both periods. Edible biomass increased by up to 69% under high irrigation and 23% under low irrigation conditions, confirming that the shade system can effectively increase yield regardless of the seasonal cycle. In addition, improved pollination services and physiological responses such as Shade Avoidance Syndrome (SAS) were observed, without compromising food quality (e.g. chlorophyll, carotenoids, antioxidants) in the hot season, while there was little difference in the coldest season. In the tomato crop, although the number of fruits under PV panels decreased, the fruits were larger, had higher water content and better Brix levels under normal irrigation, in line with commercial quality standards. These results suggest that agrivoltaics can support crop adaptation to climate stressors such as drought and heat waves, while maintaining or improving market-relevant traits. Crucially, agrivoltaic systems significantly reduce water use, by up to 50%, without compromising productivity or nutritional quality, representing a resilient and sustainable agricultural practice. These findings highlight agrivoltaics as a key strategy to address the twin challenges of energy transition and food security, contributing to the Sustainable Development Goals and the 2030 Agenda by optimising water resources, enhancing agro-ecosystem services and increasing resilience to climate change.

Evaluation of gadolinium effects on *Solanum lycopersicum* L. cultivated in a hydroponic system

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Gadolinium (Gd) is a lanthanide used in medicine as contrast agent in magnetic resonance imaging. Gadolinium-based contrast agents (Gd-BCA) are expelled through urine and discharged into surface waters. Currently wastewater treatment plants can't remove efficiently Gd-BCA leading to increasing concentrations into the environment. For this reason, our study aimed to assess the effects of Gd on *Solanum lycopersicum* L. (cv-Microtom) plants. The plants were grown in two hydroponic systems: the first one (control plants) with a Hoagland solution and the second one (treated plants, Gd150 µM) with a modified Hoagland solution and Gd. After about three months, morphometric parameters (*e.g.* leaf area, stem length and biomass, root length and biomass) were analysed and the content of Gd and calcium (Ca) in the biological samples were determined. Also, the antioxidant enzyme response was evaluated. Treated plants showed an inhibition of growth with reduced leaf area, stem length and root biomass. The effects of Gd were particularly evident on root system: the morphology of treated plants was altered by the exposure, with a significantly reduced root length. At the same time, while CNT exhibited both smaller and larger roots, Gd150 µM had only roots with a smaller diameter. The production and the weight of fruits also changed due to Gd exposure. Acid digestion of biological samples revealed how Gd was mostly accumulated by roots, while small amounts were found in leaves and stems. Hydrogen peroxide content didn't show statistically significant differences among treatments both in leaves and roots; instead, catalase activity was higher in the leaves of control plants, while it was higher in the roots of treated plants. Malondialdehyde levels were found in leaves, particularly of control plants. The results obtained so far suggest that Gd negatively affected plants growth, in particular roots growth and morphology. Thinner roots are more prevalent in both CNT and treated plants, although the CNT group exhibited even larger diameters. Direct exposure to Gd also changed oxygen reactive species production in roots which showed the highest concentration. Only malondialdehyde had higher concentration in leaves that are naturally a primary site of production. In conclusion, tomato plants were severely affected by the exposure to Gd, and plants growth and morphology were strictly compromised due to the stress condition.

Developmental dynamics of *Arbutus unedo* L. flower: morphological and molecular evidence

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Arbutus unedo L., also known as strawberry tree, is a Mediterranean plant belonging to the Ericaceae family. The complete development of its bell-shaped and pinkish-white flower takes a very long time (8-12 months), compared to other species. This unusual phenomenon suggests the induction of finely regulate molecular processes able to induce a biological slowdown; thus, the present contribution aims to bridge this gap never investigated before.

Seven different flower stages (St1-St7), from meristematic buds to anthesis, were investigated. Histological observations allowed to characterize the growth phases of the reproductive structures, highlighting how anthers required less time than ovules to differentiate and the existence of a prolonged quiescent state for the latter.

To understand whether phytochemicals could play a signalling role in this context, due to their potential effects on hormones, proteins and DNA, total and specific quantitation of these compounds was performed by spectrophotometry and targeted (HPLC-DAD) and untargeted (GC-MS) metabolomic approaches. The content of the investigated secondary metabolites tended to decrease from St1 to St7, except flavonols which showed a zig-zagged trend. This evidence was confirmed by qPCR assays used to estimate the expression profile of genes involved in phenylpropanoid and flavonoid biosynthetic pathways.

By fluorescence histochemical investigations, flavonols (i.e., kaempferol and quercetin) were observed to be accumulated in a time- and space-dependent manner, even changing their subcellular localisation. Indeed, a specific distribution of these compounds within the nuclei of ovule and ovary mature tissues were detected, maybe due to their possible role in the modulation of gene expression.

In parallel, as flowering process requires a tight control of the DNA expression mechanisms, the regulatory network modulated by microRNAs (miRNAs) and involved in the control of bloom time and induction of floral competence was explored. The expressed miRNome from 4 flower stages (i.e., St1, St3, St5 and St7) was subjected to Next Generation Sequencing. A total of 161 miRNAs were identified and 34 of them resulted significantly differentially expressed among the samples. After validation of this evidence by qPCR, bioinformatics tools were applied to predict the putative mRNA targets for these miRNAs and to carry out a Gene-Ontology enrichment analysis.

Altogether, these data suggest that the maturation dynamics of the megagametophyte, potential unconventional functions of flavonols, and the predicted miRNA-induced molecular processes may represent key factors influencing the timing of development in *A. unedo* flower.

Sulfur metabolism and chromium tolerance in *Scenedesmus acutus*: the role of OAS-TL and Cysteine Desulfhydrase activities

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In plant and algal cells, the sulfur metabolic pathway is critical for mitigating damage induced by heavy metals, a process known as sulfur-enhanced defense (SED). Previous research conducted on two strains of *Scenedesmus acutus*, wild type (wt) and chromium-tolerant strain (Cr-t), evidenced a transient tolerance increase in both strains after a period of sulfur starvation, related to the enhancement of sulfur uptake following nutrient re-supply and leading to an increase in cysteine content, significantly higher in the Cr-t strain [1]. At the core of sulfur metabolism is the cysteine synthase complex (CSC), composed by a hexamer of Serine Acetyltransferase (SAT) and two dimers O-acetyl-Serine (Thiol) Lyase (OAS-TL) [2], present in different cell compartments with different roles in cysteine homeostasis. While SAT in association with OAS-TL synthesizes OAS, the free OAS-TL dimer catalyzes cysteine synthesis from O-acetyl-serine (OAS) and sulfide produced by Sulfite Reductase (SIR). In *S. acutus*, we identified one SIR, two SAT and four OAS-TL isoforms showing different basal expression levels and inducibility by sulfur starvation being *SaSAT1*, *SaOAS-TL4*, and, notably, *SaOAS-TL2* the most inducible forms. Enzymatic assays confirmed increased OAS-TL activity following sulfur deprivation in both strains and a decrease after nutrient re-supply. SaOAS-TL4, phylogenetically closer to terrestrial plants, exhibited the highest basal expression and strongest induction under sulfur starvation, while *SaOAS-TL2* was not only strongly induced, but it was also the only OAS-TL isoform not expressed constitutively or expressed at near-undetectable levels. Phylogenetic analysis confirmed that SaOAS-TL2 is the most structurally divergent isoform, lacking the SAT-binding site and exhibiting differences in the active site responsible for sulfide interaction. These characteristics suggest its potential role as a free dimer or as an L-cysteine desulfhydrase supporting the recycle of organic sulfur during starvation. Its presence in non-photosynthetic organisms supports the hypothesis that this may be the cytosolic or mitochondrial isoform. Extra plastidic sulfide production can indeed be substained by OAS-TLs desulfydrase side activity [3] and from the activity of specific DES enzymes which can contribute to recycling organic sulfur. The analysis of L- and D-cysteine desulfhydrase activities revealed an increase following seven days of sulfur deprivation in both strains. Across all tested conditions, D-cysteine desulfhydrase activity was consistently higher than that of L-cysteine desulfhydrase, with the difference being more pronounced in the Cr-tolerant strain, especially under sulfur starvation. Notably, the expression level of the gene encoding D-cysteine desulfhydrase was significantly higher in the Cr-t strain than in the wt, suggesting a greater presence of D-cysteine in the Cr-t strain as a possible strategy of organic sulfur accumulation to be mobilized in response to stress.

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Influence of Endophytic Fungi Isolated from Mint on the Growth of Tomato Seedlings

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Plants demonstrate remarkable adaptability to diverse environments, primarily due to their plasticity and interactions with microorganisms, particularly bacteria and endophytic fungi. This study investigates the effects of four non-mycorrhizal endophytic fungi isolated from water mint (*Mentha aquatica*) on the growth of tomato seedlings (*Solanum lycopersicum*). In this experiment, tomato seedlings were inoculated with the mycelium of the selected endophytes, and their growth was evaluated through measurements of fresh and dry biomass, as well as root morphology. The results revealed that three of the endophytes significantly enhanced the dry weight of the tomato plants, while only one endophyte resulted in a significant increase in fresh weight. Additionally, two endophytes reduced root length, whereas the other two promoted the development of lateral roots. Specifically, isolate RT13 emerged as the most effective in increasing both fresh and dry weight. This study underscores the potential of non-mycorrhizal endophytes to modulate plant growth, suggesting that direct contact with fungal mycelium could serve as a valuable inoculation method in agricultural practices and laboratory studies. Furthermore, the study also examined the impacts of fungal exudates on the development of tomato plants. The findings emphasize the necessity for further research to investigate how these morphological changes may enhance plant resilience to environmental stressors.

Plants and Bacteria Interaction in the Soil: Analysis of the Emitted Volatile Compounds

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Plants are naturally associated with beneficial microorganisms that play a crucial role in their growth and health. Specifically, bacteria inhabiting the root endosphere and rhizosphere promote the growth of the host plants, increase their stress tolerance and protect them from pathogens. In addition to those already present in seeds, specific bacterial strains are recruited by seedlings during growth, forming dynamic endophytic and rhizospheric communities that are shaped by environmental conditions. Although various factors are known to contribute to these biological interactions, the mechanisms underpinning communication between plants and bacteria in the soil, essential for root colonization, remain poorly understood. This study aims to identify the volatile compounds (VOCs) emitted by roots and bacteria in soil and to investigate their role as signaling molecules mediating plant-bacteria communication, which is fundamental to establishing specific endophytic and rhizospheric communities during seedling development. To achieve this, a rectangular steel pot (60 cm in length) was filled with autoclaved sand. *Brassica juncea* seeds were sown at one end, while a consortium of selected plant growth-promoting bacteria (PGPBs) was inoculated at the center of the pot. A distance of 10 cm separated the inoculated area from the plant growth area. Teflon tubing was installed to explore the soil and collect emitted VOCs into glass vials from three zones in the pot: the plant area (PA), the inoculated area (IA), and the area opposite the plants containing only sandy soil (SA). Volatile compounds emitted by roots and bacteria were identified over time. VOCs in the vials were trapped by head-space solid phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS). To assess potential VOC-mediated bacteria migration in the soil, soil samples from the PA, IA, and SA zones, as well as intermediary regions between PA and IA (PA-IA) and between IA and SA (IA-SA), were collected at the same time points as the VOC analyses. Bacterial communities in these soil samples were quantified using fluorescence microscopy and characterized via high-throughput 16S rRNA gene Illumina sequencing. Additionally, the initial bacterial seed community and the root-associated endophytic and rhizospheric bacterial communities established by the end of the experiment were analysed.

Preliminary results indicate that during the first 24 hours post-sowing, when the majority of seeds germinate and radicles elongate, viable bacteria were exclusively identified in the IA and PA zones. At this stage, VOCs were also detected solely in these two zones; however, their profiles differed significantly between PA and IA. By 72 hours, viable bacteria were additionally observed in the PA-IA, IA-SA, and SA zones, suggesting the migration of PGPBs from the central region toward the extremities of the pot. At this point, the diversity of VOCs increased, and based on their putative functions, these compounds could be categorized into two groups: (1) those involved in plant-microorganism interactions and (2) those associated with plant development. By the conclusion of the experiment (5 weeks), specific VOCs were detected within the roots, indicating the establishment of a symbiotic relationship. The bacterial community composition across different soil zones, the rhizosphere, and within the roots will provide insights into how *B. juncea* modulates its root microbiota and the functional roles of the emitted VOCs.

Specificity of Elementomic Profiles and Photosynthetic Efficiency in Two Red Chicory (*Cichorium intybus* L.) Cultivars in a Coastal Environment

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Red chicory (*Cichorium intybus* L. var. *latifolium*) is a widely cultivated crop in Northern Italy, representing a key element of the local cultural heritage and an economically relevant product, particularly due to the Protected Geographical Indication (PGI) associated with specific varieties in the Veneto Region, including Chioggia and Treviso cultivars. Originating from PGI sites, the cv. Chioggia and, more recently, the cv. Treviso are now also cultivated along the Emilian coast below the Po River delta. Considering the strong specificity of the sandy territory, two cultivars could differ in their elementome, which depends on the soil-to-plant continuity and overall plant performance.

Plants of Chioggia "precoce" and Treviso "precoce" were analysed in the field (Mesola, Ferrara) in December 2023 with respect to some photosynthetic parameters using two portable fluorimeters, MultispeQ and Handy-PEA, and were subsequently sampled for laboratory analyses of trace and ultra-trace elements in different plant organs (roots, stem, outer leaves and inner leaves) in comparison with soil values. Elemental analyses were performed with inductively-coupled plasma mass spectrometry – triple quadrupole (ICP-MS/QQQ).

Both varieties exhibited soil-to-root bioconcentration factors (BCF) greater than 1 for P, Mo, and Cu. However, the cv. Chioggia showed a significantly higher BCF for Mo (~9) compared to cv. Treviso (~5), which suggests a more efficient response of the former against the Na enrichment in the coastal environment (Busoms et al., 2023). Moreover, cv. Chioggia was particularly efficient in transferring Group II elements of the periodic table (Mg, Ca, Sr, Ba) and transition metals (Ni, Cr) to the leaves. In the inner leaves, both varieties exhibited a preferential translocation of essential elements such as Mg, K, P, Ni, and Zn. Collectively, in leaves of cv. Chioggia the geochemical results indicated higher concentrations of elements critical for photosynthesis, including Mg, Fe, Cu, P, and K. This was consistent with the functional data, revealing in cv. Chioggia an overall higher performance than cv. Treviso, including an enhanced excitonic connectivity of photosystem II (PSII) and a higher efficiency of photosynthetic electron transport.

Among (ultra)trace elements, the profile of the rare earth elements (REEs) was also analysed. Devoid of any specific metabolic role, REEs tend to be absorbed proportionally to their concentration in soil and, therefore, can facilitate a comparison of the cultivars' absorption capacities. The REE profiles showed a preferential uptake and translocation of light REEs (LREEs) over heavy REEs (HREEs), especially in cv. Chioggia, which exhibited slightly higher BCF values for LREEs, confirming a more efficient elemental acquisition than cv. Treviso.

In conclusion, the specificity of photosynthetic and geochemical traits in the two cultivars is strongly indicative of the better adaptation of cv. Chioggia to the Emilian sandy coastal environment than cv. Treviso. Contrastingly, the PGI regulation for the latter expresses a preference for soils with a clay component and in a state of decalcification. We argue that the process of empirical selection that has been going on for decades in Mesola on the cv. Treviso is promoting its specific adaptation to the saline, slightly alkaline coastal soil and is likely to lead to a distinct cultivar.

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Assessment of Genetic and Morphological Diversity in *Tilia* spp. inside the Campus of the University of Salerno (Italy).

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The alteration of habitats, due to continuous and impactful anthropogenic activities, poses a significant threat to the survival of both plant and animal species but also of all living beings in general. These multitude of anthropogenic activities have attracted increasing attention of the scientific community just to propose new solutions to preserve the natural environment, and, therefore, improve the strategies to envelope the biodiversity. The aim of our study was to evaluate the biodiversity within the *Tilia* genus present inside the Campus of the University of Salerno (Italy) through the combined use of molecular markers as the simple sequence repeats (SSRs) and the biometric analysis of informative morphological traits. The research involved 60 georeferenced linden trees, grouped into twenty Sampling Units (SUs) identified within the study area. Genetic characterization was carried out via DNA extraction followed by PCR amplification using six polymorphic nuclear microsatellite markers (Tc915, Tc31, Tc11, Tc6, Tc937, Tc7). The alleles detected at each SSR locus were encoded into a binary presence/absence matrix and subsequently subjected to numerical taxonomy analysis using dedicated software tools. A phylogenetic similarity dendrogram was constructed to evaluate the genetic similarity/diversity among linden individuals. The samples clustered into two main groups, one of which was further subdivided into two subgroups, revealing an overall similarity of 75% among all individuals. Morphological analyses were conducted based on specific morphological characters, including leaf margin and shape, fruit shape and size, apical bud size and color. The morphometric trait analysis showed the presence of three Tilia species and one hybrid were identified and more specifically as: *T. cordata, T. platyphyllos, T. dasystyla* and *T. × europaea*. The patterns of genetic similarity revealed by means of molecular markers were consistent with the results obtained with the morphological traits. SSR analysis indicated a low level of polymorphism present in the studied linden population. Despite the limited genetic diversity, notable differences were observed in the species frequency. Trees identified as *T. platyphyllos* and *T. cordata* were less frequent compared to *T. × europaea* and *T. dasystyla*. This concordance was further supported by the variations in lichen biodiversity observed on the bark of the trees. As well-documented by several studies, the presence and frequency of epiphytic lichen can be influenced by bark pH. In the investigated area, which is a remarkable phenomenon for the "place" where it happened in, as in the case of SU2 and SU15, dominated by *T. dasystyla*, characterized by low lichen biodiversity. Conversely, a higher lichen biodiversity was recorded in those sites where *T. × europaea* was the prevalent one. Our study contributes to the assessment of genetic diversity present among the collected linden individuals of the UNISA Campus and highlights the importance of implementing measures to enhance plant species diversity in urban environments but also use plant species which can serve as more indicative biomonitors.

Bioprospecting the Italian Flora for The Discovery of New Bioactive Phytochemicals and the Exploration of Chemodiversity in Different Plant Clades

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With more than 11,000 taxa of vascular[1,2] and non-vascular[3] plants and a high level of endemism, the Italian flora is among the richest and most diverse in Europe. Spanning over 400 plant families, this remarkable biodiversity is reflected into a wide variety of plant specialized metabolomes, which constitute a largely unexplored molecular reservoir with a significant potential for bioprospecting. Furthermore, this diverse chemical landscape represents a valuable framework to investigate the chemo-evolutive dynamics in land plants, research still limited by the structural and biosynthetic complexity of plant metabolites.

In the frame of the National Biodiversity Future Center, we launched a large-scale bioprospecting plan aimed at exploring the phytochemical diversity of the Italian flora with two main purposes: the discovery of novel bioactive compounds/phytocomplexes for human health and sustainable agriculture, and the comparison of plant metabolomes within specific evolutive clades to gain more information on the diversification of plant metabolism.

Over a three-year sampling campaign, we collected more than 700 species – about 75% native and 25% alien – from botanic gardens, nurseries and open-field. This collection is a simplified model of the Italian flora, in which, at least for higher plants, we included all Italian plant families according to their real relative amplitudes (e.g., for the Angiosperms, in order: Asteraceae, Poaceae, Fabaceae, Rosaceae, etc.). The untargeted metabolomics analysis through UPLC-HRMS guides the selection of the species with the most interesting phytochemical profiles towards a downstream bioactivity screening program focused on non-communicable diseases (e.g. neurodegenerative diseases, cancer, metabolic syndrome, etc.) and crop enhancement and protection. Currently, we have profiled about 300 species and some of them have already shown, for instance, promising results against colorectal cancer (*Gratiola officinalis*[4]) and glioblastoma (*Succisa pratensis*[5]), and anti-genotoxic activities (*Cistus monspeliensis*[6]). In parallel to the phytochemical profiling, we are generating data for the relative comparison of the plant metabolomes from species belonging to the following clades: Asterids, Rosids, Monocots, Magnoliids, Gymnosperms, Lycophytes and Monylophytes, Bryophytes. Preliminary results for the Rosid clade (about 160 species in the collection) are shown.

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Engineering of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 for the Photocatalytic Production of Hydrogen

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In recent years, global warming and the energy crisis driven by fossil fuel usage have underscored the urgent need for the development of renewable energy sources. Hydrogen, as a green and zero-emission energy carrier, presents a promising alternative to fossil fuels and offers a viable solution to current global challenges [1]. Nevertheless, conventional methods, including water electrolysis powered by renewable energy, still suffer from limited thermodynamic efficiency and infrastructure costs [2]. In this scenario, the project “Enzimi artificiali per la produzione fotocatalitica di idrogeno in batteri fotosintetici” (ART-2-HYDROGEN), conducted by the University of Parma, aims to develop a sustainable and autonomous system for hydrogen production using engineered photosynthetic cyanobacteria. The primary objective of the project is the realization of a laboratory-scale prototype capable of efficiently producing molecular hydrogen using only visible light as an energy input. This approach offers a promising route towards scalable, eco-friendly hydrogen production, circumventing the limitations associated with conventional electrochemical methods that often require substantial electrical energy input, frequently derived from non-renewable sources. To achieve this goal, a plasmid containing genes for the expression of an artificial protein anchored to their outer membrane has been produced for the purpose of genetic engineering of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002. This protein is a component of artificial enzymes on cyanobacterial outer membrane. These latter are constructed via the SpyTag/SpyCatcher system and are functionalized with photochemical and redox catalytic centers. Preliminary results indicated that the engineered *Synechocystis* sp. PCC 6803 expresses the membrane protein linked to the SpyCatcher. The photochemical and redox catalytic centers will bind to the cyanobacterial cell wall through the SpyTag, optimizing interaction with photosynthetic electron donors. By overcoming the limitations of natural enzymatic systems and directly utilizing photosynthetic reducing power, this approach has the potential to establish a new class of sustainable biohybrid devices for clean energy applications.

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A Nature-Based Tool to Design Vegetation Barriers Along Road Infrastructure to Contain Anthropogenic Atmospheric Particulate Matter: Characterization of Leaf Functional Traits

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This work investigates the relationships between the leaves of plants with their functional traits and particulate matter retention by leaves applicable to phytoremediation and highlight which plant species would perform best in phytoremediation but especially the strong relationships that exist between the plant species and the functional traits of their leaves, specifically that of Trichome density and the Trichome surface area, demonstrating their strong correlation with PM phytoremediation. This work involved also an application phase with the Italian road manager company Anas s.p.a to design a Nature-Based Solution tool for roadside buffer area vegetation design. We profiled an equation to calculate the square meters of soil to be vegetated with a specific plant species as a function of certain parameters: The LAI, the Daily PM Uptake, and Total Trichome Surface area. This analysis will allow the company to understand how many m^2 of soil in a buffer area along a road infrastructure should be vegetated in order to minimize the PM produced and emitted by vehicle traffic traveling on the infrastructure.

Biochar and Compost Amendments Reshape the Rhizosphere Microbiome in a Mature Olive Orchard

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In recent years, the shift toward sustainable agricultural practices within the framework of the circular economy has become a pressing global priority. Among the approaches adopted to support this transition, biochar and compost have gained prominence as soil amendments due to their multiple agronomic and environmental benefits. Biochar, a carbon-rich material obtained by biomass pyrolysis, has been extensively explored for its ability to enhance chemico-physical soil properties, to increase crop yield, and to play as carbon sink. Compost, generated from the biotransformation of agri-food residues, also contributes to soil health by enhancing its structural, nutritional, and microbiological characteristics, thereby supporting long-term ecosystem resilience. This study was carried out in a mature olive orchard located in Fisciano (SA), Southern Italy, adjacent to the Campus of the University of Salerno. The olive trees (*Olea europaea* L.) were approximately 50 ± 3 years old, as determined from aerial photographic evidence from 1943, 1974 (no visible trees), and 1980 (canopy development observed). The orchard, neglected for several decades, experienced unregulated vegetative growth leading to canopy closure and vertical development (6.5–7.0 m), with the consequent senescence of shaded internal branches. Over a three-year period, the study evaluated the impacts of biochar and/or compost amendments on olive tree performance. A randomized block design comprising five experimental groups with five replicates each was established. Each treatment was applied to a delineated area (9.16 ± 0.15 m^2) around selected trees, maintaining a spacing of at least 6 meters among each treatment. Treatments included: biochar (20 Mg ha^{-1}), compost (38 Mg ha^{-1}), biochar plus compost (20 + 38 Mg ha^{-1}), mineral fertilizer [$NPK(SO_3)$ / 15-5-6 (26)] at 1.6 Mg ha^{-1}, and a non-treated control. Biochar was applied once at the beginning of the experimentation, whereas compost and fertilizers were supplied annually. Metagenomic sequencing of the rhizosphere microbial communities revealed that the most pronounced differences in a-diversity occurred between the control and mineral fertilizer experimental group. Significant shifts in microbial diversity were also observed in compost and biochar treatments, especially the combined application of them when compared to the other experimental groups. Notably, a-diversity was significantly greater in the control compared to the mineral fertilizer and biochar plus compost experimental groups, suggesting that these amendments did not enhance microbial richness in this specific soil. In summary, although the application of organic amendments and commercial mineral fertilizers did not provide any advantage in terms of species richness for rhizosphere microorganisms, the different soil treatments affected the presence and relative frequencies of the less represented OTUs.

Metabolomics, Melatonin Regulation, and Microbial Interactions in Lettuce Under Phosphate Stress.

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Reducing the environmental impact of agriculture while increasing efficiency without compromising productivity is a major challenge. Achieving this goal relies on different strategies, for example the efficient management of essential nutrients, such as phosphorous, and the use of plant growth regulators and biostimulants, including melatonin. Phosphorus, a key plant macronutrient, is commonly present in soils as phosphate ions, but only a small fraction is available to plants. To address possible phosphorus deficiencies, the common solution involves the intensive use of phosphate fertilisers, which contributes to significant pollution. As a possible alternative, using soil microorganisms capable of making the phosphate more available for plants represents a promising and sustainable strategy. Melatonin, an indoleamine compound, regulates several physiological processes in both vegetative and reproductive organs, including delaying chlorophyll loss during leaf senescence and improving fruit shelf life. It also plays crucial roles in plant stress protection, making it a promising compound for improving crop resilience and sustainability in modern agriculture.

This project aims to investigate i) the genetic and metabolic responses of lettuce (*Lactuca sativa*) to a soil microbial community under phosphate-limiting conditions, ii) the role of melatonin as plant growth regulator under low phosphorus stress and in microbe-plant interactions, and iii) the genes involved in the melatonin biosynthetic pathway.

The metabolic responses have been characterised by following an untargeted metabolomics approach with a UPLC-HRMS technique, highlighting the different modulation of specific secondary metabolites under defined biotic and abiotic conditions. The results about melatonin content, its involvement as regulator and the biosynthetic genes are under investigation. Preliminary results suggest the presence of melatonin at very low levels in lettuce leaves and the first gene of the pathway has been positively identified as a bona fide tryptophan decarboxylase (*TDC*) gene, which is involved in the production of tryptamine from tryptophan.

Light-Driven Modulation of Antioxidant Systems and Growth in Lentil Seedlings Under Red and Blue LEDs

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Light intensity and spectrum not only affect photosynthesis and photomorphogenesis in plants but can also induce photooxidative stress and modulate stress responses, triggering cascades of antioxidant defences. The red and blue regions of the spectrum are pivotal in driving photosynthesis and influencing metabolism due to the absorption peaks of photosynthetic pigments and the action of photoreceptors. Light-emitting diode (LED) technology has enhanced the precision of photobiological studies by enabling the customization of light regimes, and its applications are expanding rapidly.

This study aimed to elucidate how different light environments influence physiological, biochemical, and morphological parameters. We analysed the effects of narrow-band blue (BL) and red (RL) LED lights at intensities of 100, 300, and 500 μmol m^{-2} s^{-1} (two light qualities × three light intensities × continuous lighting) on the morpho-anatomical, biochemical, and molecular traits during the early growth stages of lentil (*Lens culinaris* Medik.). Blue light had a marked effect on proline content, which reached its highest levels under BL. The expression of ascorbate-glutathione cycle enzyme genes (APX, MDHAR, and DHAR) and the corresponding enzymatic activities generally showed stronger responses in BL-treated seedlings. Enhanced vascular tissue development (xylem differentiation) was also observed through anatomical analysis at the microscopic level. The interplay between light conditions and redox balance in plants is complex and multifaceted, affecting proper growth and development. Therefore, deepening our understanding of monochromatic light effects could improve crop management by helping growers optimise light environments to enhance yield, quality, and stress resilience.

Keywords: Lentil (*Lens culinaris* Medik.); antioxidants; blue light; red light, light intensity; enzyme activity; gene expression; anatomy

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