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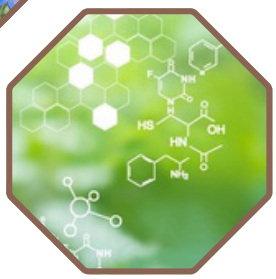
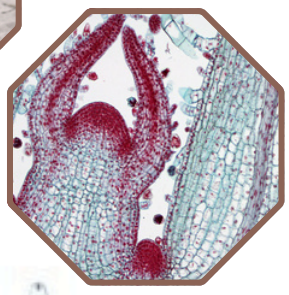

12-14 Giugno 2024
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BOOK OF ABSTRACT

Homeostasis of *cis*-OPDA amide conjugates upon stress responses

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Jasmonates (JAs) are a family of oxylipin phytohormones regulating many aspects of plant development and growth and mediating 'defense versus growth' responses. The upstream JA biosynthetic precursor *cis*-(+)-12-oxo-phytodienoic acid (*cis*-(+)-OPDA) has been reported to act independently of the JA signaling mediated by COI1 as an essential signal in several stress-induced and developmental processes. However, its means of perception and metabolism are unknown. Furthermore, OPDA, but not JA, occurs in lower plant species, such as bryophytes, exhibiting specific functions in defense and development. A few years ago, a low abundant isoleucine analog of the biologically active JA-Ile, OPDA-Ile, was detected in wounded leaves of flowering plants, opening up to the possibility that conjugation of OPDA to amino acids might be a relevant mechanism for OPDA regulation. We extended the analysis of amino acid conjugates of OPDA and identified naturally occurring OPDA-Val, -Phe, -Ala, -Glu, and -Asp. These conjugates accumulate upon wounding stress, homeostasis perturbation, and fungal pathogen infection in Arabidopsis. Members of the acyl acid amide synthetases belonging to the GRETCHEN HAGEN 3 (GH3) and the amidohydrolase IAA-LEUCINE RESISTANT 1 (ILR1) and ILR1-like (ILL) families catalyze the conjugation of OPDA to amino acids and hydrolysis of the OPDA-amino acid conjugates, respectively. Moreover, similar to free OPDA, some newly identified OPDA-amino acid conjugates show growth-inhibitory effects. Conjugation of OPDA with amino acid is a metabolic pathway that occurred early during plant evolution. We found it already operates in the lower land plant Physcomitrium patens and the gymnosperm Picea abies. Thus, similarly to other phytohormones, one level of regulation by which plants modulate OPDA homeostasis appears to be the synthesis and hydrolysis of OPDA-amino acid conjugates, which function in the temporary storage of OPDA in stress responses.

Key words: *cis*-(+)-OPDA, conjugation, hydrolysis, homeostasis, stress.

Modulating ABA response in plants through bioactive cyclic peptides

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Climate change represents a major threat to our planet, and, in the last few years, drought has become more frequent than ever before. Consequently, food production is challenged in meeting the needs of an ever-growing population projected to reach 10 billion in the coming decades. Hence, agriculture needs new and tailored solutions to boost crop productivity and enhance the resistance to environmental stresses. To accomplish this, biostimulants can be a viable solution. However, current solutions available on the market are natural product mixtures which usually have no mode of action characterization and rely on limited natural sources. By modulating specific pathways in plants, such as the abscisic acid (ABA) pathway, using small synthetic peptides, we aim to enhance plant resistance to environmental stresses. To identify these small peptides, we are employing a technology based on a combinatorial library of cyclic peptides. With the use of this library and the Yeast Two-Hybrid approach, we have identified cyclic peptides capable of binding members of the family of ABA receptors PYR/PYL/RCAR in *S. lycopersicum*, with the aim of activating the ABA pathway before drought conditions occur (molecular priming). The identified peptides will then be tested for their ability to activate the ABA pathway and induce resistance to drought in tomato plants.

Phytoremediation potential of *Ricinus communis* for diesel-contaminated agricultural soils

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Soil pollution due to petroleum hydrocarbons (PHCs) represents a serious environmental problem on a global scale, especially in agricultural areas, where PHCs can not only reduce plant growth and productivity but can also enter the food chain, potentially generating adverse effects to higher trophic level organisms. In this context, phytoremediation is considered one of the most efficient green and sustainable technologies for soil recovery.

The aim of this study was to investigate the potential of the castor bean (*Ricinus communis* L.) for the remediation of agricultural soils polluted by diesel-derived hydrocarbons. The study focused on the following 4 aspects: 1. the effect of diesel fuel on plant growth and health; 2. the contribution of root-associated microbes to plant tolerance; 3. the effectiveness of soil remediation resulting from castor cultivation; 4. the effect of soil diesel on the plant oil content and quality.

To the purpose, *R. communis* was cultivated in a diesel-contaminated farming area near Milan. A total of 9 plots (4m x 4m), characterized by 3 different concentration classes for C>12 hydrocarbons (low, medium, high) were considered. The main morpho-functional traits of castor plants as well as the abundance (SyberGreen staining and counts at the fluorescence microscope) and the structure (NGS sequencing) of its associated endophytic and rhizosphere microbial (fungi and bacteria) communities were determined at the end of the experiment. Before sowing and just after plant harvesting, the soil hydrocarbon concentration was determined for each plot. Finally, castor oil from the seeds was quantified (Soxhlet extraction method) and characterized both in terms of quality (saponification value) and composition (NMR spetroscopy). All the obtained data were statistically analysed with univariate and/or multivariate analysis.

According to the main preliminary results, a decrease of soil hydrocarbon concentration was recorded in all castor-growing plots. Castor plants were able to germinate, survive and grow, developing a deep and extended root system, even in soils contaminated by the higher concentration of C>12 hydrocarbons (>1250 mg/Kg). However, at these high concentrations, a delay in seed germination was recorded, as well as a significant reduction in photosynthetic efficiency and biomass production. Furthermore, some relevant differences in the abundance and composition of the castor associated microbiome were observed among plots. Interestingly, at the highest concentration, rhizosphere and endophytic bacteria appeared to be significantly more abundant and both the bacterial and the fungal community were richer in taxa specialized in PHC degradation and plant growth promotion. Finally, with regard to oil, despite a yield reduction proportional to soil hydrocarbon concentration increase (~ from 80% to 40%), the overall content was always rather high, without differences in quality and composition.

Overall, *R. communis* seems a valuable species to clean up diesel-polluted agricultural soils, due to its effective potential in soil remediation and the oil content of its seeds, that can be exploited for bio-energy production.

Research of drought stress molecular markers in chickpea (*Cicer arietinum* L.)

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In recent years, plants have to cope with drought and the increase in temperature, due to climate change. Investigating the adaptive response to abiotic stress will be useful to identify specific characteristics of stress resistance that could be used as morphological and/or molecular tolerance markers (Younis *et al.*, 2020). In this work we analyse the response to drought stress of an ancient and threatened variety of chickpea (*Cicer arietinum* L.), a local ecotype of the karst Murgia in the Apulian region (Southern Italy), generically referred as "Cece nero della Murgia" (Casini, 2022). This variety, known to be drought stress tolerant, has been compared to a more susceptible variety, cultivated in the same area, "Cece bianco della Murgia". The response to drought stress has been valued on 60 plants grown for 20 days in a growth chamber in the presence of 50 ml of water given every 3 days and then divided into two groups: control (watered every 3 days) and stressed (no supply of water) plants. After 20 days, half of control and stressed plants were collected in order to be analyzed. The remaining stressed plants were rewatered and grown for further 20 days with the same water supply of control plants. Morphological, physiological and molecular analyses were conducted on control, stressed and rewatered samples. Analyzing different parameters such as plant height, n° of branches, maximal roots length, n° of lateral roots, leaf fresh and drought weight, leaf area, relative water content and chlorophyll A, chlorophyll B and total carotenoids content, we observed that drought stress affects both varieties, but "Cece nero della Murgia" recovers completely after rewatering, On the other hand, "Cece bianco della Murgia" shows more severe symptoms of stress and 20 days of drought proved to be a too long period of time to allows the plants to recover, even after rewatering. These results confirm that "Cece nero della Murgia" is a drought stress tolerant variety and it can be used, compared to "Cece bianco della Murgia", to research molecular tolerance markers. The research of molecular markers has been focused on two members of *XTH*s family (*Xyloglucan endotransglucosylase/hydrolases*), *XTH29* and *XTH23*, involved in cell wall remodeling and known to be differentially expressed under stress condition. Other genes analyzed were: *LEA4* (*Late embryogenesis abundant 4*), involved in plasma membrane permeability and water absorption; *NIP6-1-like* (*Nodulin 26-like intrinsic protein*), a plant aquaporin; *PA2* (*peroxidase A2*), involved in oxidative processes. The overexpression of *LEA4* and *PA2* genes, observed after drought stress, confirmed that plants were submitted to drought stress condition. Furthermore, molecular analyses proved that *XTH29* and *XTH23* can be considered DEGs (Differentially Expressed Genes) in drought stress condition. This indicates that these genes can actually be used as molecular drought stress tolerance markers in chickpea.

Casini P. (2022). Preliminary research on seed yield and nutritional traits of desi chickpea (Cicer arietinum L.) grown in Central Italy in spring sowing. *Acta agriculturae Slovenica*, 118, 1. DOI: 10.14720/aas.2022.118.1.2058

Younis, A.; Ramzan, F.; Ramzan, Y.; Zulfiqar, F.; Ahsan, M.; Lim, K.B. (2020). Molecular Markers Improve Abiotic Stress Tolerance in Crops: A Review. *Plants* 9, 1374. DOI: 10.3390/plants9101374

Investigation on the flow of mycotoxin in plants

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The movement and transport of xenobiotics within the plant is widely covered due to its importance in ecological, physiological, phytochemical and food safety fields. Mycotoxins are secondary metabolites produced by some fungal species.
There exist over 300 varieties of these toxins (Juraschek, 2021), predominantly concentrated in agricultural regions, as the primary hosts for these fungi are specific crops such as cereals, grapevines, coffee, and fruits. It should be noted that these toxins are pervasive in all products derived from these cultivated sources and posing a substantial health risk to humans and animals throughout the world (Alkhayyat, 2014).
Mycotoxins, that can be considered xenobiotics, can be found in plant tissues in two cases: the main one is because of fungal infection, but they can also, through the root system, be absorbed directly from the soil.
However, the fate of mycotoxins within the plant has not yet been clarified.
Precisely because of their negative impact on human and animal health, these compounds have attracted wide attention in terms of biosynthesis, detection, health risks and mechanisms of action. Despite rigorous studies, the fate of mycotoxins, once released into plant tissues remains incompletely understood.
Plants have a high capacity to transform and thereby detoxify deleterious or poisonous compounds, like mycotoxins (Berthiller, 2007).
The system occurs in three stages (Righetti L., et al, 2017): first, a structural alteration is implemented on the mycotoxin, a conjugation stage, integrating actions such as glycosylation or hydroxylation; and a third phase entails storaging the altered 'masked mycotoxin' within the vacuole or cell wall.
Due to these modifications not only does it alter the structure of the toxin, but it also has the potential to modify its toxicity level, water solubility, and polarity.
We performed one experiment with three different toxins Zearalenone (ZEN), T-2, and Aflatoxin B1 (AFB1), differing in chemical characteristics, were selected for each of the experiments to investigate their possible movement and/or translocation.
As previously outlined, this concern primarily pertains to food safety, which is why we've selected Zea mays L. as our model system.
The experiment consists of a 'split root' system in which the toxins were added individually to the medium in contact with the half root apparatus (portion "D"). The portion of the root (R) was not in contact with the toxin. Both root portions and the epigeal part of the plant were analyzed by HPLS-MS.

Juraschek LM, Kappenberg A, Amelung W. Mycotoxins in soil and environment. Sci Total Environ. 2022 Mar 25;814:152425. doi: 10.1016/j.scitotenv.2021.152425. Epub 2021 Dec 21. PMID: 34952071.

Alkhayyat F, Yu JH. Upstream regulation of mycotoxin biosynthesis. Adv Appl Microbiol. 2014;86:251-78. doi: 10.1016/B978-0-12-800262-9.00005-6. PMID: 24377857.

Berthiller F, Lemmens M, Werner U, Krska R, Hauser MT, Adam G, Schuhmacher R. Short review: Metabolism of theFusarium mycotoxins deoxynivalenol and zearalenone in plants. Mycotoxin Res. 2007 Jun;23(2):68-72. doi: 10.1007/BF02946028. PMID: 23605909; PMCID: PMC4351913.

Righetti L, Rolli E, Galaverna G, Suman M, Bruni R, Dall'Asta C. Plant organ cultures as masked mycotoxin biofactories: Deciphering the fate of zearalenone in micropropagated durum wheat roots and leaves. PLoS One. 2017 Nov 16;12(11):e0187247. doi: 10.1371/journal.pone.0187247. PMID: 29145415; PMCID: PMC5690627.

The role of Endoplasmic Reticulum Quality Control in plant response to cadmium stress in *Arabidopsis thaliana*.

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Stress conditions trigger an extra load on the protein folding machinery in the Endoplasmic Reticulum (ER), resulting in the accumulation of misfolded proteins which causes ER stress. To restore ER homeostasis, the cell increases the expression level of ER-molecular chaperones involved in ER Quality Control (ERQC) pathway, which ensures that only properly folded proteins can reach their destination, and activates the Endoplasmic Reticulum Associated Degradation (ERAD) machinery, to alleviate the burden of unfolded proteins. This whole process is controlled by the Unfolded Protein Response (UPR) signalling network (1).

Very little is known about the effects of heavy metal stress on ERQC-ERAD-UPR pathways. In our recent study, we demonstrated that cadmium (Cd) treatment induces ER stress and that knockout mutant of UPR pathway shows an increased tolerance to heavy metals in *Arabidopsis thaliana* plants (2). Now, we are investigating the role of ERQC in plant response to Cd stress. To test this hypothesis, we used the *A. thaliana* mutant, *rsw3*, which has reduced activity of the ER alpha-glucosidase II (α-GII), a key enzyme that admits newly synthesized glycoproteins into ERQC for their folding and releases them once they reach the correct folding. Phenotypic analyses showed an increased tolerance of *rsw3* mutant to chronic Cd stress, while biochemical and molecular data suggest that *rsw3* seedlings do not perceive the stress condition, differently from wild type plants. These results might open new avenues for a better understanding on the impact of heavy metal stress on ER homeostasis and glycoproteins folding machinery.

1) Liu J.X, and Howell SH. Managing the protein folding demands in the endoplasmic reticulum of plants. New Phytol. (2016), 211, 418–428;
2) De Benedictis M; Gallo A; Migoni D; Papadia P, Roversi P; Santino A. Cadmium treatment induces endoplasmic reticulum stress and unfolded protein response in *Arabidopsis thaliana*. Plant Physiol. Biochem. (2023), 196, 281–290.

Unravelling cellular and molecular mechanisms induced in mint species by exogenous gallic acid

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Nowadays, the research of eco-friendly phytostimulants represent one of the most important modern challenges to substitute the use of toxic fertilizers and pesticides in agriculture. *Mentha* is a genus of aromatic plants belonging to the Lamiaceae family and possessing a great economic interest, since its essential oil is widely employed in food, pharmaceutical and cosmetic industries.

The scientific evidence recently published by our research group has documented how gallic acid (GA) possesses the ability to stimulate the production of essential oil in *M. spicata* (common mint), enriching it with highly bioactive secondary metabolites and even inducing tolerance in plants subjected to drought stress. Thus, in the first part of the present contribution, we aimed at clarifying the possible mechanism of action of GA on common mint by profiling its miRNome. We detected that 8 microRNAs significantly changed their expression after drought and/or GA treatment. Among them, miR397a showed, by bioinformatic analyses, to be the putative regulator for the transcript of the Laccase-2, a key enzyme in lignification. Thus, we carried out several molecular investigations for validating this correlation, to understand if the phytostimulating effect of GA could be linked to Laccase-2 activity.

In parallel, we also studied the effect of GA on the metabolome of another mint species, *M. arvensis* (corn mint). The data demonstrated that GA was able to increase the content of total simple phenols, flavonoids and terpenoids, especially after 21 days of treatment and with a medium-low dose (50 μM) of phytostimulant. The hydrophilic and lipophilic fractions of the phytocomplex from the mint samples was profiled through chromatographic techniques (HPLC-DAD; GC-MS), demonstrating that GA promoted the accumulation of some specific bioactive metabolites, such as menthol, the main component of *M. arvensis* menthol chemotype. Indeed, the gene expression analysis of 5 enzymes involved in the biosynthetic pathway of menthol confirmed that GA positively influenced the production of this monoterpenoid.

In conclusion, GA could be proposed as a potential phytostimulant for several crops, also taking into consideration the environmental sustainability (e.g., extraction from plant waste matrices).

Comparative ultrastructural and functional study of chloroplasts in *Cichorium intybus* L. cv. Chioggia and Treviso

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Red chicory (*Cichorium intybus* L.) is an important and widespread winter crop in Northern Italy, including cultivations of the two cultivars, Chioggia Precoce and Treviso Precoce, on the East coast of Emilia Romagna. It belongs to the Asteraceae family and presents toothed leaves arranged in a rosette, though with a great morphological diversity based on the cultivar. Particularly, the leaves developing from voluminous compact heads may suggest the occurrence of morpho-functional specificities in the chloroplasts.

To date, specific information regarding the variation of chloroplasts among different cultivars of chicory is lacking; therefore to obtain a comprehensive characterization, we combined ultrastructural and chlorophyll fluorimetric analyses of the abovementioned two cultivars. Plants cultivated in neighboring fields in the province of Ferrara were analyzed between December 2023 and January 2024, corresponding to the peak of the vegetative growth phase; in particular, the new fully expanded outer leaves were analyzed.

Using TEM, it was found that the chloroplasts of cv. Chioggia had a normal elliptical shape and a rather developed thylakoid system. Instead, the chloroplasts of cv. Treviso had a globular shape and an extremely heterogeneous internal structure, even within the same cell. The thylakoid system was formed by associations of long arrays of single thylakoids and grana of extremely variable size. Additionally, small "thylakoid circles" were present. Given the strong structural anomalies, the chloroplast functionality was analyzed in the field with a Handy-PEA chlorophyll fluorimeter and multiparametric MultispeQ spectrometer; in addition, chlorophyll fluorescence quenching was analysed through a modulated fluorimeter (PAM). Interestingly, there was no evidence of inadequate photosynthetic functionality attributable to the chloroplast anomalies observed in cv. Treviso; for some parameters, it appeared even more efficient than cv. Chioggia. To independently validate this result, analysis of the δ^{13}C-isotopic ratio was performed and compared among roots, stems, and leaves in the cultivars, showing a generally more favorable result in cv. Treviso than in cv. Chioggia, indicating a probably better response to stress in the former.

The chloroplast structural anomalies found in leaves of cv. Treviso thus do not have a negative impact on the photosynthetic functionality of the organelle and may instead represent a specific adaptation emerged during the cultivar selection process.

This research was allowed by PhD fellowship granted by EUROPEAN SOCIAL FUND P L U S - The ESF+ 2021-2027 Programme of the Regione Emilia Romagna

Effects of gadolinium on growth and morphology of *Solanum lycopersicum* L. plants grown in hydroponic system.

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Gadolinium (Gd) is a chemical element belonging to the group of Lanthanides, also known as Rare Earth Elements (REEs). Nowadays, REEs are widely used in many anthropogenic activities (*e.g.*, electronic devices production, medicine, glass and ceramic industries, agriculture, etc.) causing an environmental dispersion due to mining extraction and an improper disposal. The effects of REEs on the environment and living beings are not clear yet, therefore REEs have been recently classified as contaminants of emerging concern. Gadolinium, in particular, is commonly used in medicine as a contrast agent in magnetic resonance imaging, leading to water contamination. Our study aims to assess the effects of Gd on the growth of *Solanum lycopersicum* L. (cv-Microtom) grown in hydroponics. Seeds of tomato, after germination, were transferred to a hydroponic apparatus containing either the Hoagland solution (control plants, CNT) or an its modified variant added with Gd (treated plants, Gd150 μM), and treated for about 70 hours a week. After three weeks, plants were collected and the main morphometric parameters (leaf area, stem length, root biomass and length) were acquired. The analysed parameters showed statistically significant differences between the two plant groups (CNT and Gd150 μM). Gd150 μM plants were characterized by a lower growth, resulting in reduced leaf area, stem length, root biomass and length. The root system of CNT plants averaged approximately 31 meters in total length, whereas Gd150 μM plants only 4 meters. The root length inhibition primarily impacted the emission and elongation of lateral roots, specifically those with a diameter less than 0.4 mm. Particularly, these finer roots were significantly reduced when compared to those of CNT. This was reflected in different values of root tissue density (RTD) and specific root length (SRL). In fact, RTD was higher in Gd150 μM plants (0.062 g cm^{-3}) compared to CNT ones (0.044 g cm^{-3}), while SRL was lower when compared with CNT, 0.8 m g^{-1} versus 2.8 m g^{-1}, respectively. Our results suggest that long exposures to Gd negatively affects *Solanum lycopersicum* L. growth and root morphology, coherently with what is present in the scientific literature.

Over-expression of *ZIP1;2* in *Populus alba* Villafranca clone under Zn excess

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Abstract

Industrialization and human activities have led to a rise in the levels of air, water and soil pollution. Such pollution is produced by substances that are harmful to flora and fauna, including both micro- and macro-nutrients necessary for plant growth and survival (Priya *et al*, 2023). This study examined zinc (Zn), which, when present in plant organisms at concentrations above the physiological threshold, can be potentially toxic (Vhahangwele *et al*, 2021). Studies with sublethal doses (Di Baccio *et al*, 2011, Romeo *et al*, 2014) have demonstrated poplar's ability to absorb and tolerate high concentrations of the above essential metal involving even evolutionarily conserved proteins. For these reasons, poplar appears to be one of the model-plants in terms of tolerating and testing the effects of heavy metals (Romeo *et al*, 2014).

Results have shown the *UPr51* gene to be overexpressed in *Populus* x *euramericana* clone I-214 roots, treated with sublethal doses of Zn (1 mM) (Di Baccio *et al*, 2011). The *UPr51* gene belongs to the family of ZIP transporters involved in the uptake of Zn and other metals, including cadmium, iron and manganese (Hall & Williams, 2003, Sharif *et al*, 2021). *UPr51* is a homologue of the *Populus alba ZIP1* gene. The *P. alba* Villafranca clone was used to produce transgenic lines by overexpressing the two isoforms of the *UPr51* gene. Firstly, the nine transgenic lines of *P. alba* (four for isoform 1 and five for isoform 2) obtained by *Agrobacterium* transformation were characterized. For each line, gene expression levels and the integrated copy number were studied. The most highly expressed line of the gene studied was cultured *in vitro* for two months using WPM (McCown's woody plant medium) containing a sublethal dose of zinc (1 mM $ZnSO_4$). At the end of the growth period, the transgenic plant was compared with wild-type plants grown in the same conditions.

Bibliografia

Di Baccio, D., Galla, G., Bracci, T., Andreucci, A., Barcaccia, G., Tognetti, R., & Sebastiani, L. (2011). Transcriptome analyses of Populus x euramericana clone I-214 leaves exposed to excess zinc. *Tree physiology*, *31*(12), 1293–1308. https://doi.org/10.1093/treephys/tpr106Z

Hall, J. L., & Williams, L. E. (2003). Transition metal transporters in plants. *Journal of experimental botany*, *54*(393), 2601–2613. https://doi.org/10.1093/jxb/erg303

Priya A.K., Muruganandam M, Ali SS, Kornaros M. Clean-Up of Heavy Metals from Contaminated Soil by Phytoremediation: A Multidisciplinary and Eco-Friendly Approach. Toxics. 2023 May 2;11(5):422. doi: 10.3390/toxics11050422. PMID: 37235237; PMCID: PMC10221411

Romeo S, Francini A, Ariani A, Sebastiani L (2014) Phytoremediation of Zn: identify the diverging resistance, uptake and biomass production behaviours of poplar clones under high zinc stress. Water Air Soil Poll 225:1813–1825

Sharif, R., Raza, A., Chen, P., Li, Y., El-Ballat, E. M., Rauf, A., Hano, C., & El-Esawi, M. A. (2021). HD-ZIP Gene Family: Potential Roles in Improving Plant Growth and Regulating Stress-Responsive Mechanisms in Plants. *Genes*, *12*(8), 1256. https://doi.org/10.3390/genes12081256.

Vhahangwele M., Philani L.M. & Memory T. (2021). Sources of Heavy Metals Pollution. *Remediation of Heavy Metals*, Environmental Chemistry for a Sustainable World 70, https://doi.org/10.1007/978-3-030-80334-6_17

Auxin response is required for Megaspore Mother Cell (MMC) differentiation.

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The female gametophyte formation takes place in the ovules and consists of two main steps: megasporogenesis and megagametogenesis. Megasporogenesis begins with the differentiation of the Megaspore Mother Cell (MMC), which, upon meiotic division, forms four haploid spores. Three spores degenerate, whereas the fourth gives rise to a female gametophyte after three rounds of mitosis. SPOROCYTELESS/NOZZLE (SPL/NZZ) is required for MMC differentiation; indeed, the *spl/nzz* mutant fails to develop the MMC, resulting in sterile plants. SPL/NZZ is required to establish auxin maxima at the tip of the nucellus by positively regulating PIN1 expression (Bencivenga et al., 2012). To understand how the auxin concentration is translated into a cellular response leading to the formation of MMC I have analysed the putative role of selected factors involved in auxin response: SHY2/IAA3, which belong to the AUX/IAA family, and ARF5 and ARF9, which belong to the Auxin Response Factors (ARFs) family. The results obtained suggested that ARF and AUX/IAA factors are of pivotal importance in MMC specification.

Characterization of the network regulated by *SPOROCYTELESS/NOZZLE* required for Megaspore Mother Cell (MMC)

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During megasporogenesis, one sub-epidermal cell in the nucellus, named the archespore cell, acquires the identity of the megaspore mother cell (MMC). Then, MMC undergoes meiosis, forming four spores: three spores degenerate while the most chalazal one enters the megagametogenesis process, leading to the formation of the female gametophyte.

In this project, we have focused on MMC differentiation, a process that requires the activity of SPOROCYTELESS/NOZZLE (SPL/NZZ). SPL/NZZ was discovered more than twenty years ago as a fundamental factor in sporogenesis; indeed, *spl/nzz* mutant completely lacks the MMC. SPL/NZZ is a transcriptional repressor that binds to the transcription factor family TCP and then recruits TOPLESS (TPL) or TOPLESS-RELATED (TPR) to repress the transcription of target genes. Even though the SPL/NZZ function was discovered many years ago, very little is known about the molecular mechanism required for MMC specification (Wei *et al.,* 2015; Yang *et al*., 1999).

From the literature and previous experiments done in Lucia Colombo's lab, it is known that the accumulation of auxin, a hormone involved in many developmental processes, is impaired in the *spl/nzz* mutant ovule (Bencivenga *et al*., 2012). In this project, I have used two approaches to identify the genes regulated by SPL/NZZ that could be responsible, once expressed in the nucellus, for negatively regulating PIN1 expression.

To better characterize the SPL/NZZ mechanism of action, we performed a ChIP assay to identify the SPL/NZZ putative targets. One of the targets identified is *BEL1,* a gene encoding for a transcription factor that repressed PIN1 in the chalaza. Interestingly, in *spl/nzz* mutants, BEL1 is ectopically expressed in nucellus. Considering that SPL/NZZ, as mentioned above, is not able to bind the DNA, I performed a CoIP to identify transcription factors interacting with SPL/NZZ able to bind the DNA.

New details on OsFTL1 florigenic protein revealed by Proximity Labelling in rice

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The transition to the reproductive stage is a fundamental step of the plant life cycle. In angiosperms, a set of proteins, called florigens, belonging to the family of phosphatidylethanolamine-binding proteins (PEBPs), induce the differentiation of cells at the vegetative meristem to prompt the development of fertile flowers at specific photoperiodic conditions. The downstream molecular network shaping flowers formation is highly complex, resulting from a series of transcriptional, epigenetic and post-translational events.

In rice (*Oryza sativa*), the variability of flowering-controlling genes has been essential over history to increase the crop yield and adapt its cultivation to different latitudes. Nevertheless, the complex set of interactions of florigenic molecules is far from being completely understood.

We established, for the first time in adult rice plants, the Proximity Labelling (PL) proteomic technique, to analyse the interactions of the OsFTL1 florigenic molecule. PL exploits an optimized biotin-ligase fused to the protein of interest allowing the biotinylation of proteins laying in its contiguity *in vivo* and in specific organs (here, the shoot apical meristem). The subsequent selective precipitation of its proximal proteome, followed by mass spectrometry and bioinformatic analysis, returns a list of the potential interactors of the target protein. The protocol application on rice flower meristems has delivered a list of about 3000 proteins, 12 of which have been statistically identified as highly probable OsFTL1 interactors in two distinct stages of reproductive differentiation, tremendously expanding our knowledge on the properties and the localization of this protein.

Here we give a global overview of OsFTL1 interactome, along with presenting specific examples of candidate proteins which have never indicated before as interactors of a florigen. Therefore, our data point at new features of florigenic proteins, which could possibly be common to all PEBP family members.

Moreover, our research is able to provide selectable genetic markers and gene-editing targets for improving yield and environmental adaptability of cultivated rice varieties.

Alterations of the post-translational modification pathway shed light on the importance of peptide hormones in plant development

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Different small secreted peptide hormones (PHs) are involved in several plant developmental programs. They have been conserved across evolution and, despite their family specific aminoacidic structure and biological function, lots of them share the same biosynthetic pathways. To prove these PHs important roles and demonstrate that the post-translational events characterising their biosynthesis are fundamental for their biological activity, tobacco plants with impairments in PHs maturation processes have been generated.
Exploiting the artificial microRNA(amiRNA)-based technique, mRNAs for the Tyrosyl-Protein Sulfotransferase (TPST) enzyme and a proteinase (SBT6.1) belonging to the Subtilisin like-Serine Proteinase family, two PHs post-translational modifying enzymes, have been silenced in a constitutive manner. These targets are involved in the sulfation of a Tyrosine residue and the proteolytical cleavage of the PH precursor of members of different peptide families, respectively. Specifically, according to the literature, their activity is crucial for the production of mature bioactive CLV3/Embryo Surrounding Region (CLE) and ROOT GROWTH FACTOR/GOLVEN/CLE-LIKE (RGF/GLV) peptides. To verify their functions in species other than Arabidopsis, tobacco mutants generated, have been phenotyped with a particular focus on their root, hypocotyl and leaf development and their lateral meristem differentiation. In vitro grown seedlings overexpressing the SBT6.1 specific amiRNA showed reduced lengths of both hypocotyls and primary roots, which had also less roots hairs and a reduction in leaf area compared to negative control plants. The same impaired clones, grown in the greenhouse, displayed shorter internodal spacing. Similar internode reduction has been also observed in tobacco plants silenced for the TPST gene, as well as a reduction of leaf area for the apical leaves. These evident phenotypic differences between impaired and negative control plants confirmed the fundamental roles of the targeted enzymes in PHs maturation events and suggest the important contribution of these lasts in regulating different organs growth by possibly modulating cell expansion phenomena.

Ultrastructure and development of the floral nectary from *Borago officinalis* L. and phytochemical changes in its secretion

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Although Boraginaceae have been classified as good sources of nectar for many insects, little is still known about their nectar and nectaries. Thus, in the present contribution, we investigated the nectar production dynamics and chemistry in *Borago officinalis* L. (borage or starflower), together with its potential interaction capacity with pollinators. A peak of nectar secretion (~5.1 µL per flower) was recorded at anthesis, to decrease linearly during the following 9 days. In addition, TEM and SEM analyses were performed to understand ultrastructure and physiological changes occurring in borage nectary before and after anthesis, but also after its secretory phase. Evidence suggested that nectar may be transported by the apoplastic route and possibly by exocytotic processes, that is a granulocrine secretion. This evidence was corroborated by monitoring the signal of complex polysaccharides and calcium, respectively, via Thiéry staining and ESI/EELS technique. After the secretory phase, nectary underwent degeneration, probably through autophagic events and/or senescence induction. Furthermore, nectar (Nec) and other flower structures (i.e., sepals, gynoecia, and petals) from borage were characterized by both spectrophotometry and HPLC-DAD, in terms of plant secondary metabolites, both at early (E-) and late (L-) phase from anthesis. The content of phytochemicals was quantified and discussed for all samples, highlighting potential biological roles of these compounds in the borage flower (e.g., antimicrobial, antioxidant, staining effects). Surprisingly, a high significant accumulation of 7 phenolics was registered in L-Nec, with respect to E-Nec, indicating that this phenomenon might be functional and able to hide molecular (e.g., defence against pathogens) and/or ecological (e.g., last call for pollinators) purposes. Indeed, it is known that plant metabolites influence nectar palatability, encouraging the approach of specialist pollinators, deterring nectar robbers, and altering the behaviour of insects.

Multiple Arabidopsis group A bZIPs mediate shoot apical meristem differentiation at the floral transition

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The Arabidopsis group-A bZIP transcription factor family comprises 13 members, with FD notably recognized as the putative receptor for the florigen signal protein FT within the shoot apical meristem. FD is a key constituent of the Florigen Activation Complex (FAC), governing the floral transition and meristem differentiation. While Arabidopsis mutants deficient in FD exhibit delayed flowering, the severity of this phenotype is notably milder compared to mutants lacking FT, prompting an exploration of additional potential partners of FT within the FAC.

Our investigation revealed that several members of the Arabidopsis group-A bZIP family can bind both to the florigen FT and its antagonist, TFL1. These bZIP factors share a conserved C-terminal SAP motif, known for mediating interactions with FT/TFL1. Among these, we focused on ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3 (AREB3), which possesses a SAP motif highly similar to that of FD.

We demonstrate that AREB3 redundantly controls flowering time with FD, acting downstream of FT. The SAP domain is crucial for AREB3's interaction with FT and its function in vivo, as evidenced by late-flowering phenotypes resulting from SAP-specific CRISPR-Cas9-induced mutations. Microscopy localization analysis reveals partial spatial overlap between AREB3 and FD domains in the shoot apical meristem, supporting their functional redundancy.

RNA-seq data indicate negative feedback loops between certain bZIPs, with *AREB3* transcriptionally upregulated in *fd* mutants as well as in *eel* mutants (*ENHANCED EM LEVEL*). Our findings suggest that the relatively mild phenotypic effects of FD loss may stem from compensatory mechanisms involving other group A bZIPs, indicating a more complex FAC composition than previously recognized.

Expanding on these insights, we are currently investigating four group-A bZIPs (FD, its closest homologue FDP, AREB3, and EEL) by generating a range of SAP-specific CRISPR mutants in various combinations (single, double, triple, and quadruple mutants). These mutants exhibit diverse flowering time phenotypes and altered plant and flower architecture compared to the wild type, offering prospects for elucidating the functions of their respective genes in plant development and floral differentiation.

Unveiling the diversity of NAC genes: insights from transcriptome analysis in *Arabidopsis thaliana*

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Fruits are mature ovaries of flowering plants, usually containing seeds, which develops from the fertilized ovule after pollination. Fruits protect, nourish, and disperse seeds, thus facilitating the plant's reproductive cycle. Fruits can vary widely in structure and composition, but they are highly specialized for seed dispersal and play a crucial role human and animal nutrition.

Due to their vital role in both nutrition and biology, fruits hold substantial importance in the global agricultural economy. Consequently, there is considerable interest in dissecting the molecular processes underlying fruit growth and maturation for enhancing both yield and quality, thereby driving advancements in agricultural production.

To shed light into the molecular networks controlling fruit development and ripening in *Arabidopsis thaliana* we performed a transcriptome analysis by RNA-deep-sequencing comparing siliques at different time points. Among the thousands of genes differentially expressed we focused our attention on the NAC transcription factors family. We characterized the biological function of some NAC proteins, in particular *NAC37/NAC76/NAC105*, demonstrating that they control silique elongation by affecting secondary cell wall biosynthesis genes and influencing xylem formation.

Calcium-mediated crosstalk between plastids and the endoplasmic reticulum in arbuscular mycorrhizal symbiosis

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Plant-microbe interactions can significantly impact plant growth and physiology. Many microorganisms have no direct effects on the plant, others are pathogenic and only a few are beneficial. Among the latter ones, Glomeromycotina fungi colonize plant roots and establish the arbuscular mycorrhizal (AM) symbiosis with the majority of land plants. Calcium-mediated signal transduction pathways are activated in plant cells in response to fungal signals as part of both symbiotic and immunity cascades. Whereas Ca^{2+} signals triggered in the cytosol and nucleus have been extensively studied, the role of additional intracellular compartments has been poorly investigated so far. In this work, we engineered differentially targeted aequorin-based Ca^{2+} probes to monitor Ca^{2+} changes in the stroma of plastids and in the lumen of the endoplasmic reticulum (ER) in the roots of the model legume *Lotus japonicus*. Transient Ca^{2+} elevations triggered by chitin-based fungal signals were monitored in different genetic backgrounds to highlight the symbiotic and immunity components of the observed Ca^{2+} fluxes. Moreover, the fusion of a YFP moiety to the Ca^{2+} reporter, together with the co-expression of suitable organellar markers, will allow to monitor dynamic interactions between plastids and the ER, mediated by stromules, upon perception of the fungal molecules. The obtained data will provide first insights into the role of Ca^{2+} signalling in plastids and ER in plant-fungus communication circuits.

Steps towards the formulation of a PGPB and AMF inoculum to be used in agroecosystems of the Mediterranean area.

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Keywords: arbuscular mycorrhizal fungi, PGPB, biostimulants, agriculture

Anthropogenic activities pose a significant threat to the natural capital of the soil which includes its fertility, biodiversity and related ecosystem services. Under field conditions, plants interact with a multitude of microorganisms and the use of indigenous beneficial soil microbiota can increase their tolerance to abiotic and biotic stress. Among these microorganisms, plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) represent fundamental components of soil fertility through their role in plant nutrition, health and productivity. The main goal of the PRIMA-ProSmallAgriMed project is to promote/support farming systems that boost the efficiency of water use and aid soil conservation and fertility in arid and semiarid climates. A special concern is given to smallholder conservation agriculture by introducing the rational employment of beneficial soil microbiota and an intercropping system relying on perennial plants, such as cactus pear, and short-term species such as *Leguminosae* and cereals. In this frame, the present work reports on some parallel activities aimed to the formulation of a mixed inoculum (AMF + PGPB): (1) the isolation, and molecular identification of AMF spores from Algeria and Tunisia that will be propagated to obtain pure lines of AMF; Also in order to find good PGPB candidates that will be later applied in field conditions, different assays, such as (2) compatibility tests concerning putative PGPB strains, previously isolated in Morocco, identified and characterized; (3) germination test of sorghum seeds (4) growth of sorghum plants inoculated with the putative PGPB; (5) an *in vivo* test on cactus pear inoculated or not with AMF. Results showed a promotion of biomass production by PGPB in inoculated sorghum plants and suggested a different developmental model in AM-inoculated or uninoculated cactus pear plants in the first months of growth.

Clathrin-mediated endocytosis during myc-factor perception in arbuscular mycorrhizas

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Clathrin-mediated endocytosis (CME) is a major endocytic pathway in plants, driving the internalization of membrane-bound receptors.
Previous studies using tyrphostin A23, a CME inhibitor, demonstrated that CME is required for the regulation of symbiotic genes in response to Nod-factors in legume-rhizobium symbiosis (Wang et al., 2015). Growing evidence indicates that this symbiosis recruited part of the signaling pathway supporting arbuscular mycorrhiza (AM). We therefore decided to investigate whether CME is also involved in AM signaling.
To this aim, we treated *M. truncatula* roots with AM fungal signals in the absence or presence of CME inhibitors (tyrA23, Dynasore), analyzed the expression for early AM marker genes and monitored nuclear Ca^{2+} spiking (a hallmark of symbiotic signaling). Symbiotic gene regulation was strongly impacted by CME inhibition. Nevertheless, no significant reduction was observed in Ca^{2+} spiking, suggesting that CME is required for gene regulation but - surprisingly - not for upstream symbiotic signaling. We will discuss the new questions opened by such unexpected results, which contrast with our current model of symbiotic signaling in legumes, and present our attempt to solve this conundrum through a laser microdissection-based approach.

Lactua sativa morphological traits are pivotal drivers of leaf-associated bacterial community diversity

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Lettuce holds a unique status as one of the main vegetables consumed raw worldwide. Studying the driving forces that shape the leaf-associated bacterial community can provide a means to examine the establishment of a beneficial microbial population essential for both plant health and human nutrition. This project aims to uncover the influence of plant determinants, such as genetic distance, leaf mineral content and leaf morphology, in impacting diversity, richness and association of lettuce leaf bacteria. To address these knowledge gaps, we conducted a large-scale experiment utilizing 131 fully-sequenced genotypes of Lactuca sativa, cultivated over three months, and investigated their leaf-associated bacterial communities, using DNA amplicon sequencing of the bacterial 16SrRNA.
Results demonstrate that genetic distance and plant morphology (defined by breeders as "variety") are responsible for 3.3% and 4,4% of the overall leaf-associated bacterial community diversity, respectively. In addition, disparities in manganese (Mn) concentration among groups of closely related genotypes, as well as variations in calcium (Ca), zinc (Zn), and phosphorus (P) among different varieties, contribute to explaining this variation. Variety's importance prompted us to incorporate extensive phenotypic traits, identifying primary influencers and mechanisms. We identified 10 leaf traits that affect the structure of the bacterial community, with heart formation, head height and shape impacting bacterial richness and evenness. Altogether, these different factors explain 13.6% of the leaf-associated bacterial diversity. Finally, the origin of the leaf bacterial community was analyzed by assessing the proportion of bacteria originating from various environmental sources. Interestingly, seed-associated bacteria were found to contribute 10% to the leaf community composition.
By investigating how variation in phenotypic appearance leads to the establishment of diverse prokaryotic communities, we disentangled previously unknown mechanisms through which plants influence the recruitment of leaf bacteria, offering new observation lens for breeders and novel insights into similar associations in other agriculturally relevant crops.

The use of compost improves biodiversity in the bacterial community of the rhizosphere of tomato plants

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Over the past half-century, the necessity to feed the ever-growing global population led to invasive agricultural practices, which, combined with human activities and industrialization, have caused extensive soil deterioration and biodiversity loss. Biotic and abiotic stresses resulting from these conditions have posed a serious threat to crop cultivation, bringing up the necessity of finding new sustainable approaches that allow the preservation of the environment and of the microorganisms present in the soil. It has been shown that soils rich in microorganisms are more fertile because of their positive biochemical activities and ability to protect plants from pathogens. Some bacterial species, among the many taxa present in the soil, live in close association with plant roots, colonizing the rhizosphere (the thin layer of soil surrounding the roots), the root's surface or the root's internal tissues without harming the plant. Among all the strategies to sustain soil biodiversity, biofertilizers offer a potential remedy. Among these, compost, a product of controlled aerobic decomposition of municipal organic waste carried out by microorganisms, is a promising solution. Recognized in agriculture for being rich in organic matter and nutrients, compost's unexplored potential as a microbial inoculum for enriching the soil and fostering plant health necessitates further investigation. This work, a collaboration with S.E.S.A S.p.A., demonstrated that when compost is used to fertilize tomato plants the bacterial communities associated with the plants have more bacterial species than those of the plants treated with chemical fertilizer. Moreover, compost is naturally rich in Bacillus species, and the plants treated with compost displayed a microbiota significantly enriched in Bacillus species. These results highlight the potential beneficial effects of compost on plant-associated bacteria. This research contributes to developing sustainable agricultural practices and soil ecosystem health, aligning with the EU's 2030 sustainable development goals.

Cross-kingdom RNA interference adds a new layer of communication to the arbuscular mycorrhizal symbiosis

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Cross-kingdom RNA interference is a mechanism of interspecies communication where small RNAs (sRNAs) are transported from one organism to another; these sRNAs silence target genes *in trans* by loading into host AGO proteins. Current knowledge on RNAi-based mechanisms modulating the arbuscular mycorrhizal (AM) symbiosis, from both the plant and the fungal side, is limited. We demonstrated that the AM fungus *Rhizophagus irregularis* is equipped with peculiar RNAi machinery characterized by a single Dicer-like and an unusual high number of Argonaute-like (AGO-like) and RNA-dependent RNA polymerase genes. The *in silico* characterization of the small RNAome of *R. irregularis*-colonized roots of *Medicago truncatula* suggested that some fungal sRNAs can potentially target host plant genes through cross-kingdom RNAi. Specifically, the fungal sRNA *Rir-2216* has several potential target transcripts in the *M. truncatula* transcriptome. Among them, we focused on a transcription factor, *MtWRKY69*, belonging to the WRKY family, since many members are known to regulate plant responses to biotic factors. Using different complementary approaches, we found that *Rir2216* is loaded into an AGO1 silencing complex from the host plant *M. truncatula*, leading to cleavage of a host target transcript encoding for the MtWRKY69 transcription factor. *MtWRKY69* is downregulated in arbusculated cells in mycorrhizal roots and *MtWRKY69* overexpression led to a reduced AM colonization level.
Our results suggest that the fungal sRNA *Rir-2216* is transported from the fungus to the plant root where it associates with plant AGO1 leading to the silencing of plant target genes modulating fungal colonization, and provide the first evidence of cross-kingdom RNAi in the AM symbiosis.

Role of Nitric Oxide in root formation and development in rice plants exposed to arbuscular mycorrhizal fungi

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Nitric oxide (NO) is a signaling molecule involved with different plant developmental processes, including its interaction with phytohormones in root growth and development and stress responses (Altamura et al., 2023)[1]. Interestingly, a study reported a NO increase in *Medicago truncatula* roots in response to arbuscular mycorrhizae fungi exudates (Calcagno et al., 2012)[2]. Furthermore, it is known that rice plants in the presence of AMF develop a higher number of large lateral roots, implying their importance in the interaction with the symbiont (Chiu et al., 2022)[3]. However, the specific role of NO in plant-fungal mutualistic interactions remains unknown.
This study proposes an investigation of NO signaling in the early responses of the interaction between arbuscular mycorrhizal fungi, *Rhizophagus irregularis*, and *Oryza sativa* L., by conducting a morphological analysis on the formation and development of rice large lateral roots and of the corresponding presence of a NO signal after exposure to AMF. After seven days of exposure, we observed a significant increase in the number and formation of secondary roots and primordia on the large lateral roots compared to the control. Furthermore, we observed a NO signal enhancement in the primordia and primary large lateral roots of the treated plants compared to the control group. Moreover, we noticed a signal pattern in the elongation zone of the primary large lateral roots of the treated group. In contrast, in the large lateral roots of the control group, the signal was diffused without a proper pattern.
In conclusion, our findings suggest that the presence of AMF favors not only the formation but also the development of secondary roots from the large lateral roots, with NO interacting in the process. In particular, the distribution of the signal suggests its role in the elongation of the roots. However, more results are needed to suggest the potential importance of NO as a signaling molecule in plant-fungal symbiotic interaction.
Bibliography:

1. Altamura, M.M.; Piacentini, D.; Della Rovere, F.; Fattorini, L.; Falasca, G.; Betti, C. New Paradigms in Brassinosteroids, Strigolactones, Sphingolipids, and Nitric Oxide Interaction in the Control of Lateral and Adventitious Root Formation. Plants 2023, 12, 413. https://doi.org/10.3390/plants12020413

2. Calcagno, C., Novero, M., Genre, A. et al. The exudate from an arbuscular mycorrhizal fungus induces nitric oxide accumulation in Medicago truncatula roots. Mycorrhiza 22, 259–269 (2012). https://doi.org/10.1007/s00572-011-0400-4

3. Chiu, C. H., Roszak, P., Orvošová, M., & Paszkowski, U. (2022). Arbuscular mycorrhizal fungi induce lateral root development in angiosperms via a conserved set of MAMP receptors. Current Biology, 32(20), 4428-4437.

The evolution of the land plant life cycle

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keywords: gametangia, gametophyte, haploid, diploid, sporangia, sporophyte.

Plants alternate between two multicellular life stages with different ploidy levels: the haploid gametophyte and the diploid sporophyte. The two inflection points in this alternation of phases occur in the gametangia (fertilization) and the sporangia (meiosis). The fundamental sexual reproductive structures of the gametophyte are the archegonium and antheridium (together called gametangia) and produce egg and sperm, respectively. While a salient feature of the diploid sporophyte is the development of the sporangium, the fundamental reproductive structure of all land plant sporophytes. The sporangium is where meiosis occurs to produce spores, marking the transition between the diploid and haploid phases of the plant life cycle. We are studying the development of gametangia and sporangia across land plants, with a focus on ferns, particularly the model fern *Ceratopteris richardii*. In ferns these two life stages are morphologically distinct and completely independent from each other. Although analyses of patterning provide strong working hypotheses, contributions from molecular genetics and functional studies will be necessary to determine the complete story of how these structures have evolved in land plants. Using bulk RNA-seq of six developmental stages, we developed a time series transcriptome atlas for the gametophyte *C. richardii*. This new resource has allowed us to understand broad patterns of gene expression changes throughout gametophyte development, investigate dynamics of shared vs. unique gene expression between life stages, and identify promising targets for further functional analyses. Our candidate approach focusses on TALE Homeodomain (TALE-HD) proteins and other transcription factors. We have also used bulk RNA-seq in vegetative and fertile sporophytes to investigate the expression of candidate transcription factors. Despite the differences in sporangia cell patterning between *A. thaliana* and *C. richardii*, we found homologous genes pattern sporangia in both species. This suggests that there is a common developmental genetic module that patterns all sporangia and provides a framework for understanding the evolution and development of sporangia. Finally, we analyze the relationship between patterns of expression between life stages and patterns of gene duplication in plant transcription factor families. These comparative analyses will allow us to better understand the evolution and development of plant life cycles.

FUTUREGRAIN: exploring genetic diversity among rice varieties in agricultural systems against global warming.

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Global warming is having a huge impact on world agriculture and, more specifically, on Italian crops. The combination of low water availability and super-optimal temperatures negatively affects the reproductive processes of plants, preventing normal seed development and consequently restricting both crop yields and grain quality. In Italy in particular, climate change is affecting many local farmers who are unable to maintain their plantations, closing crops and reducing national production.

Commercial rice crops are vulnerable to alterations in soil salinity or drought, which influences seed germination and the establishment of future progeny. Some old rice varieties, compared to commercial lines, show a higher resistance or sensitivity to water and salt stress during germination. These varieties show a corresponding de-regulation in the expression of specific genes, both during germination and seed development.

Recently, GWAS studies have identified genetic regulators associated with developmental signals, oxidative stress and metabolic regulation, potentially involved in drought and heat stress tolerance in rice grains. These players may contribute to protect plants from abiotic stress damage, ensure growth performance under stress by activating signalling cascades for response mechanisms, or induce protection of protein structure at the cellular level during osmotic and oxidative stress.

In this project we propose a detailed physiological, molecular and developmental characterisation of specific ancient and modern rice lines. We will also perform nutritional prfilling of local ancient varieties in order to determine potential lines that can be used in future breeding programmes for more sustainable, resilient and nutritionally optimal production in the field. Our aim is to understand, at molecular, cellular and developmental levels, the role of genes in seed quality and plant establishment, as well as their possible involvement in abiotic stress tolerance, especially in response to heat and drought.

Morphoanatomy from flower-to-fruit transition in Andean Loranthaceae

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Loranthaceae (Santalales) is a widespread family of root and stem hemiparasitic plants with a staggering diversity in floral morphology, especially in the Neotropics where there are small flowers (5 mm long), medium flowers (3-5 cm long), and long flowers (25 cm long). This flowers have unique modifications, such as: (1) massive hypanthium and reduced carpels, (2) ovules reduced to the embryo sac; (3) migration of the embryo sac nuclei from the ovary to the style and ectopic fertilization in the style, followed by the repositioning of the proembryo(s) to the ovarian region; and (4) fruits developing from inferior ovaries fused with the hypanthium, with abundant viscine. Other atypical features recorded include the presence of dimorphic embryos (i.e. embryos have foliose or prismatic cotyledons) in the genus *Psittacanthus* and the absence of endosperm described in *Psittacanthus* and *Aetanthus*.

Conventional serial sectioning of flowers and fruits at different developmental stages of *Aetanthus colombianus* A.C. Sm., *Tristerix secundus* (Benth.) Kuijt, and *Gaiadendron punctatum* (Ruiz & Pav.) G. Don. were made in the following manner: Flowers to preanthesis to late anthesis and developing fruits were dehydrated in a series Ethanol-Histochoice, ending in Paraplast Plus. Specimens were serially sectioned rotary microtome and stained with safranin-astra-blue.

The anatomical sections allowed us to revisit previous interpretations and assess the homology of these unusual features. We have concluded that the flower to fruit transition of these species go through: (1) migration of the nuclei of the megagametophyte reaching the third distal portion of the style where fertilization takes place; (2) formation of a secretory parenchyma associated with the vascular bundles of petals and stamens; (3) differentiation of the sclerenchymatic ovarian base that delimits the megagametophyte in the flower and the proembryo in the fruit; (4) development of the pendular embryo, facing the schlerenchymatic tissue, with a conspicuous hypocotyl, and formation and of the viscin in the hypanthium; (5) development of two foliose cotyledons surrounded by massive endosperm in the absence of a seed coat; and (6) delimitation of the fruit *sensu stricto* by the vascular bundles of the gynoecium, completely surrounded by the hypanthium. This work lays the foundation for comparative evo-devo studies on the genetic bases for ovule reduction in hemiparasitic neotropical Santalales.

Keywords: endosperm, inferus ovary, memelon, ovule, pelvis, viscin

Genetic and functional traits limit the success of colonisation by arbuscular mycorrhizal fungi in a tomato wild relative

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To understand whether domestication had an impact on susceptibility and responsiveness to arbuscular mycorrhizal fungi (AMF) in tomato (*Solanum lycopersicum*), we investigated two tomato cultivars ('M82' and 'Moneymaker') and a panel of wild relatives including *S. neorickii*, *S. habrochaites* and *S. pennellii* encompassing the whole Lycopersicon clade. Plants were inoculated with the AMF *Funneliformis mosseae* and biometric and molecular analyses were carried out.
Most genotypes revealed good AM colonisation levels while, by contrast, both *S. pennelli* accessions tested showed a very low mycorrhization rate, but normal arbuscule morphology, and a negative response in terms of root and shoot biomass. This behaviour was independent of fungal identity and environmental conditions. Genomic and transcriptomic analyses revealed in *S. pennellii* the lack of genes identified within QTLs for AM colonisation, a limited transcriptional reprogramming upon mycorrhization and a differential regulation of strigolactones and AM-related genes compared to tomato. Donor plants experiments suggested that the AMF is perceived as a cost for *S. pennellii: F. mosseae* could further proliferate in the roots only when it was part of a mycorrhizal network. Overall these results suggest that genetics and functional traits of *S. pennellii* are responsible for the limited extent of AMF colonisation.
These unique features make *S. pennellii* an unprecedented model to study the molecular mechanisms which regulate the extent of AM colonisation in plant roots. We suggest that the use of tomato wild relatives as a source of new alleles requires deeper analysis which also considers the plant-beneficial microbe interaction.

DNA barcoding to identify *Quercus cerris*: a new perspective to monitor the root systems of a species for urban afforestation and reforestation programs.

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In the active urban environment, amidst concrete and asphalt, plants play a crucial role in ecological balance and human well-being. Roots act as anchors, enabling plants to withstand storms and cope with the environmental challenges also in urban life, such as soil compaction and water scarcity. But they are also the conduits through which plants interact with the surrounding soil, absorbing nutrients, and water vital to the plant survival. However, the study of roots in urban is particularly challenging due to their hidden nature, compounded by the complexity of the urban environment. Molecular techniques offer promising ways of distinguishing tree species and allow detailed analysis of plant-environment interactions, early stress or pathology detection, and targeted management strategies development. In this perspective, innovative molecular-based approaches have the potential to cope with the difficulties in studying roots in urban contexts and can be essential to better understand the health and vitality of species such as *Quercus cerris*, which are widely suggested and used for afforestation and reforestation programs. This study, funded by the National Biodiversity Future Centre-NBFC project (CUP: H73C22000300001) under the National Recovery and Resilience Plan (NRRP) - NextGenerationEU, Mission 4 Component 2 Investment 1.4, aims to use DNA barcoding to identify oak species and their ecological interactions in urban contexts. Initially, seven candidate DNA barcodes (ITS, ITS2, matK, rbcL, psbA-trnH, TrnL-TrnF, and RpoC1) will be evaluated to identify the most suitable DNA barcode for *Quercus* species. This selection process integrates both theoretical considerations and empirical evidence, providing a robust basis for DNA barcode screening and species discrimination in plants. Sequences with the highest genetic divergence within the oak genus will then be selected and primers designed using automated methods and bioinformatics tools. The primers are then validated by BLAST analysis. In silico tests show that a molecular system using at least two primer pairs is sufficient for the molecular identification of *Q. cerris*. The next steps include in vitro validation of the identified primers followed by their application on roots from intertwining field samples. Through accurate monitoring by molecular identification of oak, other plant roots, and possibly pathogens, targeted interventions can be taken to protect and promote urban biodiversity based on a full understanding of the interactions occurring in the “hidden half” of the urban environment.

Comparative plastomics of plantains (*Plantago*, Plantaginaceae) and new insights into DNA barcoding and phylogenetic relationship

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The *Plantago* genus consists of over 250 species belonging to the Plantaginaceae, the plantain family. Several *Plantago* species hold significant value in the nutraceutical and pharmaceutical sectors but their derivatives can suffer from contamination and adulteration affecting their usefulness and safety. Here, we aimed at a comprehensive analysis involving the sequencing, assembly and annotation of the complete chloroplast (cp) genomes of three *Plantago* species, namely *P. lanceolata* L., *P. argentea* Chaix and *P. atrata* Hoppe. This study aimed to achieve several goals: (i) a comparative exploration of the *Plantago* cp genomes to gain insights into fundamental genome structure, codon usage bias, repetitive structures, RNA editing sites, and substitutions; (ii) the identification of mutational hotspot regions, that might be used for the development of robust and cost-effective DNA markers, primed to be effectively used for species identification and DNA barcoding; (iii) the establishment of a comprehensive phylogenetic framework highlighting the relationships amongst species, thereby facilitating inferences about the taxonomic status of *Plantago* within the subfamily Plantaginoideae, derived from the complete cp genomes. The outcomes of this study provide crucial information for investigations into taxonomical classification, genetic diversity across distinct populations, and the discrimination of closely related species within the genus *Plantago*.

Exploring Soil-*Quercus cerris* interactions along a soil fragmentation gradient in Italian Urban Soils

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Urban ecosystems and biodiversity, which are essential to human well-being, have been seriously threatened in recent years by the combined effects of climate change and human activity. According to recent studies, interactions between plant roots and the biotic and abiotic elements of soil below ground, can shape biodiversity and ecosystem dynamics in important ways that are frequently overlooked. To explore the importance of these understudied interactions in urban environments, a controlled experiment was conducted using young seedlings of *Quercus cerris* (Turkey oak) grown in three distinct urban soils collected from various sites in the city of Campobasso (Molise region, Italy) representing a gradient of fragmentation. The collected soils were utilized to set up rhizoboxes for growing *Q. cerris* seedlings over a period of two weeks. After this growth phase, each soil sample (bulk soil and rhizosphere longitudinal surface) was subjected to 2-D soil zymography to investigate the formation of enzyme hotspots and the spatial distribution of three key enzymes: leucine aminopeptidase (related to the nitrogen cycle), β-glucosidase (related to the carbon cycle), and acid phosphatase (related to the phosphorus cycle). The 2-D zymogram analysis showed the spatial heterogeneity of soil enzymatic activity along the fragmentation gradient, with the most fragmented soil samples showing the highest enzymatic activities and hotspot level. The differences can be explained with two interdependent elements that seems to follow the fragmentation gradient: stress conditions and soil organic pollutants (SOPs). SOPs may act as microorganisms' substrates in bulk soil, resulting in an increase of the enzymatic activity. SOPs may simultaneously stress oaks, inducing them to produce enzymes and exudates in the rhizosphere in order to reduce the stress conditions. In addition, also the microbial communities characterized by 16S rDNA gene sequencing, revealed variations in their composition among the three sites and between the bulk soils and the rhizosphere of the same site. These microbial communities and the Vmax and Km values of the enzyme kinetics were correlated with a heat map to soil physiochemical parameters, showing significant correlation with some of them. To further correlate these processes, a comprehensive analysis of all these investigations are currently underway, The final aim of this ongoing study is to shed light on the function of root system and how they can mitigate the urban threats.

Large-scale bioprospection of the Italian flora: from drug discovery to the study of metabolome diversification in land plants

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During their history, plants have evolved various physiological adaptations and a vast arsenal of biomolecules to thrive in diverse environments and coexist with other living organisms. The extensive diversity in structures, functions, and bioactivities exhibited by plant specialized metabolites represents a valuable resource for bioprospecting, i.e. the exploration of biodiversity for new resources of social and commercial value. At the same time, it poses a significant challenge in studying the chemo-evolutionary dynamics affecting biosynthesis and diversification of specialized metabolites among the various taxa of land plants.

In the frame of the National Biodiversity Future Center, dedicated to the monitoring, conservation, restoration, and valorization of biodiversity, we set up a large-scale bioprospection plan to cover the huge phytochemical diversity expressed within the vascular and non-vascular Italian flora (more than 11,000 taxa[1,2]). We selected about 700 species on a phylogenetic basis to create a core collection in which all Italian plant families are represented in accordance to their relative amplitudes (e.g., for the Angiosperms, in order: Asteraceae, Poaceae, Fabaceae, Rosaceae, Caryophyllaceae, Brassicaceae, Apiaceae, Lamiaceae, etc.). Plants were sampled from Italian botanic gardens, nurseries, and open fields from various geographical regions of Italy. Among the vascular section of the collection, about 75% of the species are native (comprising 53 endemic species), and 25% are alien (including 48 casual and 92 naturalized species, of whom 38 are invasive). The collection is being currently characterized through UPLC-HR-MS, and the species with the most interesting phytochemical

profiles are entering a downstream bioactivity screening program focused on non-communicable diseases (e.g., neurodegenerative diseases, cancer, metabolic syndrome, etc.) and crop enhancement and protection. This screening aims to identify specific phytochemicals that can be exploited to produce drugs, nutraceuticals, cosmetics, and products for more sustainable agricultural practices.
In parallel, comparative data generated from the untargeted metabolomics analysis of our collection will show how plant-specialized metabolites chart within the various lineages of the Italian flora, providing further knowledge to understand metabolome diversification in land plants.

[1] Portale della flora d'Italia - Portal to the flora of Italy, 2023.1. (https://dryades.units.it/floritaly/; retrieved on 21 March 2024)

[2] Muschi ed epatiche d'Italia - Mosses and liverworts of Italy (https://dryades.units.it/briofite/index.php; retrieved on 21 March 2024)

Characterization and valorization of autochthonous bean (*Phaseolus vulgaris* L.) and lentil (*Lens culinaris* Medik) ecotypes

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Autochthonous ecotypes or local varieties are plant genetic resources characterized by high genetic diversity, specific adaptation to the environmental conditions of the cultivation area (i.e. tolerance to biotic and abiotic stresses), and the presence of specialized metabolites (terpenoids, flavonoids, alkaloids) and health-promoting compounds. However, local varieties are highly endangered, mainly due to their replacement by commercial varieties. Untargeted metabolomics is a strong approach that allows the investigation of a wide range of metabolites 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 ecotypes from different villages of Molise region (Italy) - Capracotta, CA; Rionero Sannitico, RS; and Agnone, A - in comparison to one ecotype from Umbria region (Italy) - Castelluccio di Norcia, CS - and three autochthonous bean ecotypes from Molise region (Italy) - Ciliegino bean, CI -, Basilicata region (Italy) -, and Spain - Spanish Ciliegino bean (CI SP) -, previously characterized from a genetic and morphological point of view. Untargeted metabolomics, performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) allowed the detection of 344 and 662 differential accumulated metabolites (DAMs) for bean (Be) and lentil (Le) ecotypes, respectively. The annotation of the DAMs, by consulting home-made spectral and MS libraries (e.g., the GNPS Public Spectral Library) and a molecular network approach (MetGem and Cytoscape software), allowed 47.2% and 54% of them to be assigned to different metabolic categories (12 for Be and 13 for Le). Flavonoids were the most represented metabolic category (66 for Be, 87 for Le), followed by amino acids and derivatives (59 for Be, 61 for Le), and cinnamic acids (22 for Be, 50 for Le). Significant variations in the metabolite composition of Be and Le were also observed through the combination of univariate and multivariate statistical analyses. According to the PCA scores plot, A, CA, and RS lentil ecotypes were separated from CS by the PC1 (56 % of variance). Similarly, CI and CI SP were separated from SMR by the PC1 (58 % of variance). Two clusters for Be and Le, respectively, were revealed by hierarchical cluster analysis, indicating the distinctiveness of the traditional varieties. Additionally, a partial least squares-discriminant analysis (PLS-DA) found 15 variables important in the projection (VIP) scores of metabolites, belonging to flavonoids, amino acids, and terpenoids for Le and flavonoids for Be. The enrichment analysis is in progress to assess the presence of enriched metabolomic categories, allowing the selection of ecotypes-specific metabolic features. In addition, the antimicrobial, and antioxidant activity of both legume extracts has been evaluated and analyzed in the light of the distinct metabolic profiles of each ecotype to better understand the beneficial/ health-promoting effect of some bioactive compounds.

Structural characterization and hygroscopic expansion of wild oat awns

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Wild oat seed dispersal depends on two hygroscopic dispersal units, called awns, capable of bending and twisting following changes in environmental humidity. These movements favor the dispersal of the seed capsules on and into the ground. The awns can be divided into an active hygroscopic region, called the base beam, and an inactive one, called the bending tail. Although, a previous investigation has observed the orientation of the cellulose microfibrils (MFA, microfibril angle) inside the awn(*1*), a study analysing the internal structure of the awn and the hygroscopic transversal swelling behavior of the cells in relation to the role and movement of the awn has yet to be conducted.

Sections of the *Avena Sterilis* L. awns were analysed histologically by comparing the active twisting base beam with the non-humidity responsive bending tail to detect the most evident structural differences. The results demonstrated a different structure between the two sections, with the base beam formed by cells with thick secondary cell walls either with symmetric or asymmetric deposition, suggesting a mechanical role besides the hygroscopic one. In contrast, in the bending tail sections, we observed the loss of the thick secondary cell walls, confirming its inactive hygroscopic role. Subsequently, the hygroscopic swelling behavior was analysed only in the active base beam sections at different relative humidity conditions. A differential expansion of the cells was observed and depended on the cell localization and composition within the awn section. An expansion gradient was visible from the internal to the external layer of the section. Furthermore, the external layer cells with the most expansion were stained with Alcian blue, a marker for acidic polysaccharides like pectin. This suggests that these compounds favor the transversal hygroscopic swelling in the direction of minor stiffness. Overall, the results of this study suggest a different role between the base beam and bending tail, with a more mechanical one, besides hygroscopic, for the base beam and a flexible role for the bending tail. Furthermore, the differential transversal hygroscopic expansion of the cells favored by the presence of polysaccharides may suggest a diverse contribution to the expanding behavior of the awn.

1. T. Lindtner, A. Y. Uzan, M. Eder, B. Bar-On, R. Elbaum, Repetitive hygroscopic snapping movements in awns of wild oats. *Acta Biomater.* 135, 483–492 (2021).

Assessment of lichen biodiversity and species distribution in an urban area: the case study of the University of Salerno (Italy).

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Lichens are symbiotic associations among fungi (mycobionts), mostly ascomycetes, and green algae or cyanobacteria (photobionts). The lichen symbiosis is one of the most successful example whereby fungi fulfill their requirement for carbohydrates, with nearly one fifth of all known fungal species being obligate lichen-forming species. Unlike plants, lichens do not have a protective outer layer and hence absorb nutrients directly into their thalli from the rainwater and atmosphere**.** Epiphytic lichens are one of the most valuable biomonitors of environmental pollution. They can be used as sensitive indicators to estimate the biological effects of pollutants by measuring changes at the community or population levels, or as accumulative monitors of persistent pollutants, by assaying their trace element content (Loppi et al., 2004). The aim of this study was to evaluate, for the first time, the epiphytic lichen biodiversity and species distribution in the area of the University Campus of Salerno, localized in the southern Italy and covering a total surface of 1.200.000 m^2. Epiphytic lichen thalli were collected in 20 square plots 33x33 m, from May 2022 to April 2024 in the established sampling units (SUs) of the University Campus. In this context, the lime trees with subacid bark were used. The different lichen species were characterized in the laboratory by means stereomicroscope, optical microscope, and chemical reagents commonly used in lichenology studies. Color, morphology and different structures of thalli were described. A high abundance of nitrophyc (*e.g., Xanthoria parietina*) crusted (*e.g., Lecidella elaeochroma*) and foliose (*e.g., Punctelia subrudecta*) macrolichens were identified (20). The lichen biodiversity index (LBI) ranged between 17 and 103, and an interesting trend was revealed along the SUs. From the SU located near the bus station (LBI value of 44), toward the internal area of the campus (LBI value of 68), an increasing gradient in the LBI was detected, suggesting an improvement of the air quality. However, the species of epiphytic lichens identified are recognized for their ability to tolerate major atmospheric pollutants, such as NH_3 and nitrogen oxides. The LBI values recorded in the large part of the SUs can be attributable not only to a great naturalness of the site but also to a different influence of the winds which carry pollutants along the different areas of Campus. Although the study area is located in an urban context, the lichen biodiversity was very high and indicated a good degree of naturalness of this area.

References:

Loppi, S., Frati, L., Paoli, L., Bigagli, V., Rossetti, C., Bruscoli, C., & Corsini, A. (2004). Biodiversity of epiphytic lichens and heavy metal contents of Flavoparmelia caperata thalli as indicators of temporal variations of air pollution in the town of Montecatini Terme (central Italy). *Science of the total environment*, *326*(1-3), 113-122.

Development of an early warning tool based on DNA barcoding for invasive plant detection in the urban area

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Invasive plants can compromise the ecological balance and threaten local biodiversity by invading green areas and public spaces. Prevention is crucial to avoid the introduction and spread of new invasive plants. With the aim of developing a new early warning tool for invasive plants detection, eight invasive plants listed in the EU blacklist have been selected (i.e., *Ludwigia grandiflora, Elodea nuttallii, Myriophyllum aquaticum, Pontederia crassipes, Ailanthus altissima, Heracleum mantegazzianum, Impatiens glandulifera, Pueraria montana*), and species-specific primer pairs have been designed on DNA barcoding common region (i.e., *rbcL, matK, psbA-trnH* and ITS2). Subsequently, mixes with typical urban spontaneous plants and invasive species were created in laboratory. Tests demonstrated the primer pairs' ability to uniquely identify the invasive species in the mix, making it possible to detect their occurrence within 24 hours. This rapid detection capability will enable environmental operators to intervene promptly to contain the spread of invasive plants before they can cause significant damage to the local ecosystem. This tool could have a significant impact on the protection of local biodiversity and the integrity of urban habitats.

Pollen development, ultrastructure and recognition in *Ginkgo biloba*

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For a real understanding of seed plants evolution, it is essential to expand our knowledge about their reproductive mechanisms. While the majority of research on this topic has focused on angiosperm model systems, it is important to extend our attention also towards gymnosperms, which constitute a significant proportion of seed plant diversity. Among gymnosperms, *Ginkgo biloba*, with its key phylogenetic position, offers a window into the early evolutionary history of spermatophytes.
Recent studies have highlighted the pivotal role of pollination in *Ginkgo* ovule development. The observation that unpollinated ovules undergo abortion highlights the significance of pollen-ovule interactions in *Ginkgo* reproduction. However, the underlying molecular mechanisms remain largely unexplored. To unravel the nature of the determinants mediating pollen-ovule recognition, a deeper understanding of *Ginkgo* pollen formation and ultrastructure is necessary.
Ginkgo pollen consists of four cells and is enveloped by a double wall, which exhibits uneven thickenings. Notably, pollen morphology and ultrastructure undergo drastic changes depending on the hydration state. We have analyzed such structural changes via electron microscopy: hydrated pollen tends to assume a more or less spherical shape, while dehydrated pollen adopts a distinct elongated boat-like shape, due to the uneven thickness of the wall.
We have formulated several hypotheses about the nature and the localization of the pollen determinants. Probably multiple levels of communication are necessary for an effective recognition between pollen and ovules. These may include: (1) signals deposited on the exine surface, (2) signals at the level of intine, exposed after the decoating process that *Ginkgo* pollen undergoes upon hydration, (3) signals synthesized *de novo*. To shed light on these aspects, we aim to conduct chemical analyses on the pollen, including assessments of carbohydrates and proteomic content, also in comparison to pollen collected from other gymnosperm species.
Male cone development in *Ginkgo* progresses through several stages, that should be considered to dissect the molecular and cellular events shaping pollen production. We have identified three key developmental stages for in-depth molecular analysis: (1) winter buds, containing cones with the sporogenous tissue in a hibernating state; (2) newly opened buds, containing cones with actively dividing microspores; and (3) male cones just before dehiscence, containing mature pollen ready to be released. Molecular analyses, including immunolocalization of phytohormones on these three stages, have provided initial insights into the role of auxin in male cone development. These findings shed light on the intricate hormonal regulation underlying *Ginkgo* reproductive processes. Furthermore, ongoing RNA sequencing analyses aim to elucidate the main gene networks orchestrating *Ginkgo* reproductive processes and their interaction with the hormonal signaling.

Unraveling the role of *APOSTART1* and *2* in female gametophyte progression in *Arabidopsis thaliana*

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Apomixis is asexual reproduction through seeds. Considering that apomixis does not naturally occur in major crop species, its introduction into agronomically relevant plants would enable fixation of the complete genome of elite hybrid genotypes, leading to efficient and consistent production of high-quality grains, fruits, and vegetables. Notwithstanding the importance of this process and the big effort invested in studying apomictic reproduction, many aspects of this reproductive strategy are yet to be fully understood. We previously identified a gene, *PpAPOSTART*, as a promising candidate to determine apomixis in in the apospourous *Poa pratensis.* Interestingly, it is specifically expressed in female reproductive tissues, and it exhibited delayed expression in apomictic ovules. In *Arabidopsis thaliana*, the most related genes are *APOSTART1* (At5g45560) and ENHANCED DISEASE RESISTANCE 2 (*EDR*2; renamed APOSTART2, At4g19040). Analysis of the generated double mutant infers a role of these genes in female gametophyte development. Furthermore, a putative role of APOSTART1 and 2 in association with the endoplasmatic reticulum (ER) and mithocondria was shown and discussed.

Unravelling mechanism of apomictic reproduction in Hieracium species.

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Hieracium L. (Asteraceae) is one of the most diverse plant genera among the angiosperms. Its extreme morphological variability is caused by complex reproductive strategies, massive interspecific hybridization in the past, and polyploidization. Variation in ploidy levels is associated with differences in modes of reproduction, few sexual diploids and numerous apomictic polyploids are known.

In sexual flowering plants, a diploid precursor cell inside the ovules called a megaspore mother cell (MMC) undergoes meiosis to produce four haploid cells. One spore survives and gives rise to the mature female gametophyte containing the haploid egg cell. After fertilization by the haploid pollen sperm cells, the egg cell initiates embryogenesis, and the ovules turn into seeds.

Polyploids of Hieracium reproduce instead strictly apomictically. Morphological analysis at the confocal microscopy confirmed that in apomictic Hieracium meiosis is omitted and the unreduced embryo sac originates from the MMC directly through mitosis, a phenomenon also known as diplosporic apomixis of the Antennaria-type. Moreover, apomictic Hieracium species are amongst the few known apomicts capable of both fertilization-independent embryo and endosperm development during seed formation (i.e. parthenogenesis).

Notwithstanding the importance of this process and the big effort invested in studying apomixis, many aspects of this reproductive strategy are yet to be fully understood. For this reason, we are also setting the base for performing whole ovary transcriptomes from apomictic and sexual species. Analysis of genes differentially expressed between sexual and apomictic accessions would provide new initial data for the comprehension of the molecular mechanisms associated with apomictic behavior that would contribute to elucidating complex evolution and play an important role in future noteworthy crop improvement.

Maternal regulation of starch metabolism plays a pivotal role during ovule and seed development

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In angiosperms' ovules, fertilization of both the egg and the central cell is required to ensure proper seed development. *ARABIDOPSIS* B_{sister} (*ABS*) and *SEEDSTICK* (*STK*) encode for MADS-domain transcription factors (TFs), which are expressed in the ovule integuments and seed coat, that are required for proper ovule differentiation and seed development. In the *abs stk* double mutant, the seed set is largely affected resulting in a few viable seeds. Moreover, the double mutant ovules show defects in integuments differentiation, together with a large amount of starch inside the embryo sac.
Although most of the *abs stk* ovules are properly fertilized, as demonstrated by the presence of the zygotes, the development of the embryo is drastically delayed and arrested a few days after fertilization in most of the developing seeds.
To unveil the roles of ABS and STK, during ovule and early seed development, we studied the *abs stk* double mutant phenotype by employing ChIP-Seqencing, transcriptomic and metabolomic approaches. This broad analysis has shown that sugar metabolism is widely targeted by these two TFs and impacted in the double mutant. Finally, by employing two different approaches, we were able to modulate the starch accumulation in the *abs stk* double mutant, partially rescuing the embryo and seed developmental defects.
Concluding, these data highlight the importance of the interplay between metabolic and developmental processes regulated by the maternal tissues on the products of fertilization.

Functional characterization of the AUXIN RESPONSE5/MONOPTEROS (ARF5/MP) in ovule development in *Arabidopsis thaliana* and *Oryza sativa*

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AUXIN RESPONSE FACTOR 5/MONOPTEROS (ARF5/MP) is involved in several plant developmental processes linked to auxin signalling, such as root elongation, ovule development, and embryo formation. As with several other ARFs, ARF5/MP is regulated by Alternative Splicing (AS) events. By studying the role of ARF5/MP in ovule development, we have identified Arabidopsis as an AS event that retains the eleventh intron, generating an isoform that could work in auxin minima (Cucinotta et al.,2021).
Another AS isoform lacking part of the 5'UTR and of the first exon was also annotated in Arabidopsis. Through CRISPR-Cas9 technology, we generated and characterised mutants with a premature stop codon downstream of the third ATG, mimicking the protein that could be obtained by AS in Arabidopsis. Interestingly, these Arabidopsis mutants did not present a strong phenotype except for a disorganised Quiescient Centre (QC) in the primary root.
Our research is focused on the investigation of the putative evolutionary conservation of the role of the alternative splicing isoform in rice with respect to Arabidopsis.

Genetic and hormonal network regulate ovule development in rice (*Oryza sativa* L. cv. Nipponbare).

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Ovule is the female reproductive plant organ and play a pivotal role in sexual reproduction. In particular, it orchestrates essential processes such as megasporogenesis, megagametogenesis, fertilization, embryogenesis and culminates in seed production. Seeds ensure the continuity of plant generations and directly impact yield in seed-crop plants. Therefore, to understand ovule formation and plant reproduction are crucial for potential biotechnology applications aimed to enhance crop yield. Rice (*Oryza sativa* L.) is one of the most significant seed-crop species, globally providing essential caloric requirements for billions of people. Thus, investigating rice ovule development is highly pertinent and offers an excellent model system for studying organogenesis and cell differentiation processes in cereals. The rice pistil contains a single bitegmic ovule and the sequence of developmental events, occurring to form a mature ovule, are well defined and comprise nine distinct stages [1]. Auxin has been shown to be of pivotal importance for both ovule initiation and developmental pattern definition in other species. Genetic studies in *Arabidopsis* revealed the fundamental role of AUXIN RESPONSE FACTOR (ARF) gene families in ovule development [2]. ARF5/MONOPTEROS (MP) and ARF3 are required for ovule initiation and female germ line development, respectively [3-4]. In particular, ARF5/MP has been identified as a master regulator of ovule development since it controls expression of several key genes involved in pistil and ovule primordia formation. Recently, transcriptomic analysis of rice ovules revealed the expression of several ARF genes, suggesting that auxin could regulated ovule development like in *Arabidopsis.* However, in rice the molecular mechanisms are still largely to be clarified.
In this context, our aimed was to investigate the crosstalk between auxin and the *ARF* genes, controlling ovule development. For this purpose, *in situ* hybridization and auxin distribution analyses were performed during different stages of ovule development. During early stages of ovule development, *OsARF11* transcripts were detected in the archespore cell and in the integument primordia. While, at the latest developmental stages, the hybridization signal was observed in the entire ovule and in the vascular bundle. Moreover, *OsARF1* was also detected in the integument and in the ovule at the latest stages. Concerning auxin immunolocalization, no auxin accumulation was observed in the nucella during female gametophyte formation, but a distinct signal was present in the ovary. In conclusion, our preliminary results show different expression domains for *OsARF* genes and auxin in the latest ovule developmental stage. Furthermore, they provide interesting new insight on the role of both *ARF* genes and auxin during rice ovule development.

[1] Lopez-Dee et al., Developmental Genetics (1999) 25, 237-244;
[2] Larsson et al., Journal of Experimental Botany (2013) 64,2619-2627;
[3] Galbiati et al., the plant journal (2013) 76, 446-455;
[4] Su et al., Plant Cell (2020) 23, 2842–2854.

Interaction between environmental stresses on *Phaeodactylum tricornutum*: applications for green economy

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Microalgae are proposed for multiple biotechnological applications, e.g. as feed for aquaculture, thanks to their ability to produce high-value molecules, such as proteins, lipids and polyphenols. Among microalgae, diatoms produce biomass rich in lipid and fucoxanthin, have a high growth rate, and are the only known organisms to produce a porous silica cell wall. This unique cell wall is itself a promising material, currently being studied as a nanomaterial for different applications, e.g. membrane filtration **[1]**. *Phaeodactylum tricornutum* is peculiar among diatoms thanks to its ability to change morphotypes under different environmental conditions, with only one morphotype being silicified. It is also widely known that abiotic stress can enhance the accumulation of valuable metabolites **[2]**. Thus, *P. tricornutum* plasticity makes it an optimal candidate for multiple biotechnological applications.

In this work, we studied light and salinity stress interactions on the growth of *P. tricornutum*. Algae were grown under three different light setups (White, Red-enriched and Blue-enriched LED light) in undiluted and 10% diluted artificial seawater enriched with f/2 media. Sodium metasilicate pentahydrate was added at a final concentration of 100 µM as a source of silica. Cell density and PSII maximum quantum yield were monitored to assess growth status. Moreover, morphological differences during exponential and linear growth phases were observed thanks to transmission electron microscopy (TEM). Changes in biomass composition were evaluated by considering pigments and lipids concentrations. These tests are the preliminary phase needed to choose the best environmental conditions for multiple biotechnological applications such as the production of membranes for wastewater filtration and the large-scale production of *P. tricornutum* as a feed for aquaculture.

References:

[1] C. Zhang *et al.*, 'Poly(lactic acid)/artificially cultured diatom frustules nanofibrous membranes with fast and controllable degradation rates for air filtration', *Adv Compos Hybrid Mater*, vol. 5, no. 2, pp. 1221–1232, Jun. 2022, doi: 10.1007/s42114-022-00474-7.

[2] U. Suparmaniam *et al.*, 'Abiotic stress as a dynamic strategy for enhancing high value phytochemicals in microalgae: Critical insights, challenges and future prospects', *Biotechnology Advances*, vol. 70, p. 108280, Jan. 2024, doi: 10.1016/j.biotechadv.2023.108280.

Acknowledgements

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 04 Component 2 Investment 1.5 – NextGenerationEU, Call for tender n. 3277 dated 30/12/2021 Award Number: 0001052 dated 23/06/2022

Use of microalgae as biostimulants for fruit plants: a field study on *Fragaria x ananassa*

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Bio-based solutions are gathering more and more interest, not only for addressing environmental challenges, but also as sustainable and viable alternatives to chemical products. The use of microalgae as biostimulants in agriculture represents an innovative strategy to enhance growth, productivity, and resilience of agronomically interesting plants. Microalgae are photosynthetic microorganisms rich in nutrients, bioactive compounds, and secondary metabolites that can positively influence plant development. Microalgal cultures offer numerous advantages over traditional chemical biostimulants, including environmental sustainability and biodegradability [1].
Fragaria x ananassa is a plant of agronomic and economic interest due to the pleasing flavour of its false fruits (strawberries) and their recognized value as reservoirs of bioactive compounds renowned for their antioxidant properties.
The aim of the present study was to assess the effectiveness of a microalgal product (in detail, the exhaust culture medium of two microalgae strains, *Neochloris oleoabundans* and *Chlorella protothecoides*) as biostimulant for the growth, productivity, and fruit quality of strawberry plants. The investigation was conducted in collaboration with Consorzio Italiano Vivaisti (CIV, San Giuseppe di Comacchio, Ferrara, Italy). The centre provided the strawberry plants, allowed field experimentation, and performed some analyses on the harvested fruits, enabling the evaluation of a potential biotechnological application of microalgal products in the agricultural sector. Based on previous studies by the research group, *Fragaria* plants of the cultivar KLODIA® CIVH725* set up in an outdoor field were selected for treatments. The product was applied both through the soaking of seedlings at transplanting and through foliar applications. The characteristics of plants and false fruits, as well as physiological parameters related to photosynthetic efficiency, are currently underway. However, the preliminary results obtained show differences in the total number of flowers and fruits between the plants belonging to the control and treated groups, while maintaining a similar photosynthetic efficiency. Therefore, these results suggest a potential increase in the productivity of plants treated with microalgae-based biostimulant, a hypothesis that will be further evaluated upon the collection and analysis of false fruits.

[1] B.K. González-Pérez, A.M. Rivas-Castillo, A. Valdez-Calderón, M.A. Gayosso-Morales, Microalgae as biostimulants: a new approach in agriculture, World J Microbiol Biotechnol 38 (2022) 4. https://doi.org/10.1007/s11274-021-03192-2.

This research received financial support from the University of Ferrara and Camera di Commercio, Industria, Artigianato e Agricoltura of Ferrara through a CCIAA-2022 fund granted to CB.

A dexamethasone-inducible gene expression system in *Cannabis sativa* suspension cultures for the production of secondary metabolites

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During their evolution plants developed many biosynthetic pathways to synthetize a wide array of molecules, known as secondary metabolites. Plants' biodiversity is still today a huge resource for drug discovery, but also for secondary metabolites uses in nutraceutical, cosmetic and agricultural applications. The development of reliable productive processes that exploit plant cell cultures as biofactories could constitute a sustainable source of fine chemicals for pharmaceutical, cosmetic and food industry. Among the plants used for their secondary metabolites *Cannabis sativa* is a source of many molecules of growing commercial interest for their bioactive properties. Cannabinoids are synthetized mostly in the female inflorescence, where they are secreted in the storage cavity of the trichome gland, to avoid their toxic effects on the cell. To explore the possibility of using *Cannabis* cell suspension cultures to produce secondary metabolites, we generated stably transformed cell lines expressing transcription factors involved in trichome differentiation. These genes were expressed using the dual component expression system pOp6/GR-LhG4, where the transcription is activated in presence of dexamethasone (DEX). The effect of different DEX treatments on transcriptional activation was tested using the reporter genes GUS and RUBY, the latter being a reporter system that causes the production of the red pigment betalain. GUS activity and betalain quantification showed that gene expression in transformed cells is induced by DEX and in a concentration-dependent manner. Repeated induction also had a positive effect on the induction. Moreover, measurement of GUS activity allowed to screen for cell lines that showed the best induction response and to select them for subsequent gene expression and metabolites analyses. Some of the trichome-related transcription factors were also expressed in tobacco plants to confirm their effect on trichome differentiation and thus on secondary metabolites production. The set up of an effective inducible system in *Cannabis* cells allows the generation of cell lines transformed with genes that could otherwise have a negative impact on culture growth when expressed constitutively, opening the way to potential biotechnological applications.

Identification and quantification of different bioactive compounds in *Cynara cardunculus* L.

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Climate change and overpopulation are responsible of the over-exploitation of natural resources. Furthermore, the development of sustainable materials fits the need to support and implement good practices of Circular Economy. Plant can be key actor of the change to a more sustainable world. In particular, agro-industrial residues are a possible source of bio/pharmaceutical molecules of interest and biomass that can be used to produce innovative and smart materials.

The objective of this project is to recover and further valorise a significant number of bioactive molecules and other potentially value-added components, such as fibrous extraction residues, from Globe artichoke (*Cynara cardunculus* L., Violetto di San Luca variety), for scientific and commercial purposes in the Emilia-Romagna region. In fact, artichoke is well known for its benefits to human health and only the heart and some inner bracts are traded for food, while 60–85% of the total processed dried matter of the vegetable is discarded.

The present study focuses on identification and setting up of an integrated array of sustainable cascade of technologies, to recover and convert a significant amount of bioactive molecules like (poly)phenols, inulin and terpenes.

Leaves, outer part of stems and external bracts were separately dried and grinded. Metabolites were extracted by different methods, including water processes, solvents and the use of NaDES (Natural Deep Eutectic Solvents).

The biochemical composition of the extracts has been characterised (total polyphenols, reducing sugars and anti-oxidant activity) by means of spectrophotometric assays and inulin has been quantified by a Jasco V-730 spectrophotometer. Identification and quantification of specific (poly)phenols was determined by high-performance liquid chromatography (HPLC-DAD) analyses.

Results showed hot water as a good extraction method for polyphenols especially for the stem organ, while leaves and bracts show a similar result at a lower temperature.

NaDES has revealed a good extraction method for reducing sugars but laborious during its preparation due to its high viscosity.

The final result of this study will be the obtainment of a small number of extracts to be used in the cosmetic and nutraceutical fields and of compostable material to be used as packaging and all applied technologies are going to be selected with the aim of reducing the environmental impact of the final processes.

This study received funding from the European Union - NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – Missione 4 Componente 2, Investimento 1.5 – D.D. 3277, 30/12/2021, title: Ecosystem for Sustainable Transition in Emilia-Romagna, proposal code ECS00000033 - CUP J33C22001240001).

Histological and chemical characterization of Pompia fruit

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"Pompia" is the popular name of an ancient Citrus ecotype belonging to the Rutaceae family, probably of hybrid origin, endemic and cultivated in North-Eastern Sardinia. The fruit represents the most peculiar morphological character of this plant: it is a medium-large, deep yellow hesperidium, with a characteristic deformed knobbly peel, and a thick mesocarp, which can weigh up to 500–700 g. Despite the remarkable pharmaceutical potential of its essential oil profile, little taxonomic and genetic information is available for this species, and its nomenclature and phylogeny are still debated among researchers. Due to the characteristic irregularity of the fruit, with pronounced roughness, Moris (1837) proposed the botanical name (not rejected) *Citrus medica* var *monstruosa*. Recently, the scientific name *Citrus limon* var *pompia* Camarda var *nova* was assigned in 2013 by Camarda et *al*., and in 2019, Luro et *al*., adopted the ancient name *C. medica tuberosa* Risso & Poiteau. The difference between these studies lies in the classification as lemon cultivar (Camarda et *al*., 2013) rather than a citron one (Luro et *al*., 2019).

In recent years, research on Pompia fruit gained interest for its relevance in Sardinian biodiversity as important ecotype to preserve, and for its organoleptic and nutritional properties. Both Pompia's essential oil and peel extracts demonstrated various antioxidant, antimicrobial, antifungal activities, and represent the most thoroughly investigated component of the fruit (Pinna et *al*., 2019; Rosa et *al*., 2019; Rosa et *al*., 2022, Usach et *al*., 2020). To the best of our knowledge, however, there are no studies on the histological characterisations of the peel, and on the analysis of Pompia pulp, while only one study provides a preliminary chemical characterisation of its juice, identifying seven compounds (Barberis et *al*., 2020).

Therefore, this research aims at the full characterisation of Pompia fruit by chemical and histological characterisation. The histological analysis was performed on full peel samples (flavedo and albedo), providing useful insight into morphology, shape and size of the glands and their localisation. The chemical analysis was performed by Nuclear Magnetic Resonance (NMR) and chromatographic techniques: High-Performance Thin Layer Chromatography **(**HPTLC), High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS), and Gas Chromatography-Mass Spectrometry (GC-MS). The results revealed 18 compounds exclusively in the pulp and juice: the polar fraction consisted of flavonoids and phenolics acids, particularly hesperidin and naringin, while the volatile fraction was predominantly constituted by the monoterpene limonene.

In conclusion, this study presents a comprehensive characterization of Pompia juice and peel, highlighting its chemical complexity and structural diversity. The findings contribute to a deeper understanding of this endemic citrus, laying the groundwork for further exploration of its biological activities, deepening its nutritional and cosmetic potential.

Evidence of nanoCaCO3@PAE internalization in protoplasts and leaves of tobacco seedlings

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Plants are constantly exposed to abiotic and biotic stresses, a problem amplified by the incidence of climate change. These stress conditions have significant impacts not only on agriculture, but also on the most vulnerable endemic species, generating serious consequences on crop productivity and biodiversity loss (Semeraro *et al.*, 2023). The emerging field of plant nanobiotechnology offers a promising avenue for improving plant responses to various stresses, both abiotic and biotic. Despite considerable interest, the uptake of nanoparticles by plants remains a controversial issue; the cell wall constitutes a physical and biological barrier that could limit the uptake of nanoparticles into plant cells. The primary cell wall exhibits intrinsic porosity, varying from about 3 to 6 nm depending on the plant species, its molecular organization, stage of cell differentiation and environmental conditions. However, recent research has shown that the nanoparticles themselves can alter the porosity of the cell wall by interacting with its polymeric components and causing the existing pores to expand or new ones to form. This study examined the effects of a phenolic extract obtained from pomegranate peels (PAE) coating calcium carbonate nanocrystals (nanoCaCO3@PAE) during internalization processes in protoplasts obtained from tobacco leaves (Baldassarre *et al.*, 2022) .Protoplasts incubated with nanoCaCO3@PAE, observed by confocal microscopy, showed a significant increase in the number of endosomes, labeled with FM4-64, compared with control protoplasts, suggesting an increase in internalization events in the presence of the nano-coating extract. Analysis of the autofluorescence spectrum emitted by the phenols showed, in the nanoCaCO3@PAE-treated protoplasts, punctiform intracellular structures, distinct in size from chloroplasts, but similar to the FM4-64-labeled endosomes. These results indicated the internalization of nano-coating extracts into the cells. To confirm these observations, further investigations were conducted on tobacco leaf epidermis by spraying an aqueous solution of fluorescent calcium carbonate nanocrystals (nanoCaCO3@FITC) on the leaves. After 48 hours treatment, streaps of epidermal cells observed under confocal microscopy clearly confirmed the entry of fluorescent nanoparticles into the cells; in fact, small green fluorescent dots were visible near the internal face of the plasma membrane. To monitor the kinetics of fluorescent nanoparticle internalization, tobacco leaves were transformed with PGIP2-RFP, a chimeric protein that follows the endocytic pathway. After its secretion into the cell wall, PGIP2-RFP initially localizes to endosomes and then to the vacuole. Our observations show that nanoCaCO3@FITCs are able to cross the wall and plasma membrane, first localizing in structures identifiable as endosomes, as confirmed by co-localization with PGIP2-RFP, until accumulating in the central vacuole. The stable presence of nanoCaCO3@PAE in the vacuole was confirmed by evaluating the content of total soluble phenols in treated tobacco leaves, revealing an almost twofold increase compared with the control 15 days after treatment. In summary, these data indicate the effective internalization of nanoCaCO3@PAE in both protoplasts and epidermal cells of tobacco leaves, also highlighting the proper targeting of nanoparticle-transported molecules into intracellular compartments.

Baldassarre, F., Vergaro, V., De Castro, F., Biondo, F., Suranna, G.P., Papadia, P., Fanizzi, F.P., Rongai, D., Ciccarella, G. (2022). Enhanced Bioactivity of Pomegranate Peel Extract following Controlled Release from CaCO 3 Nanocrystals. *Bioinorg Chem Appl.* 2022, 6341298. DOI: 10.1155/2022/6341298.

Semeraro, T., Scarano, A., Leggieri, A., Calisi, A., De Caroli, M. (2023). Impact of Climate Change on Agroecosystems and Potential Adaptation Strategies. *Land* 12,1117. DOI: 10.3390/land12061117

Allelopathic potential of *Sorghum bicolor* L. in the management of *Cyperus rotundus* L.

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Cyperus rotundus L. is considered the world's worst weed as, due to its rapid growth, competitive use of resources, and persistence in the soil, it has become the most widespread, troublesome, and economically damaging weed of tropical and subtropical countries. *C. rotundus* exercises its invasiveness mechanism through competition of essential resources, further inhibiting crops growth and development by the release of substances with allelopathic action. The resulting economic losses encompass the reduced crop yields and quality, but is also reflected in an increase in production cost, and decreased in market value. Effectively managing *C. rotundus* presents significant difficulty, partly attributable to its diverse propagation methods, encompassing seeds, rhizomes, and tubers with varying levels of dormancy. Furthermore, chemical control methods may exacerbate risks to human and environmental health. The emergence of adverse effects, such as shifts in weed populations, weed resistance, and unintended toxicity, resulting from the heightened reliance on synthetic herbicides in various cropping systems, underscores the need for research into environmentally safe and sustainable weed management strategies that also reduce production costs. A possible effective, green, and economically valuable managerial control could be represented by the implementation of biological control by means of the utilization of plants with highly allelopathic activity. In this sense, our study aimed to investigate the allelopathic interactions between *C. rotundus* and other plant species. A first set of *in vivo* experiments investigated the effects of two different genotypes of *Sorghum bicolor* L. (Bianca and Tonkawa) on *C. rotundus* growth and development. Plants were co-cultured for 34 days in a controlled environment. Morphological observations and Nuclear Magnetic Resonance (NMR)- based metabolomic analyses were conducted to assess interactions and metabolites involved in the allelopathic mechanisms. Results highlighted the competitive advantage of *S. bicolor* with respect to *C. rotundus*. From morphological observations, Tonkawa appeared to be more efficient in reducing *C. rotundus* germination and growth compared to Bianca genotype. This result was confirmed by metabolic comparison between the two genotypes: *C. rotundus* plants in co-culture with *S. bicolor* Tonkawa showed significantly higher levels of metabolites involved in stress response such as GABA, ethanolamine, UXP, phenylalanine, tryptophan, Unsaturated Fatty Acids (UFA), omega-9 fatty acids and proline. Based on these results a second set of experiments has been planned and is currently underway to validate the findings and implement metabolomic data with Mass Spectrometry (LC-MS) analysis.

Alternative methods for high value secondary metabolites productions: from artemisinin to other potentially bioactive compounds

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Plant secondary metabolome contains a huge and various numbers of metabolites which contribute to a broad spectrum of bioactivities, from antimicrobic to anticancer properties, giving them a prominent role in the modern medicine. For instance, artemisinin, a sesquiterpene lactone isolated in the leaves of *Artemisia annua,* is considered as one of the most potent antimalarial and it is recommended from WHO as first choice treatment of malarial disease. Despite its effectiveness, artemisinin yield from plants is rather low, ranging from 0,01% to 1,5% DW in the leaves, highlighting the necessity to find alternative approaches to increase the production.
In this frame, Plant cell culture (PCC) and Vertical Farming (VF) techniques represent an attractive and sustainable alternative if compared to the traditional methods of phytopharmaceutical production that, often, still rely on the harvesting of plants from the wild. Due to the possibility of an optimization of the growth conditions, both PCC and VF allow the establishment of controlled and continuous production of a highly standardized product, independently from seasonal and climatic changes.
In this work, we have investigated the application of alternative methods for secondary metabolites production, with a focus on artemisinin. Adaptation and suitability of an artemisinin high-yielding *A. annua* variety to be grown in a VF system was explored. We also evaluated the impact of different light quantity on the synthesis of artemisinin and biomass accumulation. Parallelly, various PCC lines from *Artemisia* spp. were established and characterized, through the use of an UPLC-HR-MS platform. Growth medium with different composition and hormones have been employed in particular to *A. annua* derived cell cultures to investigate the effects on artemisinin accumulation.

Metabolic Profiling of Green Coffee Beans from Diverse Geographical Origins

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The determination of plants' metabolic profiles can be extremely useful for a better understanding of the molecular mechanisms through which they can adapt to varying growth conditions.
This study focuses on the characterization of bioactive compounds in green coffee beans samples (*Coffea arabica* L.) as molecular markers for the assessment of their geographical origin. Sourced from six different countries worldwide (Brazil, Guatemala, Honduras, Ethiopia, Rwanda, and India) the samples were provided by *illycaffè* S.p.A. in Trieste.
The identification and quantification of bioactive compounds were conducted with focus on biogenic amines, free-form amino acids, caffeine, and phenolic compounds like ferulic acid and chlorogenic acids (5-Caffeoylquinic acid, 3-Caffeoylquinic acid, and 4-Caffeoylquinic acid). The analyses were performed with High-Performance Liquid Chromatography with Fluorescence Detection and Diode Array Detection methods.
Descriptive and exploratory data analyses were employed to identify chemical patterns that differentiates the samples according to the country of origin. Principal components analysis revealed a distinct clustering of the Brazilian samples and a separation of the Ethiopian from the Honduran and Guatemalan samples, which exhibited similar chemical patterns.
Further research will focus on highlighting the known influence of environmental factors such as soil nutrients, temperature, water availability and light intensity on gene expression and regulatory pathways involved in the biosynthesis of these compounds.

This study was carried out within the Agritech National Research Center and received funding from the European Union - NextGenerationEU (Piano Nazionale Di Ripresa e Resilienza (PNRR) – Missione 4 Componente 2, Investimento 1.4 – D.D. 1032 17/06/2022, CN00000022).

Plant protein extraction and characterization for a more sustainable future

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According to United Nation projections, the world's population is expected to increase by nearly 2 billion people over the next three decades. However, our current dietary habits, especially the excessive consumption of meat and animal products, will soon be unsustainable. In fact, meat production has a major impact on environmental pressure related to food production, and besides, relying solely on animal proteins will not be sufficient to ensure food security. Hence, there is an urgent need to explore alternative protein sources. Plant proteins represent a promising option, yet, as today, a significant portion is diverted to animal feed, leading to inefficiencies and waste. Direct consumption of plant proteins by humans would not only address this waste but also reduce land and water usage.

The present study aims to explore the benefits of plant proteins and peptides as a valid alternative to meat proteins, suitable for both animal feed and human consumption. The work fits into the framework of the AgriLoop project, a collaborative initiative that unites partners from Europe and China.

Initially, an optimization of protein extraction by enzymatic digestion was performed on tomato seeds. Seven different proteases and five different plant cell-wall hydrolytic enzymes were tested. Subsequently, a two-step approach was implemented using the most effective cell-wall hydrolytic enzyme followed by digestion with the best proteases. The resulting digestates underwent through analysis, including the assessments of protein, total polyphenols and reducing sugars contents. The optimal protocol involves the only addition of a protease, as the two-step approach fails to improve protein yield with respect to the protease addition alone. Bromelain, Protamex, and Trypsin were selected for further steps. The protocol was then applied to other feedstocks (tomato peels, whole tomato pomace, brewery spent grain and grape pomace) to determine their suitability for protein extraction. Tomato seeds and brewery spent grain were selected to proceed with the next steps.

Additionally, alkaline and neutral solubilization followed by acidic precipitation were evaluated as a protein isolation method. Preliminary results suggest that, on tomato seeds, alkaline extraction has a higher efficiency compared to neutral extraction. However, a significant portion of peptides, particularly in brewery spent grain, likely derived from previous fermentation processes, appear to remain unprecipitated.

SDS-PAGE were conducted on the most promising samples to gain insight into the molecular weight of proteins and peptides successfully extracted. Next steps contemplate peptide sequencing and the assessment of biological activities to better understand the best qualities of the extracted proteins and peptides.

AGRILOOP (2022-2026) was funded by European Union's HEU research and innovation programme, the UK Research and Innovation fund under the UK government's HEU funding guarantee (GA n. 101081776) and The National Key Research and Development Funds of China.

GIGANTEA negatively regulates the accumulation of ABA-related transcription factors

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Plants combine external and internal cues to synchronize their cellular reactions over specific time periods. An instance of this is the interplay between signals of water scarcity and the circadian rhythm, which governs stress responses to optimize resource-intensive processes only when necessary, thus minimizing energy expenditure.
The protein GIGANTEA (GI), active around midday, has been identified as a controller that dampens the transcriptional responses mediated by abscisic acid (ABA). However, the exact mechanisms behind this regulation remain unclear. Cis-motif analysis of gene promoters regulated by GI reveals a significant presence of ABSCISIC ACID RESPONSIVE ELEMENT-BINDING FACTORs (ABFs) binding elements, which are activated by ABA. Given ABFs' tendency to accumulate in the morning, we investigated whether GI interferes with their function in ABAmediated transcription.
Through biochemical and molecular assays, we observed increased ABF1/3/4 protein levels in GI mutants, particularly in the afternoon. This suggests that GI actively inhibits their accumulation. Additionally, we found that GI physically interacts with several members of the clade A bZIP family, which includes ABFs, implying a direct regulatory role.
In summary, our findings propose a model where GI orchestrates ABA signaling responses throughout the day, prompting further exploration into the chromatin-level regulation of this integration.

Study on the effect of iron-based nano fertilisers as an alternative to commercial fertilisers

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The use of chemical fertilisers in agronomy is essential to meet global food demand; through them, soil nutrient deficiencies can be supplemented, thus improving plant growth and productivity. However, we cannot overlook the environmental impact that these chemicals generate due to their chemical composition and the amount used, which affects soil and water eutrophication, alteration of soil physico-chemical properties and microbiome composition. The chemical composition of common fertilisers includes molecules such as EDTA, DTPA or EDDS that allow micronutrients to remain stable and bioavailable to plants in aqueous solution. However, once released into the environment, these molecules can cause various environmental damages even at low dosages. Alternative strategies that ensure sustainability and resilience in crop management are urgently needed. Recent studies propose the use of nanoparticle-based fertilisers as a viable alternative to commercial fertilisers. In this study, we developed a soil fertilisation method based on the use of two different types of nanoparticles,magnetite and iron phosphate nanoparticles smaller than 100 nm in size. *Solanum lycopersicum* plants were watered regularly with suspensions containing different concentrations of nanoparticles (5-50-100-500-1000ppm) from seedling germination until fruiting. Results show that plants treated with magnetite (Fe_3O_4) and iron phosphate ($FePO_4$) nanoparticles increased their productivity in terms of total weight of harvested fruit by more than 200% compared to plants treated with tested commercial fertilisers. An ionomic approach based on multi-element analysis in ICP-Mass was applied to study the effects of nanoparticles on the soil, roots, leaves and fruit of *Solanum lycopersicum* plants. A different accumulation of elements, between the plants treated with nanoparticles and those treated with EDTA, in the soil and plant matrices analysed was observed.plant roots treated with nanoparticles show a higher bioaccumulation of iron than plants treated with EDTA and EDTA-Fe. A different bioaccumulation of Ca, Zn and K was also found in the fruit of plants treated with both nanoparticles compared to plants treated with EDTA and EDTA-Fe. These differences can be attributed to the paramagnetic properties of the nanoparticles, which act as carriers by helping the transport of some elements in the plant and preventing the accumulation of others. In EDTA-treated plants, for example, a higher accumulation of arsenic was found than in nanoparticle-treated plants. In conclusion, we can suggest that the nanoparticles tested show beneficial effects on the productivity of tomato plants and also provide a valid alternative for the transport of iron in the plant.

Exploring fucoxanthin biosynthesis in *Thalassiosira rotula*: stress responses and regulatory genes

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Fucoxanthin, a potent bioactive compound, shows promising health benefits and biotechnological applications due to its anti-obesity, anti-inflammatory and anti-cancer activities, making it highly sought after in functional foods, supplements and pharmaceuticals [1]. Diatoms, the most abundant photosynthesizing microalgae, are renowned for their efficient fucoxanthin production, serving as sustainable sources for large-scale extraction. Advancements in biotechnology and cultivation methods enable the optimization of fucoxanthin yield, facilitating its widespread utilization in improving human health and addressing diverse industrial needs. In this context, this study aims to elucidate the mechanisms responsible for enhancing fucoxanthin production in the centric diatom, *Thalassiosira rotula*, under stress conditions. The strain was originally isolated from sediment samples collected at the Long-Term Ecological Research Station Mare Chiara in the Gulf of Naples. Our primary goal is to comprehend the variations in fucoxanthin biosynthesis throughout different growth phases. Instead, it is well-known that environmental stressors, as alterations in nutrient availability and light intensity, can trigger an increase in fucoxanthin accumulation in diatoms [2]. To optimize fucoxanthin production in *T. rotula*, we supplemented the culture medium with nitrate while simultaneously reducing light intensity [3]. Firstly, we assessed the impact of these treatments on cellular health by evaluating growth and morphometric parameters, such as dry weight, area and apical length of cells. Moreover, to understand if other metabolites could be interested in response to these stresses, confocal microscopy allowed us to investigate lipid presence using Nile red staining. Another key point is evaluating fucoxanthin content employing a rapid spectrophotometer method and comparing it with high-performance liquid chromatography (HPLC). Alongside these aspects, it's interesting to our study understand the role of key regulatory genes involved in fucoxanthin biosynthesis pathways in diatoms. Genes encoding phytoene synthase, phytoene desaturase, and β-carotene ketolase have been identified as key determinants of fucoxanthin accumulation [1]. Therefore, we will be investigating their expression levels in different stages and after various treatments of interest. In conclusion, this study aims to highlight stress-induced responses, analytical methodologies, and the genetic basis associated with fucoxanthin production in *T. rotula*. Understanding the regulatory mechanisms governing fucoxanthin biosynthesis and optimizing stress-induced enhancement strategies hold significant potential for advancing diatom-based biotechnological applications.

[1] Seth et al., Algal Research 2021, 60, 102475;

[2] McClure et al., Algal Research 2018, 29, 41-48;

[3] Afonso et al. Foods 2022, 11, 568.

Protective effect of *Ocimum basilicum* L. essential oil on *Lactuca sativa* L. treated with cadmium

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In recent years, essential oils (EOs) arose as a sustainable and effective alternative to conventional chemical treatments in response to HMs in plants. These natural molecules can enhance plant resilience under stress. In the present work, the ability of EOs of the aerial parts of *Ocimum basilicum* L. cv "Prospera" to improve the response of plants to HMs, was investigated in lettuce (*Lactuca sativa* L.) subjected to Cd. The chemical profile of the essential oil (EO) was investigated by GC-MS, reporting a composition by oxygenated monoterpenes, with *endo*-fenchol (21.5%) and eugenol (20.4%) as the main constituents. The EO-induced tolerance to different Cd concentrations (360 µM and 720 µM) was studied analyzing ultrastructural damage, antioxidant response and changes in expression of genes involved in the abiotic stress response. Our results indicated that exogenous application of basil EO helps to preserve the correct ultrastructure of plastids and ameliorates damages induced by Cd. Furthermore, reduces the production of ROS and beneficial regulation of the activities and of the molecular expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) were reported. In conclusion, these results clearly indicate the protective capacity of basil EO on the cytological organization and in modulating the redox state through the antioxidant pathway by reducing Cd-induced oxidative stress.

Pectin Methylesterase Secretion Pathway and Stress Response: Analysis on *At*PME12, *At*PME18 and *At*PME34

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The plant cell wall (CW) is a dynamic and complex network of cellulose microfibrils embedded in a matrix of hemicelluloses, pectins and small amount of proteins. CW remodeling has a crucial role for the plant defense (Bellincampi et al., 2014; Bacete et al., 2018). The pectin methylesterases (PMEs), a class of CW proteins, play an important function in plant growth and development. They modify the CW leading to its stiffening and/or loosening by cleaving the high methylesterified homogalacturonan (Me-HG) into partially de-methylate homogalacturonan (De-HG). It is known that PMEs are involved during biotic stress like *Botrytis cinerea or Pseudomonas syringae* infection (Del Corpo et al., 2020) but their role in response to abiotic stress is uncertain. Since matrix polysaccharides are synthesized in the Golgi apparatus while CW proteins in the Endoplasmic Reticulum (ER), it is clear that their secretion route has a pivotal role in the synthesis and remodeling of the CW. However, the secretion mechanisms of the different CW components remain, still, unclear. To deepen these aspects, we have studied three PME genes: *At*PME12, *At*PME18 and *At*PME34 by linking them to the fluorescent protein GFP and/or RFP and following the secretion routes of the obtained chimeras in tobacco (*Nicotiana tabacum*) leaves. Bioinformatic analyses have revealed the presence of a Signal Peptide for *At*PME12 and the absence of it both for *At*PME18 and *At*PME34. It has been predicted the presence of a Transmembrane Domain (TD) for *At*PME18 and *At*PME34. After the transient transformation of the *N. tabacum* leaves, the secretion pathway of the fluorescent constructs was observed under confocal microscope. It emerged how, unlike *At*PME12, that follows a Conventional Protein Secretion (CPS) pathway, *At*PME18 and *At*PME34 follow an Unconventional Protein Secretion (UPS) route bypassing the Golgi. The mechanisms of how these PMEs reach their final destination will be discussed. The overexpression of PMEs has evidenced a strong de-methylesterification of homogalacturonans (De-HGs). In response to abiotic stress such as water stress (drought and flooding), thermal stress (42°C and 4°C) and salt stress (75 mM and 250 mM), it was observed an important modification of the PMEs' expression levels. This study highlights the important roles for PMEs not only in the remodeling of CW during developmental stages, but also in response to abiotic stresses due to the environmental changes.

Bacete L, Mélida H, Miedes E, Molina A (2018) Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. Plant J **93**: 614–636

Bellincampi D, Cervone F, Lionetti V (2014) Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. Front Plant Sci. doi: 10.3389/fpls.2014.00228

Del Corpo D, Fullone MR, Miele R, Lafond M, Pontiggia D, Grisel S, Kieffer-Jaquinod S, Giardina T, Bellincampi D, Lionetti V (2020) AtPME17 is a functional *Arabidopsis thaliana* pectin methylesterase regulated by its PRO region that triggers PME activity in the resistance to *Botrytis cinerea*. Mol Plant Pathol **21**: 1620–1633

Hormonal treatments to mitigate salt-stress effects on two commercial hybrids of *Sorghum bicolor* (L.) Moench: a comparison between IAA and eBL exogenous application effects

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Soil salinization is increasingly affecting global food security, reducing the sustainability and productivity of cultivable land worldwide. It particularly affects the yield of traditional European crops, requiring to evaluate new agricultural strategies such as the cultivation of osmotic-resistant crops [1]. *Sorghum bicolor* (L.) Moench, thanks to its C4 metabolism, is naturally able to live and be productive also in harsh environments such as those characterized by high levels of salinity. Hormones are involved both in plant physiological development and response to stress. Auxins are a class of hormones crucial for root system development and abiotic stress response, either alone or in combination with other hormones such as brassinosteroids (BRs) [2]. The exogenous application of BRs is proven to be effective in alleviating salt stress since they can improve chlorophyll content, photosynthesis parameters, antioxidant compounds content and activity, mitigating the effects of salt stress. Furthermore, BRs are able to decrease ion toxicity and increase the total amino acid content under salt stress [3,4]. BRs are provento be effective also in seed treatment, the priming with 24-epibrassinolide (24-eBL, the main form of active BRs) improves germination rate, biomass production, root system development and antioxidant reactions [5]. This study aimed to evaluate if different concentrations of exogenous auxin (3-indol-aceticacid,IAA) and 24-eBL were able to mitigate the effect of salt stress on two commercial hybrids of sorghum, Bianca and Tonkawa (provided by Padana Sementi Elette S.r.l.). The hormonal treatments chosen for the preliminary analyses of germination rate, root and shoot biomass and root apparatus morphology were at concentrations of 10μM and 1μM for IAA and 0.1μM, 0.4nM, 0.04nM for 24-eBL, added to the cultural medium, and two priming treatments consisting in 8h of seeds soaking in two solutions containing respectively 1μM and 0.1μM of 24-eBL. The NaCl concentration used was 150mM, based on a previous work. The preliminary results excluded the use of exogenous IAA, because, at least at the tested concentrations, it did not seem able to alleviate salt stress in both sorghum hybrids, while the different eBL concentrations tested seemed to mitigate the salt stress effects. In fact, all the eBL treatments implemented root and shoot biomass both at day 6 and day 10 from sowing. The number and the length of adventitious roots in the presence of salt was enhanced by the treatments with 1μM eBL priming and 0.04nM. The priming with 1μM eBL resulted particularly effective in stimulating the germination in the presence of salt, yet 24h after sowing, and implemented the number and the length of the adventitious roots already at day 6 from sowing and in both genotypes. Results suggest to deepen our knowledge on the germination mechanism of this species in the presence of salt and on the effects of the eBL priming by histological analyses on the early embryonic developmental phases and expression analyses of genes involved in seed dormancy breaking.

[1] Zhang et al., 2019. *Plos one*, 14(12), e0227020; [2] Della Rovere et al., 2022. *Int. J. Mol. Sci*., 23(2), 825. [3] Anwar et al., 2018.*Biol.Res.*, 51; [4] Wu et al., *Front. Plant. Sci.*, 8, 1017; [5] Biswas et al., 2023.*Plant Stress*, 100186.

The role of tomato cell wall enzyme prolyl 4-hydroxylase in phenotypic aspects of germination and seed developmental stress response: new insights from RNA interference lines.

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Proline, one of the twenty fundamental amino acids, plays a crucial role in the structural composition, flexibility, and resistance of proteins. Its hydroxylation, catalyzed by the enzyme prolyl 4-hydroxylase (P4H), results in the formation of hydroxyproline. In plants, hydroxyproline is a key molecule involved in cell wall biosynthesis, growth regulation, stress response, and fruit maturation. Notably, P4Hs are involved in regulating various physiological processes in different plant species. For instance, in *Arabidopsis thaliana*, P4Hs regulate root hair elongation, while in *Solanum lycopersicum*, they play essential roles in cell division and fruit abscission. Specifically, it was reported that the lack of proline hydroxylation by a prolyl 4-hydroxylase 3 (P4H3) enzyme results in a delayed fruit abscission phenotype in tomato, suggesting a putative role for this post-translational modification in fruit development regulation.

The aim of our study is to identify the developmental characteristics of RNA interference mutants targeting the *P4H3* gene. Comparative analyses between iRnaP4H3 mutants and wild-type plants reveal significant alterations in plant development. Importantly, the mutant resulted in irregular seed formation and germination dynamics. We have evaluated the influence of P4H3 on seed stress response mechanisms, demonstrating its critical role from seed germination to adult plant stages.

Future efforts will be made in our lab to understand the mechanistic and structural action controlled by P4Hs in providing plasticity to plants in relation to a variety of climate change challenges. The discovery of new functions, genetic tools in crops, and agronomic applications uncovered here will shed light on the fundamental role of these enzymes. These discoveries could pave the way for new strategies to improve crops by optimizing fruit quality and yield, thus contributing to long-term agricultural sustainability.

Project funded by ERAnet- CropsForChange project

The *Arabidopsis thaliana cat2* mutation is hidden by cadmium treatment and reveals an important role for CATALASE in root development

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As sessile organisms, plants have developed intricate regulatory mechanisms to respond to environmental cues promptly and effectively. Under soil pollution, the root system is the primary plant organ system undergoing physiological and morphological changes to initiate a systemic response potentially leading to adaptation. Specifically, soil contamination with the heavy metal cadmium (Cd) stands out as a significant environmental stressor due to its non-biodegradability, bioaccumulation potential, and widespread presence in waters and soils as a pollutant. Among the various strategies that plants employ to cope with stressful conditions, such as modifications of hormonal levels, gene expression, and the production of signal molecules, the enhanced synthesis of antioxidant enzymes emerges as one of the most crucial defense systems. This occurs because, in most cases, the environmental stress is translated into oxidative/nitrosative stress within the plant. In this respect, catalase is a powerful antioxidant enzyme involved in the degradation of hydrogen peroxide (H_2O_2) into water and oxygen (Sharma and Ahmad, 2014). This enzyme is primarily found in peroxisomes, organelles that also play a role in synthesizing crucial signaling molecules, e.g., nitric oxide (NO) and in the biosynthetic steps of some plant hormones, e.g., indole-3-acetic acid (IAA) (Reumann and Bartel, 2016). In *Arabidopsis thaliana*, three catalase genes have been identified, *CAT1*, *CAT2*, and *CAT3*. *CAT1* expression is predominant in pollen and seeds, *CAT2* in photosynthetically active tissues, and *CAT3* in vascular tissues and senescent leaves. Previous research has shown that while the deletion of *CAT2* significantly reduces catalase activity by 90%, knockout mutations in *CAT1* and *CAT3* have minimal or negligible effects on catalase activity (Mhamdi et al., 2010). Given these findings, we decided to investigate the role of *CAT2* gene in response to Cd exposure by growing *A. thaliana CAT2* knockout mutant (*cat2-1)* (purchased by NASC and genetically screened for homozygosity) and the corresponding *wild-type* in *in-vitro* conditions for 10 days in the presence of 60µM $CdSO_4$ (Brunetti et al., 2011). After the growth period, different analyses were conducted, focusing on the root system, including morpho-histological investigations and ROS and RNS content detection. The morphological analyses revealed that the *cat2-1* plants had a shorter primary root and a reduced number of mature lateral roots compared to the *wild-type*. Preliminary histological analysis suggests that the reduced number of observed lateral roots may be attributed to a hindered development caused by an alteration in the cellular division pattern. Furthermore, the content of all the reactive oxygen/nitrogen species (ROS/RNS) examined, including superoxide anion (O_2^-), H_2O_2, NO, and peroxynitrite ($ONOO^-$), increased in *cat2-1* mutant, indicating a role of the enzyme on ROS and RNS homeostasis. Moreover, the increased H_2O_2 levels in mutant roots, relative to the *wild-type*, indicate that CAT2 might regulate intracellular H_2O_2 levels even in non-photosynthetic tissues as are those of the roots. Finally, the presence of Cd at the tested concentration hid most of the observed effects due to *CAT2* knockout, suggesting that the role of the enzyme in mitigating oxidative/nitrosative damage resulting from the heavy metal exposure may be dependent on the intensity and duration of the stress.

Resilience and response of plant-associated microbiomes to urban wastewater in constructed wetlands: insights from rhizosphere biodiversity analysis

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Constructed wetlands (CWs) are pivotal in mitigating the adverse effects of pollutants present in the municipal wastewater on different environmental matrices. However, the dynamic of rhizosphere biodiversity in the CW ecosystem, is still underexplored. Therefore, our interest was focused on the effects of civil wastewater on rhizosphere microbial diversity in relation to different plant species. To this aim, greenhouse experiments, for assessing the resilience and resistance of the rhizospheres belonging to three plant species (*Nerium oleander* L., *Arundo donax* L., and *Juncus conglomeratus* L.), were established. Bacterial 16S rDNA and fungi ITS hypervariable regions were analysed by means of the next generation sequencing (NGS). Our results indicate plant species-specific responses in the microbiome composition due to wastewater treatments. Fungal alpha-diversity was not statistically affected by wastewater recirculation, although a slight shifts in the relative abundance of Operational Taxonomic Units (OTUs), in the case of *N. oleander* and *J. conglomeratus,* was observed. On the contrary, the alpha diversity, relative to bacterial communities was statistically reduced by wastewater recirculation. Despite this, both fungi and bacterial communities showed remarkable resilience in OTU relative abundance rebounded post stress exposure. These observations underline the impact of urban wastewater on rhizosphere microbiomes and highlight the importance of plant-microbial interactions in CWs also in the perspective of wastewater treatment improvement. Our research contributes to increase the knowledge of the effects of civil wastewater treatment on the rhizosphere community in plants usually employed in CWs.

Modelling the oscillatory modes of *Arabidopsis* leaves: An initial step to unveil the effects of vibrational stimuli on plant growth and behaviour

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The PRIN project DAMATIRA (aDvanced Analysis and Modeling of AcousTIc Responses of plAnts) delves into the response of *Arabidopsis thaliana* (L.) Heynh to distinct acoustic stimuli emitted by the Brassicaceae phytophagous insect *Eurydema oleracea* L., aiming to unravel the ecological significance of sound in the plant kingdom and explore this intriguing form of plant-insect interaction from a co-evolutionary perspective. In this context, we investigated the vibrational modes of potential sensory plant structures involved in perceiving and responding to vibrations, spanning both macroscopic (leaves) and microscopic (trichomes) levels. Using photogrammetric analysis, we obtained detailed three-dimensional images of individual basal rosette leaves at various developmental stages while preserving their connection to the stem node ─ a "rigid" constraint at the proximal leaf end significantly affecting its vibration dynamics. The images were modelled using the Finite Element Method (FEM). The model was refined by integrating actual morphometric leaf characteristics, i.e. the cross-sectional area of the petiole and leaf, measured directly using ImageJ software on scan and stereoscopic images from 4-weeks plants cultivated under tightly controlled conditions. Additionally, the average composite elasticity modulus of both the petiole and leaf blade, measured experimentally via a tensile rheometer, was integrated into the model, along with literature-derived leaf attributes, to enhance its accuracy and reliability. The improved model enabled us to predict different resonance modes for both young and mature leaves at specific frequencies. Notably, vibrations of young leaves generally occurred at higher frequencies than in mature ones, suggesting that leaves undergoing development and fully expanded ones may respond differently to stimuli, thus expanding the plant's capacity for perception. We validated our models using Laser Doppler Vibrometry (LDV), demonstrating its effectiveness in accurately measuring *A. thaliana* leaf vibrational modes with optimal spatial resolution and signal fidelity, without perturbing the system. At the microscopic level, LDV measurements within the ultrasound acoustic range (20 - 500 kHz), administered by a piezoelectric disc, revealed fundamental flexural vibrations in trichomes, primarily localized at branch joints with the neck. Additionally, oscillations characterized by complex flexural deformations along branches were observed at higher frequencies. Real recordings of acoustic stimuli emitted by *E. oleracea* while feeding on *A. thaliana* leaves served to stimulate seeds or seedlings, assessing potential impacts on germination and various phenotypic and biochemical traits. No statistically significant effects on germination rates, plant characteristics, or biochemical profiles were observed following exposure to the stimulus. Currently, the evaluation of volatile compound levels is underway, adding depth to our understanding of plant responses to acoustic cues.

microRNA165 AND 166 modulate salt stress response of the Arabidopsis root

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In plants, developmental plasticity allows for the modulation of organ growth in response to environmental cues. Being in contact with soil, roots are the first organ responding to soil abiotic stresses such as high salt concentration. In the root, plasticity relies on changes in the activity of the apical meristem, the region at the tip of the root where a set of self-renewing undifferentiated stem cells sustains growth. We show that salt stress promotes root meristem cells differentiation via reducing the dosage of the microRNAs miR165 and 166. By means of genetic and molecular analysis we show that the levels of miR165 and 166 respond to high salt concentration, and that miR165 and 166-dependent PHB modulation is fundamental for the response of root growth to this stress. Salt dependent reductions of miR165 and 166 causes rapid increase of the Arabidopsis homeobox protein PHABULOSA (PHB) expression and production of the root meristem pro-differentiation hormone cytokinin. Our data provide direct evidence of how the miRNA-dependent modulation of transcription factors dosage mediates plastic development in plants.

Understanding the Role of bZIP AREB3 Phosphorylation in Governing Drought-Induced Flowering

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Plants exhibit remarkable adaptability to environmental changes, including water availability. Flowering time flexibility is vital for drought coping strategies. Drought escape (DE) allows plants to complete their life cycle before severe water deficits, involving early flowering traits. Despite the importance of the question, our knowledge about water deficit influence on flowering time regulation is surprisingly limited. Drought signals are integrated at different levels in the flowering network by the phytohormone abscisic acid (ABA). In the leaf vasculature, ABA upregulate the florigen FLOWERING LOCUS T (FT) expression leading to early flowering. At the shoot apex (SAM) FT interacts with FLOWERING LOCUS D (FD) to induce transcriptional reprogramming. Redundantly to FD, a small clade of related bZIP transcription factors, which are post-translationally regulated by ABA, can interact with FT and promote flowering. ABA-RESPONSIVE ELEMENT BINDING PROTEIN-3 (AREB3) bZIP was taken as model as its role in flowering time regulation has already been demonstrated. This work digs into the role of phosphorylation of bZIP transcription factor AREB3 in the regulation of flowering time under water deficit conditions. Preliminary results suggest that AREB3 plays different role depending in which tissue is expressed: in the leaf it represses the flowering transition whereas in the SAM it promotes flowering. The study aims to elucidate more specifically how N-terminal ABA-dependent phosphorylation impacts AREB3 function, including its DNA binding ability, protein stability, subcellular localization, and dimerization preferences. Through complementation assays and various molecular techniques, the project seeks to uncover the molecular mechanism underlying the interaction between drought response and flowering time regulation in plants through AREB3. The investigation of phosphorylation influence on AREB3 activity will shed light on a potentially novel flowering-ABA regulated checkpoint, providing valuable insights into plant adaptation to changing climates.