

## **Riunione annuale dei Gruppi di Lavoro SBI**

**Biologia Cellulare e Molecolare**

**Biotecnologie e Differenziamento**

**Ancona 21–23 giugno 2023**

**presso Aula Azzurra “Mario Giordano” – Polo Montedago –  
Università Politecnica delle Marche  
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**UNIVERSITÀ  
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Dipartimento di Scienze  
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## Programma

### Mercoledì 21 giugno

- 13:30-14:30                    **Registrazione**
- 14:30-14:40                    **Saluto di benvenuto**
- 14:40-15:00                    **Ricordo di Giorgio Casadoro, Laura Bruno e Anna Fusconi**

### **Risposte allo stress e Biotecnologie ambientali**

#### **Chairperson: A. Norici**

- 15:00-15:15    **Phytochelatins allow intracellular and extracellular cadmium detoxification in poplar.** Vitelli V., Andreucci A., Ruffini Castiglione M., Saba A., Sanità di Toppi L.
- 15:15-15:30    **Gallic acid as a phytostimulant enhancing yield, quality, and drought acclimation capacity of *Mentha spicata* L.** D’Agostino A., Olmi L., Di Marco G., Canini A., Gismondi A.
- 15:30-15:45    **Salt stress and tomato: role of seed priming and acclimation in the amelioration of stress response.** Borroneo I., Forni C.
- 15:45-16:00    **Tissue localization and the physiological effects induced by an environmentally relevant mix of heavy metals in the liverwort *Conocephalum conicum* (L.) Dum.** Cianciullo P., Salbitani G., Maresca V., Sorbo S., Insolubile M., Loreto F., Di Fraia A., Basile A., Carfagna S.
- 16:00-16:15    **Isolation, characterization and identification of plant-growth promoting bacteria from Morocco for the production of cactus pear in intercropping systems.** Campana D.C., Zouitane I., Derraz K., Koraichi S.I., Cesaro P., Massa N., Gamalero E., El Ghachtouli N., Lingua G.
- 16:15-16:30    **Modelling the oscillatory modes of *Arabidopsis* leaves: An initial step to unveil the effects of vibrational stimuli on plant growth and behaviour.** Placi R., Albano A., Renna L., Schioppa E., Salerno G., Masi E., Spagnolo B., Marsella G., Viscardi M., Rizzi F., Nicassio F., Maffezzoli A., Scarselli G., Lenucci M.S.
- 16:30-16:45    **The role of CiXTH29 and CiLEA4 in drought stress tolerance in six varieties of *Chicorium intybus*.** De Caroli M., Curci L.M., Pecatelli G., Carrozzo S., Piro G.
- 16:45-17:00    **Physiological responses of a green and an orange *Dunaliella salina* strains to abiotic factors.** Minio M., Petrucciani A., Norici A., Gerotto C.
- 17:00-17:30                    **Coffee break**

## Sviluppo e Differenziamento

**Chairperson: L. Ferroni**

- 17:30-17:45 **Ovule pattern formation and gametophyte development in *Arabidopsis thaliana* required the tuned activity of WUSCHEL-RELATED HOMEODOMAIN 9.** Petrella R., Gabrieli F., Cavalleri A., Schneitz K., Colombo L., Cucinotta M.
- 17:45-18:00 **Analysis of the thylakoid system in the giant chloroplast of *Selaginella martensii* Spring.** Colpo A., Molinari A., Boldrini P., Živčák M., Brestič M., Demaria S., Baldisserotto C., Pancaldi, S., Ferroni L.
- 18:00-18:15 **An atypical florigen-like integrator promotes reproductive meristem transitions in rice.** Bono G.A., Giaume F., Perrella G., Betti C., Fornara, F.
- 18:15-18:30 **In vitro propagation of three species of aquatic mosses (Bryophyta), anatomic description and biotechnological potential.** Anglana C., Capaci P., Barozzi F., Di Sansebastiano G.P.
- 18:30-18:45 **Pollination event affects auxin and cytokinin accumulation and distribution in *Ginkgo biloba* ovule.** Talarico E., Muto A., Moschin S., Di Marzo M., Nigris S., Chiappetta A.A., Colombo L., Baldan B., Bruno L.
- 18:45-19:00 **Spatiotemporal localization of key hormones involved in the development of reproductive structures in *Ginkgo biloba* L.** Moschin S., Offer E., Herrera-Ubaldo H., Nigris S., de Folter S., Baldan, B.

## Giovedì 22 giugno

### Composti bioattivi e Applicazioni

**Chairperson: C. Totti**

- 09:00-09:30 **Invited lecture: Opportunity and challenges of microalga cell factory.** Christophe Brunet – *Stazione Zoologica di Napoli, Italy*
- 9:30-9:45 **Toxic microalgae and phycotoxins along the Italian coast: data from the last decade.** Accoroni S., Neri F., Bacchiocchi S., Garzia A., Cangini M., Ubaldi M., Montresor M., Zingone A., Totti C.
- 9:45-10:00 **Characterization of *Neochloris oleoabundans* under different cultivation modes and first results on bioactivity of its extracts against HCoV-229E virus.** Baldisserotto C., Ardondi L., Gentili V., Rizzo R., Maietti A., Pancaldi S.
- 10:00-10:15 **In vitro gastric and duodenal digestion and antimicrobial activity of different preparations of Italian wild food plants organs.** Monari S., Escórcio R., Correia V.G., Cairrão A., Pereira C.S., Tassoni A.
- 10:15-10:30 **Quantitative determination of free and conjugated biogenic amines in green coffee beans.** Pettazzoni I., Navarini L., Ferri M., Tassoni A.

10:30-11:00

**Coffee break**

### **Risposte allo stress e Biotecnologie ambientali**

**Chairperson: L. Bruno**

- 11:00-11:15 **Cadmium toxicity is involved in the loss of gravitropic stimuli perception through PIN2- mediated auxin redistribution in *Arabidopsis thaliana*.** Greco E., Talarico E., Madeo M.L., Minervino M., Muto A., Ferrari M., Chiappetta A.A., Bruno L.
- 11:15-11:30 **The effects of salt-stress on *Sorghum bicolor* (L.) Moench root system: morpho-functional, cytohistological and metabolomic analyses.** Peduzzi A., Brasili E., Piacentini D., Della Rovere F., Patriarca A., Sciubba F., Altamura M.M., Falasca G.
- 11:30-11:45 **Cyto-histological analysis of rice roots exposed to arsenic and brassinosteroids.** Lanni F., Piacentini D., Della Rovere F., Fattorini L., Altamura M.M., Falasca G.
- 11:45-12:00 **Uncovering the effects of water activated by plasma discharge on arbuscular mycorrhizal symbiosis in *Lotus japonicus*.** Cortese E., Binci F., Nouri E., Capparotto A., Settimi A.G., Dabalà M., Antoni V., Squartini A., Giovannetti M., Navazio L.
- 12:00-12:15 **Nature based solutions for the treatment of civil wastewater in an internal area of Alexandria of Egypt: potential and innovation of a pilot constructed wetland.** Tammone C., Amer R.A., Taha T.H., Spiniello I., Guarino F., Cicatelli A., Castiglione S.
- 12:15-12:30 **Assessment of tolerance and seed germination to rare earth elements in *Chenopodium quinoa*.** Giuliano F., Castiglione S., Vasca E., Cicatelli A.
- 12:30-12:45 **Contaminants of emerging concern and antimicrobial resistance: use of green technologies for a safe civil wastewater reuse.** Gentile A., Iannece P., Spiniello I., Castiglione S., Guarino F.
- 12:45-13:00 ***Dittrichia viscosa* selection strategy based on Nip1.1 Gene for Arsenic phytoremediation.** Barozzi F., Anglana C., Capaci P., De Benedictis M., Di Sansebastiano G.P.
- 13:00-14:15 **Lunch**

### **Sviluppo e Differenziamento**

**Chairperson: C. Betti**

- 14:15-14:30 **A transcription factor coordinating flowering and stem elongation in *Oryza sativa* L.** Vicentini G., Bertagnon G., Brambilla V.
- 14:30-14:45 **Auxin dependent post-translational regulation of MONOPTEROS in the *Arabidopsis* root.** Cavalleri A., Astori C., Truskina J., Cucinotta M., Farcot E., Chrysanthou E., Xu X., Muino J.M., Kaufmann K., Kater M.M., Vernoux T., Weijers D., Bennett M.J., Bhosale R., Bishopp A., Colombo L.
- 14:45-15:00 **ERAD-mediated maturation of the regulatory protein of plant meristematic cells**

**CLAVATA 3 emerged during evolution from algae to higher plants.** Maricchiolo E., De Marchis F., Bellucci M., Perozeni F., Ballottari M., Menotta M., Fraternali D., Pompa A.

15:00-15:15 **A metabolic engineering approach to investigate the serotonin metabolic pathway of *Solanum lycopersicum*.** Gambacorta G., Negri S., Commisso M., Avesani L., Guzzo F.

15:15-15:30 **Natural allelic variation of Phospholipase C 2 hints at a crucial role for plasma membrane phosphoinositides and phosphate homeostasis.** Somoza S.C., Capparotto A., Binci F., Castelli D., Kopriva S., Busch W., Giovannetti M.

15:30-15:45 **Fruit growth and maturation is controlled by NAC transcription factors.** Periccioli L., Massafra A., Rotasperti L., Mizzotti C., Tagliani A., Masiero S.

15:45-16:00 **Insights on peptide hormones families in different plant species and their role in fleshy fruit development and ripening.** Francese C., Armellini M., Resentini F., Salvagnin U., Morbiato L., De Zotti M., Sales G., Trainotti L.

16:00-16:15 **Triploid block in plants, a postzygotic barrier full of twists yet to be revealed.** Zumajo-Cardona C., Gabrielli F., Cavalleri A., Ezquer J.I., Colombo L.

16:15-16:30 **Insights into pollen recognition and pollen-ovule cross-talk in *Ginkgo biloba*.** Offer E., Moschin S., Nigris S., Baldan B.

16:30-16:45 **Interactomics for rice flowering: a proximity labelling approach.** Chirivì D., Parrotta F., Betti C., Fornara F.

16:45-17:00 **The evolutionary importance of stomata and mesophyll traits on adaptation to climate changes.** Cinti E., Cerri M., Reale L.

17:00-17:15 **Molecular network of SPOROCYTELESS/NOZZLE (SPL/NZZ) controlling Megaspore Mother Cell (MMC) differentiation in *Arabidopsis thaliana*.** Cavalleri A., Manrique S., Ruiu A., Movilli A., Lopez F., Astori C., Spanò M., Bruzzaniti G., Colombo L.

17:15-17:45 **Coffee break**

17:45 **Riunione amministrativa**

20:30 **Cena sociale (*Ristorante Il Giardino*)**

## Venerdì 23 giugno

### Risposte allo stress e Biotecnologie ambientali

**Chairperson: M. De Caroli**

- 09:30-09:45 ***Caulerpa prolifera* and its bacterial coating: a promising association for coastal marine water phytoremediation of diesel hydrocarbons.** Caronni S., Busnelli A., Comotti A., Quaglini L.A., Franzetti A., Gentili, R., Montagnani C., Citterio S.
- 09:45-10:00 **Preliminary study of Mediterranean diatoms species to improve the production of fucoxanthin and the challenge to their long-term preservation.** Madeo M.L., Ferrari M., Greca T., Bruno L., Chaerle P., Chepurnova O., Vyverman W., Montresor M., Romano G., Cozza, R.
- 10:00-10:15 **Functionalized biochar as alternative fertilization methods for sustainable agriculture.** Petrucelli V., De Rosa D., Petrilli I., Badiali C., Di Palma L. Pasqua G., Brasili E.
- 10:15-10:30 **Winter season outdoor cultivation of an autochthonous *Chlorella*-strain in a pilot-scale prototype for urban wastewater treatment.** Benà E., Giacò P., Demaria S., Marchesini R., Benini A., Benetti L., Pancaldi S., Baldisserotto C.
- 10:30-10:45 **The characterization of the rhizosphere in urban soils: an integrated view for non-disruptive methods supported by bioinformatics.** Sferra G., Fantozzi D., Gillini A., Bucci A., Naclerio G., Montagnoli A., Trupiano D., Scippa G.S.
- 10:45-11:15 **Coffee break**

### **Simbiosi e Biodiversità**

**Chairperson: G. Lingua**

- 11:15-11:30 **One actor, many roles: Mildew Locus O (MLO) at the crossroads of plant-microbe interactions.** Binci F., Rago G., Di Nuzzo E., Somoza S.C., Navazio L., Giovannetti M.
- 11:30-11:45 **A safe and affordable protocol for plant DNA barcoding applicable in educational contexts.** Scapin A., Trainotti L.
- 11:45-12:00 **The effect of a soil microbial synthetic community on *Lactuca sativa* phosphate homeostasis depends on plant genotype.** Capparotto A., Salvucci P., Sello S., Sudiro C., Altissimo A., Giovannetti M.
- 12:00-12:15 **Study of the effects of compost on soil microbial community to improve soil biodiversity and plant health.** De Biasio N., Cipriani R., Zanardi W., Bonato T., Baldan B., Nigris S.

## **Composti bioattivi e Applicazioni**

**Chairperson: F. Guzzo**

- 12:15-12:30 **Isolation and production of biologically active extracts from by-products of grape pomaces of *Vitis vinifera* L. Cabernet Sauvignon (origin of South Kazakhstan).** Shakiryanova Z.M., Tassoni A., Saparbekova A.A.
- 12:30-12:45 **Phylogenetic and computational approaches to better understand diversity and potential of *Vigna unguiculata* (L.) Walp. Bowman-Birk protease Inhibitors.** Panzeri D., Toini E., Vertemara J., Silvestri G., Labra M., Grassi F.
- 12:45-13:00 **Optimization of anthocyanins production in tobacco cells.** Armellin M., Carpi A., Md. Abdur Rahim, Marin A., Dal Monte R., Trainotti L.
- 13:00-13:15 **Chiusura dei lavori e saluti**

## Phytochelatins allow intracellular and extracellular cadmium detoxification in poplar

<sup>1,2</sup>**Vitelli V.**, <sup>1</sup>Andreucci A., <sup>1</sup>Ruffini Castiglione M., <sup>2</sup>Saba A., <sup>1\*</sup>Sanità di Toppi L.

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Plants have evolved a set of mechanisms that control and respond to the uptake and accumulation of both essential and non-essential metals, including their prompt chelation and sequestration in the vacuolar compartment by thiol ligands, such as glutathione (GSH) and phytochelatins (PCn) [1, 2]. Similar mechanisms might well be thought responsible for transporting metal complexes – in particular thiol-cadmium complexes – across the plasma membrane, with the consequent release of this metal into the extracellular environment. This hypothesis was recently confirmed in axenically-grown gametophytes of the liverwort *Marchantia polymorpha* L., when exposed to three different cadmium concentrations over five exposure-times [3]. In fact, GSH and PCn can jointly accomplish intracellular and extracellular cadmium detoxification in this bryophyte. Given the above, the main aim of the work we are going to present is to verify whether the jointly intra- and extracellular defense responses towards cadmium stress, observed in the haploid gametophytes of *M. polymorpha*, can also occur in the model-tracheophyte *Populus alba* L. (poplar), in particular in the root system of axenically-grown *in vitro* plants of the Villafranca clone. In short, the results that will be extensively presented and discussed show that in poplar roots cadmium simultaneously stimulates an intra- and extracellular detoxification-response, based on GSH and PCn augmented synthesis. Specifically, in relation to the extracellular response, 10, 20 and 36  $\mu\text{M}$  cadmium induced a progressive increase in the concentration of PCn released in the culture medium, and a comparable intracellular response occurred as well. Thus, both in bryophytes and in tracheophytes, an intriguing new role played by thiol peptides as intracellular and extracellular cadmium-detoxifying agents, might now be assumed. Future studies will concentrate on the complex path through the plasma membrane, particularly on metal-thiol transporter inhibition mechanisms. Overall, these studies could help us understand the main mechanisms underpinning various phytomonitoring and phytoremediation techniques [4], and accordingly help improve them from an applied standpoint as well.

### References

- [1] Sanità di Toppi, L., Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environmental and Experimental Botany*, 41: 105-130.
- [2] Bellini, E., Betti, C., Sanità di Toppi, L. (2021). Responses to cadmium in early-diverging streptophytes (charophytes and bryophytes): Current views and potential applications. *Plants*, 10: 770.
- [3] Bellini, E., Bandoni, E., Varotto, C., Sanità di Toppi, L. (2023). Glutathione and phytochelatins jointly allow intracellular and extracellular detoxification of cadmium in the liverwort *Marchantia polymorpha*. *Environmental and Experimental Botany*, 209: 105203.
- [4] Li, M., Leso, M., Sanità di Toppi, L., Varotto, C. (2022). Phytochelatin synthase de-regulation in *M. polymorpha* indicates cadmium detoxification as its primary ancestral function in land plants and provides a novel visual bioindicator for detection of this metal in the environment. *Journal of Hazardous Materials*, 440: 129844.

## **Gallic acid as a phytostimulant enhancing yield, quality, and drought acclimation capacity of *Mentha spicata* L.**

**D'Agostino A.**, Olmi L., Di Marco G., Canini A., \*Gismondi A.

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Drought is an abiotic stressor that limits plant growth and yield, and whose occurrence is associated with the global warming. Nowadays, several methods are applied for improving the productivity of crops, including chemical fertilisers, herbicides, etc. Unfortunately, these approaches are linked to adverse side-effects on environment and human health. Thus, in a context of a growing human population and a steep rise in food demand, an eco-friendly alternative to the unsustainable agricultural practices might be represented by biostimulators. For instance, gallic acid (GA) has been recently proposed as an agent favouring plant response to different environmental pressures.

Since ancient times, *Mentha* L. species (Lamiaceae family) have represented important sources of medicinal and aromatic compounds and, still today, their economic value is significant in food, cosmetic, pharmaceutical, and herbal field.

According to this premise, the present research aimed at investigating GA as a potential phytostimulant enhancing yield, quality, and drought acclimation capacity of *Mentha spicata* L. (also known as spearmint). To do this, plants were treated with several concentrations of GA (25-750  $\mu\text{M}$ ) and exposed or not to water deficit stress (50% field capacity) for 21 days (14 h light/10 h dark; light intensity: 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The effects of the treatments were assessed at morphological, biochemical, and molecular level.

First, morphometric parameters (e.g., stem length, leaf counting) and water content of the plants were measured, together with the total content in phenolics and terpene compounds. Our results revealed that low doses of GA were able to stimulate the accumulation of terpenes in both drought and control conditions. However, liquid chromatography analysis showed that GA concentration did not change significantly among the several samples, suggesting the possibility that the biostimulant exerted its effects by a molecular signalling induced starting from the roots.

To better understand the modifications determined by the treatments on synthesis and storage of the lipophilic metabolites in the plants, the following steps were achieved: i) counting of trichomes; ii) histochemical staining of trichome content; iii) estimation of essential oil yield; iv) qualitative and quantitative characterization of the mint oil by gas chromatography-mass spectrometry.

Finally, a transcriptomics analysis (RNA-seq) was performed by Illumina Next Generation Sequencing platform. Preliminary data revealed the modulation of the expression of about 1700 genes, some of which involved in chromatin remodelling and ribosome synthesis.

Although other investigations are still on-going, the evidence collected until now would suggest that GA may represent a potential phytostimulant for *M. spicata*, promoting yield and quality of its essential oil, even in presence of drought conditions.

## Salt stress and tomato: role of seed priming and acclimation in the amelioration of stress response

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The lack of rainfall, high temperatures, poor snowfall during the winter seasons, are the main factors that lead to drought, related to the climate changes with dramatic consequences, for the agriculture and for the supply of drinking water. Besides the limited water availability, it has been detected an enhancement of salts in the water, due to the seas flowing back into the branches of river deltas and, in coastal areas, it infiltrates increasingly low aquifers due to excessive drawing from irrigation wells. It is well known that plants irrigated with saline solutions undergo osmotic and oxidative stresses, which affect their growth, photosynthesis and yield. Therefore, the use of saline water for irrigation, in addition to the increasing soil salinity, is one of the major threats to crop productivity worldwide. Among the horticultural crops, tomato (*Solanum lycopersicum* L.) requires a lot of water for cultivation, although high humidity leads to higher incidence of pests and diseases and fruit rot. Dry climates are therefore preferred for tomato production. To improve crop tolerance to abiotic stresses seed priming and acclimation, can represent useful tools. Seed priming is a pre-sowing treatment, which consist of soaking the seeds in a priming agent, followed by drying the seeds to avoid rootlet emergence. A priming agent can provoke abiotic stress to seed, inducing a cross-tolerance to different stresses. While an acclimation to stress can be obtained through a gradual exposure of the plant to stressful conditions, leading the plant to an adaptation to stress, therefore to a better performance. Based on the economic importance of tomatoes, an experimental greenhouse trial was conducted on salt-sensitive cherry tomato (cv. Principe Borghese), to determine the effects of combined treatment of seed priming and acclimation on salt stress response. Chemical priming of seeds was performed by treating seeds with polyamines (PAs): 2.5 mM putrescine (PUT) 2.5 mM spermine (SPM) and 2.5 mM spermidine (SPD). Germinated seeds, primed and non-primed (controls), were sown in non-saline soil; after 2 weeks, the plants were irrigated with saline and non-saline water for 4 weeks. At the end of the growth period, physiological and biochemical analyses were performed. The positive effects of combined treatments were evident, when primed plants were compared to non-primed plants, grown under the same conditions. An increase in antioxidant activity and reducing power of primed plants was observed, using the DPPH, FRAP and PFRAP assay. PAs increased the activity of various antioxidant enzymes, including superoxide dismutase (SOD), polyphenol oxidase (PPO), and peroxidase (POD), in particular ascorbate peroxidase (APX). In conclusion priming with PAs improved tolerance to salt stress and enzymatic antioxidant responses in all salt-exposed plants, reducing the negative effects of salinity on growth. PAs have been proved to be beneficial for detoxification of reactive oxygen species (ROS) and activation of the antioxidative machinery, ameliorating abiotic stress tolerance of tomato. These results may open new perspectives and strategies to increase tolerance to salt stress in sensitive species.

## **Tissue localization and the physiological effects induced by an environmentally relevant mix of heavy metals in the liverwort *Conocephalum conicum* (L.) Dum**

<sup>1</sup>**Cianciullo P.**, <sup>1</sup>Salbitani G., <sup>1</sup>Maresca V., <sup>2</sup>Sorbo S., <sup>3</sup>Insolvibile M., <sup>1</sup>Loreto F., <sup>1</sup>Di Fraia A.,  
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Tissue accumulation, ultrastructural alterations, oxidative stress, and effects on photosynthesis were assessed in *Conocephalum conicum* exposed to heavy metal concentrations detected in three different sites of the Savone River, representative of three situations characterized by different anthropic impacts. The pathway of conduction and accumulation of heavy metals in the thallus of liverwort was first studied, after which the biological effects in relation to the different accumulation sites along the thallus, ribs, and wings were investigated. The biological effects were studied considering: bioaccumulation (carried out by atomic absorption spectrometry), localization (assessed by X-ray scanning electron microscopy microanalysis), ultrastructural damage of photosynthetic parenchyma (observed with transmission electron microscopy), oxidative stress (ROS contents and antioxidant enzymes activities determination), photosynthetic performance (valuated by chlorophyll fluorescence). The results showed the heavy metals bioaccumulation in *Conocephalum conicum* was dependent by its concentration in the contaminated water. As for localization, non-essential metals (Pb and Cd) preferentially accumulated the nerve of gametophytes respect to the wings, and, as tissue localization, in the hyaline parenchyma. At the ultrastructural level, heavy metals caused alterations of the fine structure of the cells, inducing marked alterations of the chloroplast structure and therefore of the photosynthetic capacity. Such alterations are most evident in the nerve, respect in the wings.

## Isolation, characterization and identification of plant-growth promoting bacteria from Morocco for the production of cactus pear in intercropping systems

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Unsustainable management practices and climate change are threatening the natural capital of soils, particularly in the Mediterranean region, where increasing population, rapid land-use changes and associated socio-economic activities are imposing high pressures on the region's shallow soils. Under field conditions, plants interact with a multitude of microorganisms, some beneficial and some noxious. Among beneficial microorganisms, plant growth-promoting bacteria (PGPB) represent a fundamental component of soil fertility in natural and agroecosystems, supporting plant growth, health and productivity. Where the natural communities of bacteria need to be restored or reinforced, it is possible to inoculate plants with bacteria propagated in the laboratory. The isolation of culturable bacteria from specific areas is important to guarantee the availability of strains adapted to the environmental and climatic conditions of interest. The isolated strains must then be physiologically characterized to detect the presence of plant growth-promotion traits, which must be confirmed under controlled conditions. Inocula can finally be formulated and tested in the field. In the present work, we report the isolation and characterization of rhizospheric bacteria from three sites in Morocco, in the frame of the PRIMA project “Promoting soil fertility, yield and income in smallholder agriculture of semiarid and arid Mediterranean regions by management of beneficial soil microbiota, conservation agriculture and intercropping”, whose main aim is the improvement of small farmer agronomic practices through the promotion of the rational use of beneficial soil microbiota and their positive effects on productivity of inter-cropped perennial (cactus pear – *Opuntia ficus-indica*) and short-term species (field crops and winter-grown vegetables). Root samples of cactus pear were sampled in three different sites in Morocco differing for their climatic conditions: a humid area (Tafrant, 34.625; -5.12417, geographic coordinates expressed as decimal degrees), a semi-arid area (Fez, 34.0701; -4.95473), and an arid area (Chichaoua, 31.26365; -884411). Rhizospheric soil (adhering to the roots) was used to isolate bacteria by cultivation on LB-agar medium. A total of 77 strains were isolated (23 from Tafrant, 34 from Fez, and 20 from Chichaoua). They were tested for the following plant growth-promoting traits: solubilization of inorganic phosphate, mineralization of organic phosphate (acid and alkaline phosphatase activity), fixation of molecular nitrogen, production of indole acetic acid, siderophores, ammonia, hydrocyanic acid, and exopolysaccharides, and antagonistic activity against the phytopathogenic fungus *Fusarium solani*. In addition, also the water stress resistance was tested by growing bacteria with polyethylene glycol 6000. As a result, a subgroup of 22 strains was selected, presenting three or more PGP traits, which were further tested for ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, antibiotic resistance and molecularly identified by sequencing of the 16S rRNA gene. The plant growth-promotion activity of the individual strains is currently under analysis, growing sorghum plants under controlled conditions and will lead to the formulation of a mixed inoculum.

## **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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Plants respond to external mechanical stimuli like gravity and touch. These trigger responses (i.e. gravitropism and thigmomorphogenesis) often associated to changes in cell wall architecture, cell division planes, hormone production, and gene expression. The PRIN project DAMATIRA (aDvanced Analysis and Modeling of AcousTic Responses of plAnts) aims to investigate how *Arabidopsis thaliana* (L.) Heynh responds to specific acoustic stimuli, a subset of the mechanical ones, produced by insects to determine the ecological relevance of sound for plants and explore this type of plant-animal interaction from a co-evolutionary side. We hypothesize that acoustic stimuli propagate through the whole plant by mean of self-vibrations. Accordingly, in the first phase of our study, we plan to model the vibrational modes of the main plant sensory structures likely involved in sound perception and response using Finite Element Method (FEM) analysis and validate the model through Laser Doppler Vibrometry (LDV) at macroscopic (leaves) and microscopic (trichomes) level. Direct measurements using a tensile testing equipment, were led on several leaves from 4-week-old *A. thaliana* plants grown under strictly controlled conditions (light 200  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 8:12 h day:night, temperature 22°C, humidity 60%) to estimate the average composite modulus of elasticity of the petiole. The petiole cross-sectional area was determined using the ImageJ software on stereoscopic images. The analyses revealed an average modulus of 202  $\pm$  108 KPa. To obtain detailed 3D models of fully expanded leaves for FEM, an accurate photogrammetric analysis of individual leaves from the basal rosette was carried out without detaching them from the stem, representing a "rigid" constraint on proximal leaf end oscillation. Anatomical and functional morphometrics, along with published values of tissue parameters, were used to model the resonance modes of single leaves. Our preliminary results demonstrate that acoustic sources were a suitable solution for leaf excitation avoiding any mass and stiffness effect. Besides, we found that LDV was a proper tool for measurements of *A. thaliana* leaf vibrational modes, allowing an adequate spatial resolution and signal quality while avoiding any other system modification. Regarding trichomes, LDV measurements in the ultrasound acoustic range (20 - 100 kHz) by a piezo-actuated disc detected vibrational modes identified as transversal vibrations in their branches. The results revealed flexural vibrations, such as the fundamental mode when the deformation is localized on the branch hinges, and further complex flexural modes, shaped as sinusoidal deformations, along the branches at higher frequencies. Resonance peaks have not yet been detected; this investigation will be the goal of future studies in other acoustic ranges. The application of LDV will allow us to measure the mechanical characteristics of trichomes and develop a mechanical model by FEM.

## The role of *CiXTH29* and *CiLEA4* in drought stress tolerance in six varieties of *Chicorium intybus*

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Climatic factors, such as drought and high temperatures, drastically affect the productivity of cultivated plants. The identification of plants showing stress resistance characteristics, as well as the recognition of morphological or molecular traits associated with drought tolerance, is extremely important because progressive climate change is expected to further reduce water availability, with dramatic impacts on crop productivity. In order to identify some morphological and/or molecular markers of resistance to water stress, the seeds of six varieties of chicory (*Chicorium intybus* L.) were germinated in the presence of PEG-6000, a non-penetrating osmotic agent that lowers the water potential causing stress condition. For the same aim, six-week-old seedlings were subjected to drought stress by withholding the irrigation for a further 10 days of growth. The Selvatica, Zuccherina di Trieste and Brindisina varieties, with high vegetative development, showed a higher germination rate, greater seedling development and greater resistance to dehydration than the varieties Galatina, Esportazione and Rossa Italiana. The involvement of *LEA* (Late Embryogenesis Abundant) and *XTH* (xyloglucan endotransglucosylase/hydrolase) multigenic family in the response to abiotic stress is known (De Caroli et al., 2021; Magwanga et al., 2018), therefore the expression profiles of *LEA4* and *XTH29* under water stress conditions were studied for all chicory varieties analysed. It has been shown that chicory varieties with high basal expression of *CiXTH29* and *CiLEA4* and with a good level of vegetative development of the basal rosette, i.e. Selvatica and Zuccherina di Trieste tolerate drought better than the varieties which show the overexpression of the two genes only in response to a condition of water shortage. Other specific morphological traits characterized almost all chicory varieties during water stress, namely the appearance of lysigenic cavities and a general increase in the amount of xyloglucans in the cell walls of xylem vessels. The results obtained show that the high basal expression of *CiXTH29* and *CiLEA4*, associated with a high level of vegetative growth, could represent significant traits for tolerance to drought stress.

### References

- De Caroli, M. et al. Ride to cell wall: *Arabidopsis* XTH11, XTH29 and XTH33 exhibit different secretion pathways and responses to heat and drought stress. *Plant J.* 2021, 107, 448-466.
- Magwanga, R.O et al. Characterization of the late embryogenesis abundant (LEA) proteins family and their role in drought stress tolerance in upland cotton. *BMC Genet.* 2018, 19, 6.

## Physiological responses of a green and an orange *Dunaliella salina* strains to abiotic factors

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Microalgae occupy a variety of ecological niches and show a great diversity in their physiology and their responses to environmental factors. Despite their physiological flexibility, little is known about the physiology and photosynthetic regulation in response to stress of many algal species. In our study we are using *Dunaliella salina*, a wall-less chlorophyte that dominates hypersaline lagoons, lakes, and salt-saturated brines. Thanks to its ability to thrive in such environments, characterized by rapid shifts in salinity and nutrient depletion due to salts precipitation, *D. salina* has become a model organism for the study of stress responses. *D. salina* is of some biotechnological interest, like being intensively cultured for the commercial production of glycerol, that it accumulates as an osmoticum, and  $\beta$ -carotene. We are challenging two *D. salina* strains, one characterized by a bright green colour and the other by an orangish one, by growing them in different salinities or nutrients availability. We performed pigment quantification and *in vivo* chlorophyll fluorescence analysis, to investigate how such changes impact photosynthesis and its regulatory processes. Analysis of the allocation of resources is also being performed through (i) FT-IR spectroscopy, to assess changes in the carbon allocation between the different macromolecular groups, and (ii) elemental analysis, to check for shifts in the ratios between macroelements in the cells. Initial results showed that both *D. salina* strains successfully faced the environmental changes we probed. Maximum quantum yield of PSII was kept rather similar in most of the differently grown samples, whereas growth rate and cell composition were modulated as a function of the growth conditions.

## Ovule pattern formation and gametophyte development in *Arabidopsis thaliana* required the tuned activity of WUSCHEL-RELATED HOMEODOMAIN 9

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In spermatophytes, the sporophytic (diploid) and the gametophytic (haploid) generations co-exist in ovules, and the coordination of their developmental programs is of pivotal importance for plant reproduction. To achieve efficient fertilization, the haploid female gametophyte and the diploid ovule structures must coordinate their development to form a functional and correctly shaped ovule. Here, we have investigated the role of WUSCHEL-RELATED HOMEODOMAIN 9 (WOX9), also known as STIMPY (STIP), in *Arabidopsis thaliana* ovule development. WOX transcription factors share important roles in a wide range of processes during plant development.

WOX9 is expressed in the placenta and developing ovules and its protein probably moved from the funiculus to the epidermal layer of the chalaza and the nucellus. The fertility of the *wox9* mutant is severely compromised due to a block in outer integument formation, resulting in ovules with a radial rather than a bilateral symmetry.

Such defect has been already described for the mutant in the gene named *INNER NO OUTER (INO)* and for the gain of function mutant *phabulosa-d* that causes ectopic expression of *PHB*, a repressor of *INO*. By expression analysis and chromatin immunoprecipitation experiments, we demonstrate that in the abaxial layer of the outer integument, WOX9 positively regulates *INO* expression by directly repressing *PHB*.

Moreover, the analyses revealed that in ~94% of *wox9* ovules the female gametophyte development was arrested at the functional megaspore stage. Lack of gametogenesis initiation coupled with integument defects corroborated the hypothesis that tight regulation of WOX9 in the sporophytic tissue is required to ensure gametogenesis progression.

## Analysis of the thylakoid system in the giant chloroplast of *Selaginella martensii* Spring

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In the chloroplast of vascular plants, the most characterizing morphological feature of the thylakoid system is the segregation of granal and stromal lamellar domains, which is biochemically related to the uneven distribution of photosystem I (PSI) in the stroma-exposed domains and photosystem II (PSII) in the appressed domains. A *granum* is generally represented as an approximately cylindrical stack with an average diameter of ca. 500 nm and usually formed by 5 to 25 thylakoids depending on species and light conditions (Kirchhoff, 2019; Mazur *et al.*, 2021). In electron micrographs of the giant chloroplasts of the shade-adapted lycophyte *Selaginella martensii* the thylakoid stacking degree appears very variable within the same organelle (Ferroni *et al.*, 2016). With a chlorophyll *a/b* ratio of 2.6, a PSI/PSII ratio of 0.31, and a minute amplitude of the I-P phase in chlorophyll fluorescence transients, *S. martensii* presents typical hallmarks of shade-adaptation. The use of a biochemical method based on the treatment of isolated thylakoids with digitonin allowed an estimate of 26% non-appressed membranes. However, the morphometric analysis of the thylakoid system in electron micrographs led to a very different result, i.e., 46% fraction of non-appressed thylakoid domains. To understand this discrepancy, the digitonin-soluble and insoluble fractions were analysed by SDS-PAGE: interestingly, a part of PSI, known to be excluded from grana cores, was still found in the insoluble fraction, suggesting the presence of non-appressed domains that remained inaccessible to the detergent. A possible cause could be an unusually high structural complexity of the thylakoid system in *S. martensii*. To verify such hypothesis, the grana morphometrics was thoroughly investigated in electron micrographs. The grana were formed by 16 thylakoids per stack on average, and giant grana were a rare occurrence. However, the average length of the grana partitions was ca. 760 nm, largely overcoming the threshold of 500 nm currently hypothesized to allow a fast diffusion of long-range electron carriers (Höhner *et al.*, 2020). The slow diffusion of electron carriers was indeed confirmed by PSI redox kinetics. Furthermore, the newly introduced morphometric parameters proposed by Mazur *et al.* (2021) indicated a high degree of granum cross-sectional irregularity. We conclude that, under the selective pressure of the deep-shade environment, the lycophyte underwent a particular evolution of some structural traits of the thylakoid system, which privileged the horizontal extension of the thylakoid appressions, in place of the vertical size of grana.

### References

Ferroni *et al.* (2016) *New Phytol.* 211, 554-568.

Höhner *et al.*, (2020) *PNAS* 117, 15354-15362.

Kirchhoff (2019) *New Phytol.* 223, 565-574.

Mazur *et al.* (2021) *Front. Plant Sci.* 12, 756009.

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## An atypical florigen-like integrator promotes reproductive meristem transitions in rice

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Rice (*Oryza sativa*) is an herbaceous annual plant of Asian origin, whose flowering is accelerated under short-day (SD) conditions. Photoperiod is measured in leaves and, when conditions are favorable, florigens are produced and induce flowering. Florigens in rice are encoded by Heading Date 3a (Hd3a) and Rice Flowering Locus T1 (RFT1), both homologs of *Arabidopsis thaliana* FT. These proteins are transmitted to the Shoot Apical Meristem (SAM), where they form the Florigen Activation Complex (FAC), a multiproteic complex whose targets include genes involved in the vegetative-to-reproductive transition. Panicle, the rice inflorescence, develops from the inflorescence meristem, which generates sequentially new types of meristems, which acquires indeterminate or determinate fate in a time and position-dependent manner. Early generated meristems, of indeterminate fate, assume branch meristem (BM) identities; those ones, after having generated a certain number of other branch meristems, are transformed into determinate spikelet (SM) and floret meristems (FM). One of the key factors influencing panicle architecture is the time it takes for BMs to transform into determinate SMs: delaying the specification of determinate SMs causes indeterminate branching to repeat, leading to the production of larger and more complex panicles; accelerating the SM determination process leads to panicles with fewer branches and seeds.

Transcriptomic analysis at the SAM allowed the identification of a florigen-like gene, *FLOWERING LOCUS T- LIKE 1 (FTL1)*, the closest homolog of *Hd3a*, whose expression is strongly induced by rice florigens. Its transcription at the SAM distinguishes FT-L1 from traditional florigens, whose transcription is restricted to leaves, indicating non-canonical regulation and function in rice flowering. At the beginning of reproductive commitment, *FT-L1* transcripts and the corresponding protein localize in the outer layers of the inflorescence meristem and later accumulate in the newly formed branch meristems. Then, at the tip of these branches, *FT-L1* expression is turned off, to then reappear at the base of determinate meristem types, suggesting that FT-L1 absence might be crucial for preventing the premature determination of meristems, which interrupts branching, favoring flower formation. We have isolated different *ft-l1* mutant alleles, both in the Italian elite variety Volano, through chemically induced mutagenesis, and in the variety Nipponbare using CRISPR, and performed genetic analysis on them: the results indicate that loss of *FT-L1* causes a delay in flowering time, as well as increasing the number of secondary or higher order branches. Moreover, combining *ft-l1* alleles with *hd3a* or *rft1* further intensifies these phenotypes. Taken together, these results suggest a dual role as a positive regulator of the vegetative-to-reproductive transition and of the transition from indeterminate branch meristem to determinate spikelet meristem fate for FT-L1, proposing the existence of a triple system based on florigens and a florigen-like protein determining reproductive commitment. Ongoing research is addressing the molecular antagonism between florigenic and antiflorigenic proteins, to understand how their activities are integrated into the complex regulation of inflorescence architecture.

## **In vitro propagation of three species of aquatic mosses (Bryophyta), anatomic description and biotechnological potential**

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The use of mosses in biotechnology is an ever-expanding area of interest. Traditionally, mosses have often been used as medicinal plants and nowadays can be a great source of secondary metabolites with pharmacological activity (Mishra *et al.*, 2014). In agriculture formulations based on bryophyte extracts have been developed and used as natural pesticides, biopesticides or for their allelopathic effects (Nozaki *et al.*, 2007). Another important aspect is their application as bioindicators for their ability to accumulate polluting substances, such as heavy metals, modifying, as response, their secondary metabolism.

We showed as aquatic mosses can be used in phytoremediation/phytofiltration of polluted waters as a biofilter having the ability to absorb heavy metals such as Pb, Cd, and Cr in a short time (Papadia *et al.*, 2020) and their ability to capture inorganic nanoparticles revealing a potential tool capable of purifying water from nanostructured materials reducing the toxicity associated with the ingestion of contaminated drinking water (De Matteis *et al.*, 2021). To further expand research in the field of biotechnology, solid in vitro propagation culture protocols are needed and an extensive knowledge of *in vitro* adapted lines.

Laboratory protocols for axenic in vitro culture of moss were adapted to three species of aquatic moss: *Leptodictyum riparium* (Amblystegiaceae), *Vesicularia montagnei* and *Taxiphyllum barbieri* (Hypnaceae). The anatomic characters for their description and physiologic monitoring were described and their biotechnological potential started to be explored. Treatments with phytohormones, cytokinin (BAP) and auxin (NAA), showed a different response in the three species. Selected the best growing condition, stereoscope was used to analyze different qualitative characters, and light microscope was used to observe and measure the laminal and alar cells of the leaf with differences between the three species after that cycle induction conditions were tested to study the various life stages. Not all species adapt completely growing *in vitro* and may be suitable for different purposes. The metabolic profile was also analyzed with <sup>1</sup>H-NMR and analysis of metals and trace elements were analysed using ICP/AES to highlight the differences between the three species.

### **References**

- De Matteis *et al.* (2021). Physico-chemical properties of inorganic NPs influence the absorption rate of aquatic mosses reducing cytotoxicity on intestinal epithelial barrier model. *Molecules*, 26(10), 2885.
- Mishra *et al.* (2014). Potential of bryophytes as therapeutics. *International Journal of Pharmaceutical Sciences and Research*, 5(9), 3584-3593.
- Nozaki *et al.* (2007). Momilactone A and B as allelochemicals from moss *Hypnum plumaeforme*: first occurrence in bryophytes. *Bioscience, biotechnology, and biochemistry*, 71(12), 3127-3130.
- Papadia, P. *et al.* (2020). Aquatic mosses as adaptable bio-filters for heavy metal removal from contaminated water. *International Journal of Molecular Sciences*, 21(13), 4769.

## Pollination event affects auxin and cytokinin accumulation and distribution in *Ginkgo biloba* ovule

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The development of seed has represented a turning point in the history of land plants, leading the colonization of a variety of habitats. In Spermatophytes, the seed originates from a fertilized ovule, inside of which is an embryo sac, called the female gametophyte (FG) [1]. FG development is controlled by an intricate molecular network, in which plant hormones play a key role. In particular, auxin and cytokinin are crucial during ovule development and after fertilization for its transformation into seed [2, 3]. All these mechanisms are widely studied in Angiosperms, especially in model species *Arabidopsis thaliana*, but they are poorly explored in Gymnosperms. In this scenario, we extended the studies on *Ginkgo biloba* L., whose ovules present interesting and primitive characteristics and has been extensively described [4]. In *Ginkgo*, pollination and fertilization events are separated by a long interval (i.e. 4-5 months), in which ovule continues to grow and FG differentiation occurs, suggesting that pollination event could trigger the signal for ovule transformation in seed. In this context, the specific pathways activated/deactivated by pollination event were identified by a transcriptomic approach, using pollinated (PO) and unpollinated ovules (UO), the last ones collected from female plants isolated geographically from male plants. For both condition, three different stages were used: 1, 6 and 8 days after the end of pollination drop emission. Statistical and bioinformatics analysis, by using pairwise comparisons of pollinated *vs* unpollinated ovules libraries from the same time point, revealed key pathways modulated by pollen capture. Among these, we focused our attention on hormone metabolism pathway, mainly on auxin and cytokinin, given their important involvement in FG development. An immunolocalization of indol-3-acetic acid and *trans*-zeatin riboside was performed in both PO and UO, in order to evaluate if pollen arrival could affect their accumulation and distribution. In addition, we selected a PIN gene encoding an auxin efflux carrier modulated by pollination event and we identified the expression domains through an *in situ* hybridization approach, in both PO and UO. The obtained results highlighted a strictly connection between auxin and cytokinin distribution and pollination event before the fertilization in *G. biloba*. Moreover, polar auxin transport resulted modulated by pollination, allowing the proper FG development in PO and the degeneration of the nucellar tissue in UO, which lastly results in ovule abort.

### References

- [1] Linkies et al. *New Phytologist* **2010**, 186, 817-831.
- [2] Wang et al. *New Phytologist* **2020**, 230, 2261-2274.
- [3] Terceros et al. *International Journal of Molecular Sciences* **2020**, 21, 8161.
- [4] Douglas et al. *International Journal of Plant Sciences* **2007**, 168, 1207-1236.

## **Spatiotemporal localization of key hormones involved in the development of reproductive structures in *Ginkgo biloba* L.**

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Unravelling the genetic and molecular basis of plant reproductive development is of key importance from both the scientific and economic perspective.

Thanks to three decades of research, many master regulators of flower, fruit and seed development have been identified, downstream pathways have been discovered and the role of hormones has been investigated and integrated in the regulative networks acting during these processes.

Most of these studies have been performed on model angiosperms, therefore there is the need to investigate also gymnosperms and expand the knowledge on the diversity of the reproductive strategies that seed plants have evolved.

*Ginkgo biloba* is the only extant species of the clade Ginkgophytes. *Ginkgo* and cycads form a sister clade to the other gymnosperms occupying a key phylogenetic position in-between the extinct Palaeozoic seed ferns and the other extant gymnosperms. Given its phylogenetic position and considering the availability of genomics, transcriptomics and metabolomics data, the study of *Ginkgo* reproductive development appears of particular interest for a better understanding of seed plants evolution.

After the identification of key stages of both *Ginkgo* female (ovules, D’Apice et al., 2021) and male (pollen cones) reproductive structures, we investigated the role of hormones during their development.

In a parallel work focused on the ovules, the attention has been paid on the involvement of hormones in *Ginkgo* ovules shortly after pollination, comparing pollinated vs unpollinated ovules (Muto et al., in preparation).

Here we focused the study on early steps of ovule formation (from the first stages within the wintering buds to the pre-pollination stage) and male reproductive structures at different stages of development. Immunolocalization experiments of auxins and cytokinins can provide a comprehensive picture of the spatiotemporal distribution of these hormones during the early development of *Ginkgo* reproductive organs. Besides that, the expression patterns of specific hormone-related genes can be investigated by *in situ* hybridization studies. The results obtained will provide new interesting insights on the hormonal crosstalk regulating the development of *Ginkgo biloba* reproductive structures.

### **References**

D’Apice, G., Moschin, S., Araniti, F., Nigris, S., Di Marzo, M., Muto, A., Banfi, C., Bruno, L., Colombo, L. & Baldan, B. (2021). The role of pollination in controlling *Ginkgo biloba* ovule development. *New Phytologist*, 232(6), 2353-2368.

*Invited lecture*

## **Opportunity and challenges of microalga cell factory**

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Microalgae represent an important resource for biotechnological applications thanks to their biological and physiological properties and high diversity (specific, functional and content in secondary metabolites). Although still little developed on an industrial scale, microalgae can find applications in nutraceuticals and cosmeceuticals, as well as in the field of feeding farm animals/aquaculture, and environment or energy. Among microalgae, diatoms represent a highly relevant group in nature both from an ecological and biogeochemical point of view, with a high growth rate, but still underestimated in the field of biotechnological research.

C. Brunet's talk will present the knowledge acquired in this research field and discuss the challenges accompanying the appropriate development of the use of microalgae as a "cell factory" for the synthesis of molecules with biological activity with health benefits human or, more generally, as a crucial resource for the expansion of "blue biotechnologies".

## Toxic microalgae and phycotoxins along the Italian coast: data from the last decade

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Coastal waters are widely exploited by human activities and can be threatened by outbreaks of toxic microalgae (both benthic and planktonic). Some species are responsible for severe illnesses, caused by consumption of contaminated seafood or the formation of toxic bio-aerosols.

Since the 1970s, toxic microalgae and their toxins have been reported in Italian seawaters as well as in wild and cultivated shellfish. Data gathered by the Veterinary Public Health Institutes (IZS) and the Italian Environmental Health Protection Agencies (ARPA) from 2006 to 2019 showed that, among detected toxins, i.e., okadaic acids (OA) and analogs, yessotoxins (YTXs), pectenotoxins (PTXs), saxitoxins and derivatives (STXs), domoic acids (DAs) and azaspiracids (AZAs), OA and YTXs have been those most frequently reported. Levels of OA exceeding regulatory limits were associated with high abundances of *Dinophysis* spp. and for YTXs with blooms of *Gonyaulax spinifera*, *Lingulodinium polyedra*, and *Protoceratium reticulatum*. Seasonal blooms of *Pseudo-nitzschia* spp. have occurred all along the Italian coast, but DA has only occasionally been detected in shellfish at concentrations always below the regulatory limit. *Alexandrium* has been recorded in many more areas as compared to those where STXs were detected, only rarely exceeding the regulatory limit. *Azadinium* has been sporadically recorded, and AZAs have been sometimes detected but always in low quantities.

Among the emerging toxins (including palytoxin-like compounds (PLTXs), cyclic imines (CIs), and tetrodotoxins (TTXs), for which at now a regulatory limit has not provided by EU), PLTXs have been often detected along rocky shores associated to the blooms of *Ostreopsis* cf. *ovata*. These blooms are of great concern because of the associated problems on human health often observed (mainly skin and eye irritations) due to inhalation of sea water droplets containing aerosolized toxins and/or cells of *Ostreopsis*, or cutaneous contact with cells.

Overall, Italian coastal waters harbour a high number of potentially toxic species, with a few HAB hotspots mainly related to toxins of Diarrhetic Shellfish Poison (DSP) group (i.e., OA and YTXs). Still, rare cases of intoxications have occurred so far, reflecting the whole Mediterranean Sea conditions.

## Characterization of *Neochloris oleoabundans* under different cultivation modes and first results on bioactivity of its extracts against HCoV-229E virus

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Microalgae are proposed in several biotechnological fields because of their ability to produce biomass enriched in high-value compounds. Artificial manipulations of growth conditions (nutrients, temperature, salinity, etc.) are often linked to the variation of microalgae metabolism so that these micro-organisms are induced to modify their morphology and/or to synthesize or also accumulate different compounds frequently linked to bioactivity [1]. Regarding the health sector, microalgae are a source of various bioactive compounds like pigments, vitamins, polyunsaturated fatty acids, phenolics, polysaccharides, or proteins, which have been studied for their antibacterial, antiviral, anti-inflammatory, antioxidant, anticancer and other pharmacological activities. An emerging area focuses on natural products exploitable against viruses. Very recently, the green microalga *Neochloris oleoabundans*, mainly studied for its capability to accumulate lipids for the bio-energetic sector, has been proposed for some applications in the field of health. This work deals with the characterization of the green microalga *N. oleoabundans* cultivated under autotrophic and mixotrophic conditions as a source of whole aqueous extracts, tested as antivirals against HCoV-229E (*Coronaviridae* family). Glucose was employed for mixotrophic cultures. Growth and maximum quantum yield of photosystem II were monitored for both cultivations. Algae extracts for antiviral tests were prepared using cultures harvested at the early stationary phase of growth. Biochemical and morphological analyses of algae indicated a different content of the most important classes of bioactive compounds with antiviral properties (lipids, exo-polysaccharides, and total phenolics, proteins and pigments). To clarify which phase of HCoV-229E infection on MRC-5 fibroblast cells was affected by *N. oleoabundans* extracts, four conditions were tested. Extracts gave excellent results, mainly against the first steps of virus infection. Notwithstanding the biochemical profile of algae/extracts deserves further investigation, the antiviral effect may have been mainly promoted by the combination of proteins/pigments/phenolics for the extract derived from autotrophic cultures and of proteins/acidic exo-polysaccharides/lipids in the case of mixotrophic ones.

### References

[1] Castillo, T.; Ramos, D.; García-Beltrán, T.; Brito-Bazan, M.; Galindo, E. Mixotrophic cultivation of microalgae: An alternative to produce high-value products. *Biochem. Eng. J.* 2021, 176, 108183.

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## **In vitro gastric and duodenal digestion and antimicrobial activity of different preparations of Italian wild food plants organs**

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Many edible wild plants have both nutritional and therapeutic values due to the presence of biologically active compounds, so that they can be considered as food-medicines. The aim of the present research was to study and characterize, both for their phytochemical profile and biological activities, several Italian wild food plants collected and used, in the past and still nowadays.

A detailed bibliography search of Italian wild food plants species previously studied was performed, in order to identify the most promising species traditionally used also as medicines. A complete database was created with all information collected of each species [1]. Four of the most cited species with a high number of uses were selected and analysed: *Borago officinalis* L., *Foeniculum vulgare* Mill., *Hypericum perforatum* L., *Sambucus nigra* L. Sampling was carried out in April 2021, the collected plants were separated into different organs. Flowers, leaves and stems of *B. officinalis* and *H. perforatum*, stems of *F. vulgare*, flowers and leaves of *S. nigra* were processed. Infusion and decoction, which resulted to be the methodologies mainly associated to plant medicinal preparations [1], were prepared with the selected organs. The liquid fractions of infusions and decoctions were subjected to *in vitro* oro-gastric-duodenal digestion and the differences after simulated human digestion were detected. The digestion process was performed by simulating *in vitro* the 3 different digestion phases: oral phase, gastric phase and intestinal phase. Digestates and undigested samples were analysed by means of spectrophotometric techniques in order to assess total protein, total polyphenol, total reducing sugar contents and antioxidant activity. In general, the digested preparations contained lower polyphenol amounts and antioxidant activity respect to the not digested ones, with an average decrease of 60.3% and 50.4% respectively. Regarding the amount of reducing sugars and proteins, generally digestates resulted to have higher concentrations respect to the undigested samples, with an average increase of 134.7% in sugars and 272.3% in proteins. The antimicrobial activity of the digested and not digested samples of *H. perforatum* was also evaluated. Two different bacteria, *Escherichia coli* and *Staphylococcus aureus*, were exposed to different plant extracts concentrations (15-0.03 mg/ml) to detect the minimal inhibitory concentration (MIC). *H. perforatum* flowers and leaves showed the best antimicrobial activity (0.94 mg/ml), both for infusion and decoction not digested. Overall, the digestates didn't show activity at the analysed concentrations. These results demonstrated that *H. perforatum* is the best performing species with great nutritional value, and with the most promising human health application.

### **References**

[1] Monari, S.; Ferri, M.; Salinitro, M.; Tassoni, A. Ethnobotanical Review and Dataset Compiling on Wild and Cultivate Plants Traditionally Used as Medicinal Remedies in Italy. *Plants* 2022, 11, 2041. <https://doi.org/10.3390/plants1115204>.

## Quantitative determination of free and conjugated biogenic amines in green coffee beans

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The investigation of plant metabolic responses to environmental factors can be extremely useful for a better understanding of the physiological processes by underlying molecular mechanisms through which plants can adapt to a changing climate.

The main aim of this work was to determine the biogenic amine profiles of green coffee beans of *Coffea arabica* L. from different areas of the States of Minas Gerais and São Paulo in Brazil. The analysis of the samples of green coffee beans, supplied by Illycaffè S.p.A. (Trieste), was conducted with the purpose of evaluating any correlation between the presence and concentration of biogenic amines and the environmental parameters of the samples' origin.

Biogenic amines are ubiquitous constituents of both eukaryotic and prokaryotic cells and play a central role in the regulation of numerous biochemical and physiological functions.

The amines (putrescine, spermidine, spermine, diaminopropane, and tryptamine) were determined by high-performance liquid chromatography with fluorescence detection (HPLC-FD) after derivatization with dansyl chloride. With the aim of the determining both free and conjugated forms the analyses were conducted before and after acid hydrolysis.

Multivariate analysis was applied to evaluate the possible use of these amines as chemical descriptors of their geographical origin. Generally, putrescine was the most abundant amine in both conjugated and free form, followed by spermidine, spermine. Biogenic amines and their concentrations in the analyzed Brazilian samples did not show any striking correlation with the environmental parameters taken into consideration. Mesoclimatic and microclimatic factors, fruit formation and ripening times, as well as post-harvest processes to which green coffee beans are subjected may have directly influenced the presence and concentration of amines in the samples. By expanding the investigation with new green coffee bean samples from other geographic locations from four additional different continents, and analyzing, in addition to biogenic amines, several bioactive compounds including alkaloids, chlorogenic acids, phenolic acids and flavonoids, the objective of future research is to highlight the metabolic responses of the coffee plant to environmental factors and climate change.

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).

## **Cadmium toxicity is involved in the loss of gravitropic stimuli perception through PIN2- mediated auxin redistribution in *Arabidopsis thaliana***

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Cadmium (Cd) is a non-essential heavy metal, considered one of the most relevant pollutants, due to its high toxicity and solubility in water [1], that cause severe environmental and social problems. In plants, Cd can be absorbed by roots and transported to shoots resulting in physiological, cellular and molecular alterations that affects plant growth. Cd modulates photosynthetic activity, nutrients absorption, respiration and it can induce oxidative stress, promoting the generation of Reactive Oxygen Species (ROS) [2]. Root is the first organ that get in contact with Cd in soil, and consequently, it can be considered the primary target of this heavy metal. Recently, it has been demonstrated that short-time exposure to high Cd concentration inhibits *Arabidopsis thaliana* shoot and root growth, by modulating the expression of *WUS/WOX* homolog genes and cytokinin accumulation [3]. Furthermore, other studies highlighted that Cd could also affect root growth, by altering the SCARECROW (SCR) expression and auxin-cytokinin crosstalk [4]. In the present work we focused our attention on the effects of Cd toxicity on *Arabidopsis thaliana* root apical meristem (RAM) in both Col-0 and transgenic lines, expressing molecular markers with an important role in root growth. For this purpose, the root was exposed for short periods (24 and 48 h) at two Cd concentrations (100 and 150  $\mu$ M). The effects of Cd were explored through integrated morpho-histological, molecular and pharmacological analyses. The obtained results showed an inhibited primary root elongation. Moreover, Cd affects auxin accumulation by interfered with the PINFORMED (PINs) family, involved in polar auxin transport, particularly PIN2. In addition, our results showed that the exposure to high Cd concentrations induce an increase of ROS. Therefore, we also observed in *p35S::GFP-TUB6* an altered organization of cortical microtubules, that affects vesicular transport in *pPIN2::PIN2-GFP* roots. Also, starch and sucrose metabolism were impacted, and consequently the gravitropic root response. Globally, our results demonstrated that acute Cd toxicity induces ROS accumulation, affects auxin redistribution and microtubules orientation pattern in RAM and consequently cause an altered root gravitropic response.

### **References**

- [1] Pinto et al., Science of The Total Environment (2004) 326, 239-247.
- [2] Sanità di Toppi & Gabrielli. Environmental and Experimental Botany (1999) 41, 105-130.
- [3] Bruno et al., Plant Physiology and Biochemistry (2021) 167, 785-794.
- [4] Bruno et al., Frontiers in Plant Science. (2017) 8, 1323.

## The effects of salt-stress on *Sorghum bicolor* (L.) Moench root system: morpho-functional, cytohistological and metabolomic analyses

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Europe, like the rest of the world, is experiencing desertification, drought, loss of biodiversity, soil erosion and salinization, rise of sea level, fresh-water scarcity and heat waves. Salt stress is putting at risk food and water safety and demand in every part of the world. Nowadays over 7% of the world's lands and 20% of the global irrigated land area are salt-affected due to inefficient and uncoordinated water use. These climate issues are affecting the most cultivated European crops, such as wheat, maize, rice and barley and the countries of southern Europe are the most affected. Italy is suffering a very high yield decline and it has been estimated that most of the coastal areas in the subsoils (from 30 to 100 cm) of Italy are salt-affected. This phenomenon caused an increase in the extension of marginal areas or “less-favored agricultural areas” (LFAAs) called so because of their unfavorable biophysical characteristics, such as salinity, scarcity of water and limited rainfall, that decrease their agricultural potential (Ahmadzai et al., 2021). Even if sorghum is not usually grown in European countries, Italy is one of the biggest European producers. *Sorghum bicolor* (L.) Moench is the 5<sup>th</sup> cereal globally produced, especially in arid and semi-arid regions of the world due to its natural resistance against salinity, drought and heat and to its nutritional characteristics, it has a high protein content and is gluten-free. It has the peculiar bio-anatomical leaf adaptations of C4 plants that reduce photorespiration energy losses due to intense light, high temperature and low water supply. The higher efficiency of the C4 photosynthetic pathway confers fast-growth and high-yield characteristics, a trait that makes Sorghum a perfect crop species to rely on the ongoing climate change, enhancing marginal and altered soils and contributing to the achievement of agroecological sustainability. This study takes part in the CN2 AGRITECH PNRR project and is aimed to analyze the effects of salt stress on the root systems of two commercial sorghum hybrids, both provided by Padana Sementi Elette S.r.l., through morpho-functional, cytohistological and metabolomic analyses. The two sorghum hybrids, Tonkawa and Bianca, certified as tolerant and non-tolerant to drought, respectively, were *in vitro* cultured for 10 days in the presence of 0, 150 and 300 mM of NaCl. Metabolomic analysis was performed by using 1 H-NMR spectroscopy. Monodimensional 1 H and bidimensional 1 H- 1 H TOCSY experiments were acquired by a JNM-ECZ600 Spectrometer. The higher salt concentration reduced seed germination in both genotypes and strongly inhibited primary and adventitious root development in germinated seeds. In particular, at 300mM of NaCl the germination rate of Tonkawa hybrid showed a reduction of the 80%. Sodium chloride at 150 mM altered the root system architecture, in both hybrids, mainly affecting lateral root formation and development and adventitious root elongation, thus reducing root biomass. Tonkawa showed a higher decrease in root biomass than Bianca. These morphological results were supported by cytohistological and metabolomic analyses. In conclusion, these preliminary results show different responses of the two genotypes to salt stress. However, the Bianca genotype seems to be better adapted to higher salinity levels, suggesting that it could be considered for the recovery of marginal agricultural areas with high salt levels.

## Cyto-histological analysis of rice roots exposed to arsenic and brassinosteroids

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In recent decades, heavy metals (HM) contamination in soils has become a serious environmental issue worldwide. The term HM refers to the group of metals and metalloids with a density greater than  $5 \text{ g} \cdot \text{cm}^{-3}$ , such as cadmium (Cd), lead (Pb), and arsenic (As). The inorganic arsenate [ $\text{As}^{(\text{V})}$ ] and arsenite [ $\text{As}^{(\text{III})}$ ] are the most dangerous As species, due to their toxicity to living organisms, and their soil mobility. This latter aspect makes them easily absorbed by plants through the roots. Under aerobic conditions,  $\text{As}^{(\text{V})}$  is taken up by roots using phosphate transporters, and once inside the root, it impairs phosphate metabolism. Moreover, it adversely affects nutrient uptake, physiological and biochemical processes, and plant growth (Bali et al., 2021, *Chemosphere*, 283, 131050). Arsenite is absorbed by roots through various transporters such as Nodulin 26-like Intrinsic Protein (NIP) and it causes severe damage to the whole plant. *Oryza sativa* L. (rice) is cultivated worldwide and currently feeds more than half of the world's population. Much of the world's paddy fields are at risk due to high levels of As, which is easily absorbed by the rice roots. Numerous works have highlighted the negative effects of As on rice root system development, as well as the negative effects of this metalloid on the plant hormonal homeostasis (Ronzan et al., 2018, *Environ. Exp. Bot.*, 151, 64–75). It is also known that certain classes of phytohormones can reduce the toxic effects of As on the development of plants such as rice (Piacentini et al., 2020, *Frontiers in Plant Science*, 11, 1182). Among these hormones are brassinosteroids (BRs), steroid hormones involved in root formation and development, which appear to play a role in mitigating the negative effects of certain toxic elements (Della Rovere et al., 2022, *Int. J. Mol. Sci.* 23, 825). Recent results on the role of BRs in the response of plants to environmental stresses have opened the way for new hypotheses on the strategies implemented by plants to survive in adverse environmental contexts. Hence, this study aims to investigate whether BRs can restore the correct organization of roots at the cyto-histological level when altered by the presence of toxic elements such as As. In particular, the objective was to analyze in detail, the formation and development of rice roots following exposure to  $\text{As}^{(\text{V})}$  and  $\text{As}^{(\text{III})}$  and/or 24-epibrassinolide (eBL), which is known to be one of the most bioactive forms of BRs. Our histological analysis, performed on adventitious root regions where lateral roots are formed, confirmed that both As forms induce cyto-histological anomalies in the forming lateral root primordia (LRPs) and prevent their regular development into roots. Exogenous eBL, at  $10^{-7}$  M, induced an increase in LRP formation in comparison with the Control and regular LRP development. However, the arsenic-induced anomalies became more pronounced when the plants were simultaneously exposed to As and eBL, showing that eBL is unable to reduce most of the histological damages induced by both As species in rice roots. Nevertheless, the hormone has a positive and tissue-specific effect, because it counteracts the deposition of lignin in the cell walls of the cortical parenchyma, an event which is instead strongly induced by both the As species. These results increase the knowledge on the mechanisms of action of BRs on root system growth and on protection from the stress induced by As and pave the way to applications of these phytohormones in the cultivation of agronomically important plants such as rice.

## Uncovering the effects of water activated by plasma discharge on arbuscular mycorrhizal symbiosis in *Lotus japonicus*

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Non-thermal plasma is a cutting-edge technology that has been developed about a decade ago and that is currently attracting growing interest in view of its wide array of applications, ranging from medicine to food industry and agriculture. In plant biology, plasma-activated water (PAW) has been shown to promote seed germination and plant growth, and to increase plant resistance to biotic and abiotic stresses. Despite increasing knowledge of the beneficial effects exerted by PAW on plants, as well as of the underlying plant perception mechanisms, little information is currently available about how this emerging technology may affect the mutualistic interactions established by plants with microbial symbionts in the rhizosphere.

In this work we have investigated the potential modulatory effects played by PAW on the establishment and development of arbuscular mycorrhizal (AM) symbiosis in the model legume *Lotus japonicus*. To this aim, 2-weeks old seedlings of *L. japonicus* were transferred in sand-filled pots and then inoculated with the AM fungus *Rhizophagus irregularis*. After 10 days plants were irrigated once a week with freshly generated PAW obtained by exposing water to a pressurized air-fed plasma torch operating at 900 W. Different time intervals of water exposure to plasma and PAW dilutions were tested. Plants treated with PAWs for 4 and 6 weeks, together with their respective controls, were collected and the root apparatus was stained to visualize and quantify the presence of the different AM fungal structures via the Trouvelot method. In parallel, Ca<sup>2+</sup> measurements in *L. japonicus* roots transformed with constructs encoding cytosolic and nuclear aequorin-based Ca<sup>2+</sup> reporters were used as a rapid assay to check Ca<sup>2+</sup>-mediated responsiveness of plants to the applied PAW doses.

Preliminary results indicate that treatments with PAW, although known to trigger plant defence responses against pathogens, do not negatively affect the symbiotic interactions between *L. japonicus* and *R. irregularis*. We are currently evaluating frequency, intensity of root colonization and percentage of arbuscules in PAW-treated samples *versus* control. Interestingly, non-thermal plasma seems to induce the accumulation of lipid-containing vesicles in colonized roots, suggesting a modulation of the fungal metabolism within plant roots. Chemical analyses are ongoing to quantify total phosphorous, carbon, nitrogen and sulfur as an additional proxy of AM-mediated nutrient uptake and its interaction with PAW treatment. These data will provide insights into the potential use of PAW to improve plant mineral nutrition, to understand its impact on the establishment and functioning of AM symbiosis and further develop sustainable agricultural practices.

## Nature based solutions for the treatment of civil wastewater in an internal area of Alexandria of Egypt: potential and innovation of a pilot constructed wetland

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Agricultural land in Egypt is saline, mostly in arid regions, and billions of people are thus threatened by soil degradation and loss of food production; the management of water resources is therefore a critical issue in the adaptation to climate change. The SRTA-City working group implemented a Nature Based Solution (NBS), consisting of a septic tank completed by a sand biofilter, in one village at Banger EL (Alexandria), for the treatment of civil wastewater, in order to reuse the purified water for soil washing. Unfortunately, the effluent quality doesn't yet comply with legal standards required for reuse in agriculture. In this context, the use of phytoremediation (PR) technology as finishing treatment, through the implementation of Constructed Wetlands, represents one of the most exploitable alternatives for local communities, thanks to their environmental and economic sustainability. For this purpose, based on the previous experiences of the UNISA group [Spiniello et al., 2022], we realized an innovative PR pilot plant, of submerged horizontal flow and semi-hydroponics type, in which we tested the efficiency of bioremediation for the treatment of the effluent coming from the NBS system. The pilot plant has been carefully dimensioned, and the plant species used, *Phragmites australis*, *Thypha* spp., *Arundo donax* and *Nerium oleander*, have been selected among the ones available on site. In order to overcome PR applicability limit due to the large surfaces necessary, three strategies have been adopted to make the PR plant more efficient in biological terms: the use of natural filling media with high Specific surface (Ss), e.g. expanded clay with Ss = 2000 m<sup>2</sup>m<sup>-3</sup>, to promote the growth of large biofilms that can assist the process; acclimatation by recirculating in the filter bed an aqueous solution enriched with rhizosphere microorganisms from the selected plants; installation of a bioelectric cell to enhance the biodegradation of organic matter without supplying energy. The efficiency of the PR plant was evaluated by analyzing the following concentrations: Bio/Chemical Oxygen Demand; Nitrogen compounds; Chlorides; metals; TDS and TSS. After 48 hours of treatment, a significant abatement of all the pollutants was already observed (e.g. 79% remediation efficiency for COD, starting from a value of 111 mgL<sup>-1</sup>), which were almost totally removed after two weeks (e.g. 93% for COD), respecting the local limits for the reuse of effluent in crop irrigation. About one order of magnitude removal of pathogenic colony-forming unit for mL (*E. coli*, Staph, Yeast) was also observed, which can be completed by a solar-driven UV lamp installed downstream. In addition, toxicity and germinability tests with the model organism *Daphnia magna* and tomato seeds, respectively, confirmed a reduced mortality in contact with the effluent than the influent. The results of metagenomic analyses carried out both on wastewater and filtering media will help to understand the dynamics underlying the process and could pave the way to the isolation of bacterial consortium to be reused in future applications. Therefore, the technical innovations adopted have certainly contributed to the achievements of the objectives, with the ultimate aim of reducing the marginalization of Mediterranean inland areas.

## Assessment of tolerance and seed germination to rare earth elements in *Chenopodium quinoa*

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Rare earth elements (REEs) are a group of 17 elements composed of Yttrium, Scandium and 15 lanthanides with an Atomic Number between 57 and 71, and widely used in anthropogenic activities. Although REEs haven't a role in biological and physiological processes, previous studies have shown their capability to enhance seed germination and plant growth. *Chenopodium quinoa* Willd. is a highly nutritious crop that can successfully respond to several abiotic stresses (*e.g.*, cold, salt and drought); however, its tolerance to REEs is not clear yet. In this study we exposed seeds of quinoa (cultivar Regalona) to increasing concentrations of Gadolinium (III) acetate and Cerium (III) nitrate to assess the effects on germination, shoot and root elongation. Quinoa seeds were sterilized with a 70% ethanol solution followed by a sodium hypochlorite 1,5% treatment; after that 20 seeds were placed in Petri dishes on filter paper soaked with Ce or Gd solutions and then kept in the dark in a phytotron at a temperature of 21 °C and 60% humidity. Sterilized seeds were exposed to the following concentrations of the two assayed lanthanides: 10 µM, 30 µM, 50 µM, 70 µM, 100 µM and 150 µM. Germinated seeds were counted daily and, after seven days, germination rate, shoot and root lengths were measured. Germination index (GI), relative seed germination (RSG), relative roots elongation (RGR) and relative growth of shoots (RGS) were calculated. Previous studies demonstrate that GI's values of 80% indicates absence of phytotoxicity. A GI < 80% was observed only at the Ce intermediate concentrations (50 and 70 µM); at the lowest and the highest concentrations GI reached 100%. On the other hand, in the presence of Gd a GI > 80% was observed at 10 µM and 150 µM. The lowest GI values (31%) resulted for 50 µM exposition. RGR reached the highest values for 10 µM concentrations of both Ce and Gd (386% and 231%, respectively). RGR values < 50% were detected at 50 µM Gd exposure. Furthermore, even RGS showed the highest values at 10 µM for both Ce and Gd (107% and 152%, respectively). RGS was < 80% only in the case of 30 µM Ce exposure. Moreover, another informative parameter to estimate possible Ce or Gd interaction with the assayed quinoa seeds was calculated: the coefficient of germination velocity (CVG). A high CVG value indicates that more seeds germinate in a lesser time. It was observed that REE treatment affected positively CVG: at 10 µM, in the case of both lanthanides, CVG was almost duplicate in respect with the controls (from about 30% up to 80% and 73% for Ce and Gd, respectively). The exposition at 150 µM revealed an increment of CVG parameter for Ce (90%), or an its reduction (68%) for Gd, relatively to their respective controls (about 70%). In conclusion, this preliminary study would suggest positive effects at the lower doses of REEs on quinoa seed germination, root and shoot elongation. Therefore, their application on quinoa seeds at low concentrations could be a successful method for enhancing in particular during the early stages of crop growth and development.

## Contaminants of emerging concern and antimicrobial resistance: use of green technologies for a safe civil wastewater reuse

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Nowadays, it is commonly to find in wastewater drugs, used by humans and/or for livestock, or personal products care, both included in the so-called Contaminants of Emerging Concern (CECs), whose concentrations range from ng L<sup>-1</sup> to ug L<sup>-1</sup>. Among CECs, antibiotics can promote the selection and diffusion of antibiotic resistance bacteria (ARB) and genes (ARGs). Because of their incomplete removal by conventional treatments, CECs, ARB and ARGs are found in the effluents, posing risks to the environment and public health with particular regards to water reuse. For all these reasons, in our study we tested an innovative NBS (Nature Based Solution), the so-called Constructed Wetlands (CWs), for the secondary treatment of civil wastewater. CWs exploit the natural remediation capability of plants and microorganisms, so CWs represent an eco-friendly solution for the environmental contaminant removal so as to assure the reuse of treated water in compliance with the Italian regulatory limits imposed by D.M. 185/03. Moreover, we wanted to test the capability of CWs to reduce CECs (*e.g.*, antibiotics, ARB and ARGs concentrations) as foreseen by the ISTISAN 21/03 report. Specifically, we assayed the microorganism resistance to four different antibiotics commonly used: Ampicillin, Tetracyclines, Vancomycin and Erythromycin. For this purpose, we carried out two independent experiments based on two pilot-scale plant configurations: hybrid CW flow (inverted vertical and horizontal flow – HCW), and vertical CW flow (VCW). The experiment has foreseen: i) physico-chemical (COD, N, Cl, HM, antibiotics) and biological (BOD, coliforms, enterococci) characterization of the influent and effluent wastewater after 48 hours of water treatment; ii) treatment of an aliquot of the effluent with an UV ray irradiation; iii) analysis of the influent wastewater and effluents (after CWs, after UV) through cultural and molecular methods to evaluate the presence of ARB/ARGs and to quantify the four ARGs by real-time PCR; iv) use of the effluents (after CWs or after CWs + UV treatments) to irrigate 48 seedlings of lettuce for two weeks in order to verify the possible transfer of ARB/ARGs to the seedling rhizosphere or to their edible part (leaves). The CWs treatments resulted effective to obtain water of high quality for irrigation purposes. In fact, it was observed a reduction of the main assayed physico-chemical and biological parameters for both used CWs, in compliance with D.M. 185/03. Cultural methods showed a significant decrease of ARB after 48 hours of wastewater treatment in both CWs and their total removal after UV treatment. Moreover, the genes quantification, throughout real-time PCR analysis, carried out after the CWs treatment, alone or in combination with UV irradiation, demonstrated the ARGs removal up to 97% or 99%, respectively. Finally, cultural methods showed the presence of few ARB only in the rhizosphere of the crops irrigated with CWs, whilst they were absent in the lettuce leaves. Contrarily, crops irrigated with water treated by CW+UV showed the absence of ARB in any part of the plant and ARGs, instead, were not detected in all the rhizospheres analyzed probably because of their low amount. The innovative aspect of this study lies in the civil wastewater treatment through CWs, showing the efficacy of both CWs configurations (HCW or VCW) in water reclamation also in the light of CECs elimination and in the consequent reduction of health and environmental risks.

## ***Dittrichia viscosa* selection strategy based on Nip1.1 Gene for Arsenic phytoremediation**

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Arsenic uptake by plants occurs primarily through the root but it is not well known. *Dittrichia viscosa* uptake and translocation of As is not fully understood and some data are contradictory. In one study is reported that As is fully translocated to *D. viscosa* shoots and volatilized, while in another is show that As is stabilized in the roots. In a previous work we studied the accumulation of As[III] and As[V], confirming the high variability among individuals. We established several experimental clonal populations to perform a functional study on the aquaporin NIP1.1.

*D. viscosa* is not a hyperaccumulator plant but can grow in high drought conditions while still producing large biomass, even tolerating significant concentrations of As[III], As[V] and Cd[II]. In spite of these remarkable characteristics, adaptive modification of performances is not predictable in wild populations.

Genetic stability is a desirable trait and interest in the genetic improvement of this plant species is increasing. We test a strategy that take in consideration the use of a clonal population of *D. viscosa* with a defined NIP1.1 expression level and the *in-vitro* micropropagation technique to quickly scale-up the number of plants to perform specific HMs phytoremediation of contaminated areas.

## A transcription factor coordinating flowering and stem elongation in *Oryza sativa* L.

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Rice is a facultative short-day plant domesticated in southeast Asia from its ancestor *Oryza rufipogon*. Rice flowering starts with the transition of the shoot apical meristem (SAM) from vegetative to reproductive phase, leading to the development of a panicle, a determined inflorescence. During the vegetative growth the SAM is located at the base of the plant, while the panicle needs to be at the top of the plant to allow a correct floral development and seeds dispersion. Stem elongation takes place during floral development in response to the same photoperiodic signals inducing flowering in the SAM. Stem elongation allows the emergence of the panicle from leaves at the correct stage of flower development. During vegetative phase nodes and internodes are compressed at the base of the plant. When floral induction occurs, internodes begin to elongate driven by the activity of the intercalary meristem, located at the base of each internode. Floral transition and stem elongation are two tightly linked phenomena, but the molecular link between the two remains still largely unclear.

Recently the identification of a downregulated gene during the floral transition of the SAM could help in clarifying this relationship. PREMATURE INTERNODE ELONGATION1 (PINE1) is a transcriptional factor highly expressed in the SAM and at the base of internodes during the vegetative stage and it is harshly repressed when the flowering process begins. *pine1* knockout mutants present elongated internodes starting from seedling stage. This indicates that when PINE1 is expressed it represses internode elongation, while internode elongation occurs when PINE1 is downregulated (passing from long to short day length).

Moreover, *pine1* mutants are very responsive to exogenous GA treatment in contrast to the wild type. For this reason, we can speculate that PINE1 has a role in the GA signalling. To clarify this issue, we are currently creating double knockout mutants in which PINE1 and proteins involved in GA biosynthesis (SD1) or GA signalling (SLD1, GID1, GID2) are silenced. The ultimate scope of this experiment is to reveal a possible interaction among PINE1 and these genes.

PINE1 has two EAR motif that are commonly associated to transcriptional repression. Ear motifs are known to be associated with TPL co-repressors. Our data suggest an interaction between PINE1 C terminal EAR motif and two TPL proteins of rice. TOPLESS protein's role is to connect a transcription factor with a histone deacetylase (HDAC), who leads to a more compact chromatin structure, thus inhibiting gene expressions. We identify a TOPLESS3-HDAC15 interaction, suggesting the existence of PINE1-TPL-HDAC complex.

We are studying PINE1 expression by looking at its promoter. In particular, with a CRISPR multiplex approach, we obtained mutants harbouring deletions of different size in the promoter region. We are currently characterizing these mutations with the final goal to connect them with different levels of PINE1 expression and with the overall phenotypes.

## Auxin dependent post-translational regulation of MONOPTEROS in the *Arabidopsis* root

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Cellular responses to the phytohormone auxin are controlled by transcriptional regulatory modules composed of AUXIN RESPONSE FACTORS (ARF) and Aux/IAAs. One of the best characterised bimodular effectors of auxin signalling include ARF5/MONOPTEROS (MP) and the Aux/IAA protein BODENLOS (BDL), which interact through their PB1 domains. Auxin perception disrupts the MP-BDL repressor complex, allowing MP to promote the expression of its direct target genes. Recently, we have shown that MP activity for the control of specific developmental programs is dependent on isoforms that result from alternative splicing of the *MP* mRNA (Cucinotta *et al.*, 2021). Here we show a new mechanism determining cell-type specificity of MP output. We show that in roots there is a differential stability of the MP isoforms which depends on the auxin concentration. In regions of low auxin content, the interaction between the canonical MP and BDL through the PB1 domain triggers MP degradation by the 26S proteasome. Such mechanism allows a fine tuning of the MP regulatory output among the diverse root tissue-types, reinforcing root organisation and patterning. Our findings evidence the plasticity of the ARF-AUX/IAAs response, offering the basis for a highly dynamic auxin response model respect to the classical auxin perception paradigm.

## ERAD-mediated maturation of the regulatory protein of plant meristematic cells CLAVATA 3 emerged during evolution from algae to higher plants

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The indefinite growth capacity and the ability to form organs in plants are guaranteed by meristems, where cells are in continuous division and differentiation: these stem cell-made centers are maintained by intercellular signaling pathways (Dao et al., 2022). In the apical meristem of *Arabidopsis thaliana*, a dodecapeptide obtained by the maturation of the protein CLV3 establishes a negative feedback control against the expression of transcription factor WUSCHEL (WUS) (Brand et al., 2000). Nowadays CLV3 is considered a secretory protein, which is matured by secreted proteases at the extracellular level into the active dodecapeptide form. This matured fragment behaves as a ligand of the CLV1/CLV2 receptor complex in the shoot apical meristem (SAM) (Kondo et al., 2006). However, recent studies on tobacco suggest that the protein's maturation process does not occur in the apoplast, but probably through the intracellular ERAD (Endoplasmic Reticulum Associated Degradation) process, which is naturally used by cells to degrade unfolded proteins (De Marchis et al., 2018). Our idea is that the maturation mechanism present in tobacco cells is not active in unicellular algae and therefore CLV3 is not degraded by ERAD mechanism, but follows the classical secretion pathway and reaches the apoplast intact, without any hormonal activity. In order to confirm this hypothesis, we have produced a CLV3-expressing transgenic cell strain of *Chlamydomonas reinhardtii*, to be able to follow the maturation process of this protein in an organism that doesn't show meristematic cells. In order to validate our hypothesis and study the behavior of CLV3 protein in *C. reinhardtii*, CLV3 tobacco protoplast supernatant was collected: the effects of this medium were detected through physiological assays by using *A. thaliana* seedlings. While the medium of the CLV3 strain of tobacco protoplasts strongly affects root development compared to the WT medium conducted test, we have not seen significant differences in root growth in CLV3 algae supernatant until now. Pulse-chase analysis confirms the secretion of the 12-aminoacids active form in CLV3 tobacco protoplasts medium: the same experiment conducted on CLV3 algae strain would confirm a difference between these two organisms in the active dodecapeptide formation, suggesting that the maturation process of CLV3 regulatory protein still needs further analyzes for its characterization.

### References

- Dao TQ, Weksler N, Liu HMH, Leiboff S, Fletcher JC (2022) Interactive CLV3, CLE16 and CLE17 signaling mediates stem cell homeostasis in the *Arabidopsis* shoot apical meristem. *Development*, 149 (19).
- Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R (2000) Dependence of Stem Cell Fate in *Arabidopsis* on a Feedback Loop Regulated by CLV3 Activity. *Science*, 289(5479), 617–619.
- Kondo T, Sawa S, Kinoshita A, Mizuno S, Kakimoto T, Fukuda H, Sakagami Y (2006) A Plant Peptide Encoded by CLV3 Identified by in Situ MALDI-TOF MS Analysis. *Science*, 313(5788), 845–848.
- De Marchis F, Colanero S, Klein EM, Mainieri D, Prota VM, Bellucci M, Pagliuca G, Zironi E, Gazzotti T, Vitale A, Pompa A (2018) Expression of CLAVATA3 fusions indicates rapid intracellular processing and a role of ERAD. *Plant Science*, 271, 67–80.

## A metabolic engineering approach to investigate the serotonin metabolic pathway of *Solanum lycopersicum*

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Tryptamine (TAM) and serotonin (SER; 5-hydroxytryptamine, 5-HT) are secondary metabolites belonging to the class of tryptophan-derived compounds known as indole-alkaloids or, more specifically, as indolamines.

In the last years, a large body of scientific literature provided evidence that plant indolamines are probably involved in the regulation of many processes, including plant architecture and morphogenesis, protection against reactive oxygen species (ROS), biotic and abiotic stress responses, chronoregulation, and developmental events such as flowering and fruit ripening.

Although TAM and SER accumulate to high levels ( $\mu\text{g/g}$  fw) in edible fruits and seeds of many plant species, their biological functions in reproductive organs remain unclear and the metabolic pathways have not yet been characterized in detail.

In plants, TAM and SER are generally produced from tryptophan via consecutive decarboxylation and hydroxylation reactions catalysed by the enzymes tryptophan decarboxylase (TDC) and tryptamine 5-hydroxylase (T5H).

We recently functionally characterized a 3-member *TDC* gene family and a *T5H* gene involved in the biosynthesis of TAM and SER in the model species *Solanum lycopersicum*.

By coupling metabolomics and expression data, we revealed a complex spatiotemporal gene expression and metabolite accumulation pattern that suggested a model in which SITDC1 allows TAM to accumulate in fruits, SITDC2 and SITDC3 work together with SIT5H to convert TAM to SER, respectively, in aerial vegetative organs or in roots and fruits.

In order to modify TAM and SER levels in flowers, fruits and seeds, the traditional transgenesis as well as the CRISPR/Cas9 mediated gene knockout methods were both employed to produce several transgenic and edited tomato lines in which the normal function of the TAM and SER biosynthetic genes is altered.

## Natural allelic variation of Phospholipase C 2 hints at a crucial role for plasma membrane phosphoinositides and phosphate homeostasis

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The challenge of feeding the growing world population was partially solved during the 1960s by developing new high-yielding crop varieties and high-intensity agricultural management. However, their optimal production could only be achieved with massive use of chemical phosphate fertilizers, which is not sustainable in the long term. Phosphorus is essential for plant development, but its supply chain is fragile and its natural reserves are limited in time. For that reason, there is a growing global awareness about the need for optimization of inorganic phosphate (Pi) usage and this can only be accomplished by understanding the molecular mechanisms that regulate plant phosphate homeostasis. Two different Genome Wide Association Studies (GWAS) on two different model legume species, *Lotus japonicus* and *Medicago truncatula*, identified the phospholipase C 2 (PLC2) as a common candidate for the variation of root responses to external phosphate levels. PLC2 is a phosphoinositide-specific enzyme that cleaves plasma membrane phospholipids and releases diacylglycerol and inositol 3-phosphate in the cytoplasm. Interestingly, it was shown that PLC2 transcript and protein levels are regulated by phosphate availability.

To understand the role of PLC2 and its allelic diversity in the regulation of root responses to phosphate, using *L. japonicus* as a model organism, we studied the subcellular localization of PLC2 in root cells from wild type by expressing PLC2-GFP in hairy roots. We observed an accumulation of the protein at the plasma membrane, with a stronger and specific signal in growing root hair tips. In addition, we generated roots expressing a fluorescent indicator for phosphatidylinositol 4,5-bisphosphate, the substrate of PLC2, and we were able to confirm its accumulation in the plasma membrane of wild-type roots, whereas *plc2* mutants revealed a compromised phosphoinositide traffic. Furthermore, we selected five *L. japonicus* wild accessions that showed SNPs in the *PLC2* promoter region compared to Gifu and we generated roots expressing the six *pPLC2::GUS*, in order to study their expression when they are grown on high or low phosphate media. Our preliminary results show that the *PLC2* promoter is active in the root differentiated region, with a stronger signal coming from root epidermal cells and depending on phosphate levels. Altogether, by combining natural variation studies and molecular biology, we unveiled the impact of PLC2 on phosphate accumulation and unmasked its regulation at the transcriptional and protein level.

## **Fruit growth and maturation is controlled by NAC transcription factors**

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The fruit is a fundamental botanical organ for the protection, nutrition, and dispersion of the new progeny, which assumes an essential role in the world agricultural economy. Since fruits are important for both their role in the human diet and their biological relevance, it is of great interest to uncover the molecular processes that orchestrate fruit growth, ripening, and senescence. This will also lay the base for future improvement of critical traits, such as yield and quality. Detailed expression analyses during *Arabidopsis thaliana* valves development were performed in our laboratory, employing an RNA-seq strategy. From this data set, various candidate genes differentially expressed during fruit development were identified that can have a role in the control of *Arabidopsis* fruit development and maturation. Among all the differentially expressed genes, we focused on the NAC transcription factors family, a large family of plant-specific transcription factors involved in miscellaneous plant functions, including fruit maturation and development. Several NAC mutants, differentially expressed in the valves RNA-seq, were analyzed, and characterized to shed light on the molecular pathway that tunes the silique growth. Although NAC genes form a huge family with more than 100 members, our analyses have shown that even single mutants of some of the NAC genes can have a very strong phenotype at the level of fruit growth, ripening, or senescence. The generation of higher-order mutants will allow us to further elucidate the molecular pathway regulated by the NAC genes that control fruit development.

## Insights on peptide hormones families in different plant species and their role in fleshy fruit development and ripening

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Fruit development and ripening are complex, genetically programmed and environmentally regulated processes. They are essential to warrant the success of plants reproduction but, at the same time, they are responsible for the production of economically interesting sources of food. Despite their diversification, different plant species share common regulatory networks involving hormones, transcription factors (TFs) and different signaling molecules. Among the latter, peptide hormones (PHs), being involved both in long and short distance signaling and fulfilling a plethora of functions, are good candidates for being regulators of fruit development. Despite many studies on their occurrence in various plant species and involvement in different plant processes, they are still often overlooked in gene predictions and for their functions in fruit development. The role of the peptide hormone CLAVATA3 (CLV3) and CLAVATA3-EMBRYO-SURROUNDING REGION (CLE) peptides in regulating floral meristem and fruits size is well established, but less is known on the involvement of other peptide hormones families as ROOT GROWTH FACTOR/GOLVEN (RGF/GLV) in fruit development and ripening. To investigate the possible role of these peptides during fruit development and ripening, we looked at genomic and transcriptomic data available in public databases for species differing for fruit morphology, hormonal regulation of ripening and taxonomic position. In these selected species, PHs sequences comparisons demonstrated high conservation of the motif sequences and a contrasting high variability of the remaining portion of the propeptide sequences. Looking at their gene expression profiles, we identified genes encoding PHs transcribed at maximum levels at the early fruit developmental stages and decreasing thereafter while others with the opposite tendency. Therefore, there are some gene expression trends which suggest that they may exert common roles despite their propeptide sequences differentiation occurred during their evolution. To investigate such roles, we are taking advantage of the genomic and transcriptomic data collected for tomato (*Solanum lycopersicum*) and the tools available to genetically modify this model system for studying fleshy fruits ripening and their climacteric regulation. The preliminary experimental procedure that we are following is based on studying the involvement of more PHs classes together in tomato fruit development by exploiting what is known on the maturation events allowing their release in the biologically active form. Indeed, we are focusing on disrupting their biogenesis by inactivating in a fruit specific manner key components of their maturation pathway and post-translational modifications. By this way, the resulting phenotypic defects, along with complementation experiments consisting in the administration of specific PHs, will elucidate their function at the fruit level.

Through these studies we aim at opening new perspectives on the discovery of natural peptides that can exert common regulative functions in different fruit developmental and ripening processes. This could lead to the development of new natural and eco-friendly agrochemicals, that could be used to control different fruits maturation events and improve their quality.

## Triploid block in plants, a postzygotic barrier full of twists yet to be revealed

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The seed is formed by a protective layer, the seed coat (diploid tissue), the embryo (diploid) and the endosperm (triploid). For the proper development of the seed, crosstalk between the endosperm and the seed coat is required. It turns out that plants have developed different strategies to avoid hybridization between individuals of different species or ecotypes of different ploidy, but interestingly, they have also found a way to overcome it. In the postzygotic barrier, called triploid block, resulting from hybridization between individuals with an unbalanced ploidy level, leading mainly to non-viable seeds, the seed coat and, in particular, the innermost layer, the endothelium, plays a pivotal role. A remarkable fact is that in the model species *Arabidopsis thaliana*, maternal excess (i.e., 4x X 2x), leads to viable seeds.

We have focused here on the *TRANSPARENT TESTA 8 (TT8)* gene, expressed mainly in the endothelium, and well known for its function in flavonoid biosynthesis in the seed coat. It turns out that in the absence of TT8, unbalanced crosses, bypass triploid block and result in viable seeds. To better understand which mechanisms may regulate this postzygotic barrier, we performed a thorough morphological characterization: i) of *tt8* mutant seeds, in comparison with the wildtype, and ii) performed comparisons of seeds resulting from different balanced and unbalanced crosses (i.e., 2x X 2x; 2x X 4x; 4x X 2x; *tt8* 2x X 4x). Having observed that in *tt8* the cells of the endothelium are irregularly shaped, that the adjacent cell layer, known as *ii1'*, which originates from periclinal divisions of the endothelium, also does not show the same cell expansion as the wild type, and that the endosperm cellularizes prematurely.

Curiously, these phenotypes turn out to be very similar to seeds resulting from maternal excess crosses. Hence the relevance of exploring the role of maternal genes in seed development and triploid block and to shed light on the mechanisms of action of TT8, an important regulator of hybridization barriers in plants. Understanding hybridization as a mechanism of speciation, it then becomes fundamental to decipher the mechanisms regulating the triploid block.

## Insights into pollen recognition and pollen-ovule cross-talk in *Ginkgo biloba*

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Most of the studies on the reproduction of spermatophytes have been conducted on angiosperms model species. However, four of the five main lineages of seed plants belong to gymnosperms: Cycadales, Ginkgoales, Coniferales and Gnetales. Hence, for a complete understanding of spermatophytes reproduction, investigation on gymnosperm species can no longer be postponed. In this context, an emblematic gymnosperm is *Ginkgo biloba* due to its isolated phylogenetic position and remarkable evolutionary history. Indeed, *Ginkgo biloba* belongs to one of the oldest seed-plant lineages: it is the only extant species of the clade ginkgophytes and plant fossils testify that its morphology has been basically unchanged for at least 250 million years. *Ginkgo* is a large dioecious tree in which the development and maturation of its ovules to give seeds takes several months. The observation that unpollinated ovules abort and fall from the mother plant suggests a central role for the pollination event. Metabolomics and transcriptomics analyses conducted on ovules before and after pollination and new RNA-sequencing data on pollinated vs un-pollinated ovules revealed that DNA damage repair and DNA replication proteins are highly expressed in pollinated ovules (D'Apice et al, 2021). This suggests that pollen arrival blocks the abortive programs that are instead activated in unpollinated ovules, thus allowing the further ovule growth. Given the key role of pollination in promoting *Ginkgo* ovule development and maturation, the perception of pollen grains arrival and the specific ovule-pollen communication are crucial aspects to investigate. Some hypotheses about the location and biochemical nature of the pollen determinant can be made. First, it may be that the pollen determinant is deposited on the ornamentations of the pollen exine. Indeed, in angiosperms lipids and small peptides, deposited in the exine sculptures and part of the so-called pollen-kit, play a crucial role in pollen-pistil interactions and compatibility establishment (Hiscock et al., 2008). Alternatively, it may be that the pollen determinant is present on the intine surface, being exposed only after the hydration and de-coating processes that the pollen undergoes once captured by the pollination drop. Finally, it could be synthesized *de novo* and subsequently secreted once the grain has hydrated and starts germinating in the pollen chamber. Likely, more levels of communications may be necessary for a proper recognition. Concerning the molecular nature of the pollen determinant, it could be a small peptide or a protein, the sugar component of arabinogalactan proteins (AGPs), of which the *Ginkgo* pollen intine is particularly rich, or a non-coding RNA. Finally, the mechanical component could have a role given the dramatic changes that *Ginkgo* pollen ultrastructure and morphology undergo upon hydration (Lu et al., 2016).

### References

- D'Apice, G., Moschin, S., Araniti, F., Nigris, S., Di Marzo, M., Muto, A., Banfi, C., Bruno, L., Colombo, L., Baldan, B. (2021). The role of pollination in controlling *Ginkgo biloba* ovule development. *The New Phytologist*, 232(6), 2353–2368.
- Hiscock, S. J., & Allen, A. M. (2008). Diverse cell signalling pathways regulate pollen-stigma interactions: the search for consensus. *The New Phytologist*, 179(2), 286–317.
- Lu, Y., Zhang, L., Cheng, F., Zhao, J., Cui, J., Li, W., Wang, L., Jin, B. (2016). The morphology, ultrastructure, element distribution and motion behaviour in pollen of *Ginkgo biloba* L. *Trees* 30, 2189–2201.

## **Interactomics for rice flowering: a proximity labelling approach**

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Variability in flowering-controlling genes has been fundamental over history for adapting rice cultivation to different latitudes. The molecular network controlling floral induction in rice is highly complex and it is the result of a stratification of interactions: transcriptional, epigenetic and post-translational. A relatively new technique called Proximity Labelling (PL) allows a high-throughput identification of protein interactors by molecular engineering and has never been tested in rice plants. The goal of this research is to implement PL in rice, establishing efficient vectors and protocols while applying it to the identification of interactors of two rice flowering regulators: OsFT-L1 and Hd1. The application of PL to OsFT-L1 and Hd1 will deliver a list of the proteins laying in their contiguity. The method exploits a biotin-ligase fused to the two proteins of interest: exposure of tissues to biotin leads to biotinylation of OsFT-L1- and Hd1- interacting proteins; biotin tags allow for selective precipitation of the proximal proteome followed by mass spectroscopy analysis.

## The evolutionary importance of stomata and mesophyll traits on adaptation to climate changes

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The most recent *Intergovernmental Panel on Climate Change* report provides a range of scenarios for future CO<sub>2</sub> concentrations, depending on assumptions about future greenhouse gas emissions (IPCC, 2021). Without mitigation actions, under the most optimistic scenario, atmospheric CO<sub>2</sub> concentrations would reach 420 ppm by the end of the century, which is slightly above the highest levels recorded during the middle Pliocene, about 3 million years ago. Under the more pessimistic scenario, atmospheric CO<sub>2</sub> concentrations would reach 1100 ppm by the end of the century [1]. Therefore, it is essential to understand the impact of rising CO<sub>2</sub> on plant physiology and growth for the sustainability of ecosystems, agriculture, and food security. This is the background of the *Evolutionary implications for the development of climate resilient productive plants* PRIN project (EvoPlant), which aims to elucidate patterns of plant evolution to promote the development of climate-resilient plants through the study of stomata evolution, photosynthesis, and plant-atmosphere gas exchange.

In plant evolutionary studies there are clear evolutionary trends towards increased conductance to CO<sub>2</sub>, biochemical efficiency of photosynthesis, water transport, and stomatal control across basal groups to more recently derived angiosperms.

As part of the EvoPlant project, the purpose of this study is to analyze the correlation between leaf anatomy and physiology and stomatal patterning. We are currently completing morphological and anatomical analyses of the mesophyll and stomatal apparatus of 13 plant species representing an evolutionary pathway from lycophytes to dicotyledonous angiosperms, via ferns and gymnosperms. Specifically, we are examining the density, distribution, morphology, and size of stomata. These measurements will then be correlated with the surface area of the mesophyll cells exposed to the intercellular airspaces, the size of the mesophyll cells, the thickness of the mesophyll, the fraction of the volume occupied by the intercellular airspaces, and the total cross-sectional area of the cells that make up the mesophyll. These data will allow us to identify the most efficient combination of stomata-mesophyll traits for climate change resilience.

### References

[1] IPCC, 2021: Summary for Policymakers. In: *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 3–32.

## **Molecular network of SPOROCYTELESS/NOZZLE (SPL/NZZ) controlling Megaspore Mother Cell differentiation in *Arabidopsis thaliana***

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The female gametophyte formation take place in the ovules and consists of two main steps; the megasporogenesis and the megagametogenesis. The megasporogenesis begins with the differentiation of the Megaspore Mother Cell (MMC) that upon meiotic division formed 4 haploid spores. Three spores degenerate whereas the fourth gives rise to female gametophyte after three rounds of mitosis. SPOROCYTELESS/NOZZLE (SPL/NZZ) is required for MMC differentiation, indeed *spl/nzz* mutant fails to develop the MMC resulting in sterile plants. Here we report that SPL/NZZ is required to establish auxin maxima at the tip of the nucellus and, that in *spl/nzz* mutant. SPL/NZZ activate indirectly *PIN1* expression by repressing PIN1 repressors. During my work I have analysed the molecular network controlling the establishment of the MMC by testing putative repressor of *PIN1* such as SHORT HYPOCOTYL 2/ INDOLE-3-ACETIC ACID INDUCIBLE 3(SHY2/IAA3).

## ***Caulerpa prolifera* and its bacterial coating: a promising association for coastal marine water phytoremediation of diesel hydrocarbons**

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In the last years, hydrocarbon pollution in coastal marine waters has remarkably increased and chronic events of modest but continuous contamination have become quite frequent, especially in small harbours and touristic marinas. Bioremediation is considered one of the main natural processes for the removal of the non-volatile fraction of oil from the environment.

The aim of this work was to investigate the role of *Caulerpa prolifera* (Forsskal) Lamouroux in the remediation of seawater contaminated by diesel oil in coastal marine environment. To this aim, an experiment in controlled conditions, to test the tolerance of *C. prolifera* to different diesel oil concentrations and to evaluate its degradative ability, was carried out. Seaweed traits (thalli mortality and blade length and number *per* alive thallus), the abundance (DAPI staining and counts at the fluorescence microscope) and structure (NGS sequencing of 16S rRNA) of the bacterial community associated with its blades and the change of hydrocarbons water concentrations (gas chromatography) were considered as response variables. Several *C. prolifera* thalli collected in the field (Tavolara Punta Coda Cavallo Marine Protected Area, Sardinia) were transplanted in small aquaria and exposed for 1 month to 3 different treatments, 2 with the addition of diesel at different concentrations (0.01% and 1% v/v) and one without diesel, according to a fully orthogonal experimental design (n=3). The obtained data were statistically analysed (univariate and multivariate analysis, depending on the response variable).

No relevant difference in the mortality of the algae between the treatment with 0.01% v/v diesel and the control was observed, while a significant mortality was recorded for the treatment with 1% v/v diesel. Regarding both the blade length and number of alive individuals, the statistical analyses did not underline any significant difference between treatments; nevertheless, a decreasing trend was overall observed in 1% v/v. Moreover, a remarkably higher abundance of epiphytic bacteria was observed on algae exposed to 0.01% v/v treatment, for which a community particularly rich of hydrocarbon degrading bacteria (mainly belonging to *Vibrionaceae*) was found. Finally, also the hydrocarbon degradation appeared to be significantly higher for the aforementioned treatment.

These results suggest that *C. prolifera* and its bacterial coating can effectively be used for the remediation of small harbours and touristic marinas affected by modest hydrocarbon pollution.

## Preliminary study of Mediterranean diatoms species to improve the production of fucoxanthin and the challenge to their long-term preservation

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In the field of marine biotechnology, the diatoms, key organisms in the ecology and biogeochemistry of the oceans, are attracting great interest as a promising source of functional products of wide application in the pharmaceutical, health, cosmetics, nutrition, and nanomaterials sectors [1].

Diatoms show high inter- and intra-specific diversity and different growth conditions and/or stressors can affect the production of metabolites of interest. In this context, we focused attention on the potential biotechnological application of some marine diatom species belonging to the genera *Thalassiosira* and *Chaetoceros* [2]. In particular, a strain of *Thalassiosira rotula*, isolated from the sediment sampled at the Long-Term Ecological Research Station Mare Chiara- Gulf of Naples (Italy), was investigated to explore its good potential for the production of metabolites of interest (i.e. fucoxanthin). Preliminary tests were performed in order to: i) identify the optimum of cultivation conditions for the microalgal growth; ii) enhance fucoxanthin production. A combination of nitrate supplementation in the growth medium and low light intensity has been experimented to induce fucoxanthin production. In addition, the total concentration of fucoxanthin was assayed using a fast spectrophotometer method [3] that will be validated by HPLC analysis. Moreover, since there is a growing need for long-term preservation of the strains of scientific and/or commercial interest, we focused our attention to identify an alternative methodology to the serial subcultures, such as cryopreservation, which is becoming increasingly used in algal crop collections [4].

We are going to investigate the possibility to apply cryopreservation protocols to the different species of interest and also to different states of the diatoms' life cycle, in particular in the resistance stages (spores and/ or resting cells). Preliminary tests were performed to evaluate the dynamics of spore formation in *Chaetoceros socialis*, starting from very low concentrations and considering the influence of both cell density and the reduction of nitrogen concentration in the growth medium (N-depletion) [5,6]. The obtained results highlighted a strict connection between cell density and nitrogen depletion in inducing spore formation in a short time. In addition, several parameters in cryopreservation protocols, like the time of equilibration in the cryoprotectant (CPA), were evaluated.

### References

- [1] Sharma et al. *Frontiers in Marine Science* **2021**, 8, 63-66.
- [2] Di Dato et al. *Scientific Reports* **2019**, 9, 1-14.
- [3] Wang et al. *Marine Drugs* **2018**, 16, 33.
- [4] Stock et al. *Scientific Reports* **2018**, 8, 4279.
- [5] Pelusi et al. *Limnology and Oceanography Letters* **2020**, 5, 371-378.
- [6] Pelusi et al. *European Journal of Phycology* **2020**, 55, 1-10.

## Functionalized biochar as alternative fertilization methods for sustainable agriculture

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Over the past decade, research in environmental plant biotechnology has focused on the circular economy. Several valorization strategies have been proposed to explore food wastes and by-products as biomass suppliers to obtain different bio-based products. Among them, biochar is gaining an increasing attention among scientists as it may improve the overall performance of plants. Biochar is a stable solid obtained from thermochemical conversion of a such biomass in an oxygen-limited environment. Through a process of pyrolysis at high temperatures, waste matrices from both plant and animal source can be transformed into carbon-based mineral and aromatic compounds. However, the exclusive use of biochar may present some limitations, such as the lack of nutrient. In this context, using biochar enrichment techniques is possible to obtain biochar-based fertilizer, with great potential to improve soil fertility, to stabilize pH, to increase water-holding capacity, as well as enriching the soil with minerals with positive effects on the soil microbiota. In this work, we obtained a biochar from the waste of the licorice industry, specifically, from the woody and fibrous part of the roots resulting from the extraction process. SEM analyses were conducted to characterize its structure at the microscopic level. SEM observations showed that the biochar was highly porous with a filamentous structure. This characteristic allowed to obtain functionalized biochar with macronutrients such as nitrogen, phosphorus and potassium, to be used as sustainable alternative to common commercial fertilizers. The release kinetics of biochar in water showed that the nutrients loaded into the carbon matrix have a controlled and prolonged release over time, which makes it possible to avoid the leaching phenomena of commercial fertilizers that cause heavy environmental pollution. Functionalized biochar was used in in vivo experiments to grow *Solanum lycopersicum* plants. One month old plants were treated with different biochar/soil ratios, respectively 5, 15 and 25 percent of the total dry weight contained in a pot. The effects on both physiological and photosynthetic parameters were evaluated to test the effectiveness of different biochars as fertilizers.

## Winter season outdoor cultivation of an autochthonous *Chlorella*-strain in a pilot-scale prototype for urban wastewater treatment

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Anthropic activities lead to the production of wastewaters (WW), containing pollutants, mainly nitrogen (N) and phosphorous (P), or bacteria, like *Escherichia coli*, which can cause eutrophication or risks for human health, respectively. Thus, WWs must be depurated. In the city of Ferrara, WWs are treated at the Hera SpA wastewater treatment plant (WWTP), where traditional chemical-biological methods are employed. These WW treatments produce sludge that in turn requires further processing (thickening, anaerobic digestion, dewatering), due to its pollutants content. Unfortunately, such treatments still result in WWs rich in contaminants, so that they are recirculated in the WWTP. However, microalgae, well-known photosynthetic microorganisms, can use N and P contained in WWs, also those derived from sludge treatments [1, 2], and can be applied as bioremediation agents to minimize the recirculation of those WWs in the WWTPs, thus contributing to reduce management costs. Results from laboratory-scale experiments on microalgae-based WW treatments are generally promising. However, upscaling to outdoor conditions is still in early stages. For this study, a *Chlorella*-like strain isolated from the thickening WWs of Hera-Ferrara was used for phycoremediation tests in an 800-L outdoor prototype. The alga, already tested in a late-summer experiment, was used to set up winter-season trials to better understand how this native strain works in the geographical area of Ferrara under the harsh conditions typical of wintertime. Algae productivity, nutrients removal and *E. coli* load abatement were monitored. Results were set up by comparing two winter replicas realised in December and February. In December the algae did not grow, probably due to low temperatures and insufficient light exposure; nevertheless, N was reduced by 70%, while P remained unchanged. Interestingly, *E. coli* load decreased by 95% in only 3 days; this was linked to the production of exo-mucopolysaccharides, entrapping bacteria and causing the formation of aggregates that tended to settle. Conversely, in February, parallel to cell density increase, P was removed by 97% and N by 75%, being below law limits at the end of the trial, when also *E. coli* was totally abated. Both trials showed encouraging results highlighting how microalgae can be an alternative biological treatment for WW even in unfavourable outdoor winter season conditions.

### References

[1] Baldisserotto et al., 2020. *Plants*, 9, 1802

[2] Baldisserotto et al. 2023. *Plants*, 12, 1027

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## **The characterization of the rhizosphere in urban soils: an integrated view for non-disruptive methods supported by bioinformatics**

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Trees in urban forests are relevant both from visual and functional perspectives and they represent a green infrastructure delivering multiple services spanning from social to economic, health and ecological aspects. Despite these benefits, tree biology in urban areas has been poorly understood, especially in the optic of investigating the rhizosphere in which roots represent a part of a holobiont together with soil and microbiome. These interactions depend on plant morphological, physiological and biochemical characteristics, defined root functional traits, which affect the soil environment through a series of complex mechanisms and contribute to underground ecosystem services. Up to now, the studies of rhizosphere are still very limited by the methodological difficulties of being able to observe the roots *in situ* and *in itinere* during their development. There is a lack of consolidated and routine non-destructive techniques, especially in the case of perennial plants with extensive root systems such as wood species that populate complex and highly anthropized environments such as urban ones. Nowadays, plant rhizosphere is taking advantage of bioinformatics approaches supported by databases and by powerful algorithms able to infer knowledge from complex data or to apply modelling strategies to derive rhizosphere characterizations. These approaches are of particular relevance especially in urban areas where studies and sampling are limited by the context.

As part of the National Biodiversity Future Centre project goals, this work aims to use and integrate knowledge derived by the application of various bioinformatics and innovative non-destructive approaches to functionally characterize rhizosphere of different target species of tree plants in urban areas. In addition to deepening knowledge about rhizosphere in urban soils, we will derive practical and applicative guidelines to drive the management of urban forests or to be used in order to implement effective reforestation strategies in urban contexts.

## One actor, many roles: Mildew Locus O (MLO) at the crossroads of plant-microbe interactions

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Arbuscular Mycorrhiza (AM) symbiosis is an ancient and widespread plant-fungus mutualistic interaction. The establishment of the symbiosis is a multistep process, which is finely regulated by the host plant. Genes of the *Mildew Locus O (MLO)* family have been found to be among the most expressed ones in mycorrhizal roots, but their role in the establishment and functioning of AM symbiosis is still unknown. The MLO protein family is wide and is involved in different plant processes, such as defence, reproduction, and stimulus-driven root growth. After decades of research about their mysterious biochemical function, MLO proteins have recently been shown to act as Ca<sup>2+</sup>-permeable channels. In this work, we investigate the function of *MLO4* in different steps required for AM symbiosis in *Lotus japonicus*. By confocal microscopy we show that the localization of fluorescent reporters whose expression is driven by *MLO4* promoter and of GFP-tagged MLO4 protein largely overlap with AM fungal structures in mycorrhizal *L. japonicus* roots. We are currently evaluating whether MLO4 is involved in the activation in the host plant of Ca<sup>2+</sup>-mediated signalling pathways involved in the chemical communication with the AM fungus, by monitoring intracellular Ca<sup>2+</sup> changes induced by purified fungal molecules in *mlo4* mutant roots expressing specifically targeted aequorin chimeras. Moreover, phenotypic analyses of *L. japonicus* LORE1 insertional mutants suggest that MLO4 plays a broader role in root development in *L. japonicus*. The obtained data provide first hints about the function of MLO4 in the development and functioning of AM symbiosis in *L. japonicus*.

## **A safe and affordable protocol for plant DNA barcoding applicable in educational contexts**

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DNA barcoding is a technology that allows the genetic identification of species using specific sequence-conserved genomic regions that have enough evolutionary divergence to be univocally recognized.

It has been observed that in plants there are no single universal regions usable as barcode (unlike animals and fungi), but multiple loci are needed to ensure specificity. Among these, MatK and ITS2 are good candidates because of their presence in land plants and their capacity to differentiate plants to the species level.

Throughout the years, the protocols for DNA barcoding have been consolidated, both in nucleic acid extraction, in the selection of regions to amplify and in the optimization of the bioinformatic tools for sequence analyses. However, many of these protocols are not easily applicable in educational contexts, because of the use of harmful (like liquid nitrogen) or toxic (like organic solvents) components, the use of expensive instrumentation, and difficult and time-consuming protocols.

The goal of this project is the development and distribution of a relatively easy, harmless and feasible method for plant DNA barcoding, optimising the three main steps of the protocol: DNA extraction, marker region amplification and sequence comparison.

The final product will be an economically feasible protocol that high school/bachelor students can use, with a list of different kits and reagents that are safe and easy to manage. The protocol will be accompanied by multimedia material describing the principles of the DNA barcoding technique and video tutorials for the individual phases of the experimental procedure.

## The effect of a soil microbial synthetic community on *Lactuca sativa* phosphate homeostasis depends on plant genotype

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Phosphorous (P) is one of the most important plant growth-limiting nutrients. Despite the large abundance of P in the soil, less than 20% is present in an inorganic and thus available form for the plant. In order to face this limitation, phosphate-based fertilizers are widely used in agriculture and provided to crops. However, this P source mainly derives from phosphate rock, which is non-renewable and undergoing exhaustion. Given the ability of some soil bacteria to solubilize and mineralize the organic insoluble phosphorus and of some endosymbiotic fungi to dramatically extend the root surface able to absorb phosphate, microbial-based solutions have been pointed out as possible eco-friendly candidates for a more sustainable food production technology. The aim of this project is to study the effect of a microbial inoculum, made by two arbuscular mycorrhizal fungi and a *Bacillus simplex* bacteria, on a panel of 128 fully sequenced varieties of *Lactuca sativa* in a controlled condition of P starvation, being aware that plant genetic diversity plays an essential role in the responsiveness to microbial inoculi. The combination of physiological, metabolic and biomass parameters allowed us to reconstruct a complete picture of the response of the plants to the inoculum. Altogether about 10% of the analyzed lettuce genotypes showed a statistically significant effect on plant growth and/or plant phosphate concentration, while the whole panel demonstrated a range of phenotypic diversity in their responsiveness highlighting the pivotal role of genotype x environment interaction. The subset of *L. sativa* genotypes showing contrasting phenotypes will be further analysed for their photosynthetic pigments, free amino acids, total soluble sugars and starch content and a link to root microbial community will be investigated. Furthermore, all the phenotypic traits collected during the experiment will be used to run the genome wide association study (GWAS), in order to identify genetic loci associated with contrasting responses. To conclude, the use of microbial-based consortiums represents a step forward toward the development of more sustainable techniques in agriculture. With this study, we aim to add a new piece of knowledge on the genetic and physiological mechanisms underlying the establishment and the effects of beneficial interaction between plants and soil microorganisms.

## Study of the effects of compost on soil microbial community to improve soil biodiversity and plant health

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It has been shown that soils rich in microorganisms are more fertile, not only because of the ability of microorganisms to degrade organic matter, but also because of their positive biochemical activities and their 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 surface of the root, or the internal tissues of the root without harming the plant. For the plant, it is advantageous to host such microorganisms, and in fact, it actively participates in their recruitment, selecting species with mechanisms that are not yet fully understood. Soil microbial biodiversity is thus important for the establishment of rich bacterial communities associated with plant roots. However, soil biodiversity is threatened by intensive agricultural practices, such as abundant chemical fertilization, the use of pesticides, and deep soil tillage. Soil biofertilization, the improvement of bacterial biodiversity in the soil, due to its low environmental impact and its general effects of increasing plant health, is among the practices suggested by the EU to restore or enhance soil biodiversity, to improve the environmental sustainability of agricultural production (EU Sustainable Goals 2030). Among the possible bio-fertilizers, compost is standing, as a product of the circular economy. In fact, compost is mainly produced from urban food waste and plant residues, and it has long been used as an agricultural amendment because it is rich in organic matter and mineral nutrients. The aim of this research project, that is a collaboration between S.E.S.A spa and the Department of Biology at the University of Padova, is to investigate the effect of using compost on the soil microbial community, in order to verify if the microorganisms present in the compost can enrich the natural microbial biodiversity of the soil and interact with the plants to improve their growth and health.

## **Isolation and production of biologically active extracts from by-products of grape pomaces of *Vitis vinifera* L. Cabernet Sauvignon (origin of South Kazakhstan)**

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Grape pomace is a natural product rich in dietary fibers, polyphenols and anthocyanidins. By their chemical composition, secondary products from grape processing are valuable raw materials for obtaining a variety of new products. The quality and biological value of natural food products are determined by their chemical composition and a whole complex of integral organoleptic properties that depend on this composition. Natural anthocyanin dyes not only give color to vegetable raw materials, but also have a well-known physiological activity, in particular, coloring and antioxidant. Purified natural dyes have recently been increasingly used to improve the consumer properties of food products, in biologically active additives, in pharmaceutical preparations for the treatment and prevention of various diseases. At the same time, the composition of anthocyanins, even for the same variety of plant raw materials, is complex and variable; it depends on climatic conditions, on the maturity of berries, root crops, and the quality of agricultural work. Anthocyanins easily undergo a number of transformations depending on the conditions of extraction and analysis.

The originality and novelty of the isolation and production of biologically active extracts from by-products of local wineries in ecologically safe areas of southern Kazakhstan lies in the fact that natural environmental conditions: high temperatures and low humidity which contribute to the formation of biologically active substances with increased concentrations. Current research was aimed at the deep study of extracts from grape pomace rich - anthocyanins.

Red grape pomaces of *Vitis vinifera* L. Cabernet Sauvignon were extracted using a solvent-based (SE) method with concentrations of 70% (v/v) and solid/liquid ratios of 1:10, followed by incubation at three different temperatures of 30°C, 40°C, and 50°C for 1h, 2h, 3h, and 4h. All solvent extracts showed higher amounts of anthocyanin pigments. The maximum yield was obtained by using the optimal time of extraction (2 h at 50 C), with the highest total anthocyanin recovery obtained by means of 70% (v/v) ethanol.

## Phylogenetic and computational approaches to better understand diversity and potential of *Vigna unguiculata* (L.) Walp. Bowman-Birk protease Inhibitors

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*Vigna unguiculata* (L.) Walp., commonly named as cowpea, is a crop of rising interest in many research and agricultural fields. Cowpea originated and is nowadays very diffused in Sub-Saharan Africa, where it is cultivated by small and medium farmers. In the last few years, researchers are valorising this species because it is associated with a great nutritional profile, but also with the occurrence of many bioactive compounds. Among these Bowman-Birk protease inhibitors (BBIs) are a class of small proteins (about 70 amino acids) characterised by a conserved and resistant structure made possible by 14 cysteines that generate 7 disulphide bonds. BBIs also feature two loop domains that inhibit trypsin and chymotrypsin as physiological targets, exerting their primary defensive role against insect attack. BBIs though are demonstrated to be active against different cancer models and their activity is strictly related to their capacity to inhibit trypsin and chymotrypsin.

For this reason, we evaluated the natural genetic diversity of two genes encoding two BBI isoforms: trypsin-trypsin (BBI-TT) and trypsin-chymotrypsin (BBI-TC). Secondly, we applied phylogenetic and network techniques to understand haplotype relationships and selective pressures applied on the genes. Ultimately, computational approaches intervened to assess how the single mutations could affect the interaction energies with the physiological targets.

The genetic screening was performed on 182 accessions mainly from the African continent, producing 426 sequences for BBI-TT and BBI-TC genes. 24 and 29 haplotypes were found respectively for BBI-TT and BBI-TC genes, and the great majority of haplotypes was retrieved in wild accessions. Network analyses confirmed the previous result and visually reconstructed the possible relationships between haplotypes, but also the amino acid sequence and the mature protein sequence. Furthermore, selective analyses allowed us to identify sites that are actually under negative and positive selection. Negatively selected sites were more and more diffused, while positively selected sites were less but, in the mature protein, all near the interactive loops, suggesting the tendency to make interaction or stability more efficient. Finally, thanks to computational calculations, BBIs found by the exploration showed an interesting energy pattern highlighting, firstly, that wild genetic pool is still underestimated and, secondly, that some isoforms could be better interactors by exerting a more efficient defensive action, but also more capable in counteracting cancer development.

## Optimization of anthocyanins production in tobacco cells

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Plant polyphenols have a wide range of beneficial effects on human health and wellbeing [1]. Among them, the water-soluble anthocyanins have been associated with protection against cancer, cardiovascular, neurodegenerative and other chronic diseases. The growing demand of these molecules is fuelled also by their use as natural dyes in the food, cosmetic, pharmaceutical and textile industries [2]. In recent years breeding programs are trying to increase their contents [3], but the production of these molecules cannot rely only on agricultural products. Anthocyanins can indeed hamper plant growth and generally they are produced only in specialized cells, such as those in petals or epidermal cells exposed to stresses like UV light. The elucidation of the anthocyanin biosynthetic pathway and its regulation [3] allowed the generation of biotech plants [4] and cell cultures [2] that can accumulate high amounts of these molecules. In peach *PpMYB10.1* and *PpbHLH3* have been shown to be responsible for anthocyanins accumulation in the fruit during ripening [5] and their overexpression in tobacco induced anthocyanin pigmentation [6]. Starting from *PpMYB10.1/PpbHLH3* overexpressing plants, tobacco plant cell cultures have been established. After repeated selection of darker cells on solid medium the best clones were used to develop liquid suspension cultures. These cultures were scaled-up in a 25L pilot scale bioreactor. Pigment extraction followed by column chromatography resulted in the recovery of up to 20 grams/bioreactor of freeze-dried material, with a total polyphenol concentration of 400mg/g. The most abundant polyphenol in the extract was chlorogenic acid, accounting for about 30% of ethanol-soluble polyphenolic molecules. Anthocyanins were about 10% of the column-eluted molecules, of which the most abundant was cyanidin (cyanidin 3-rutinoside, 72.51%) followed by delphinidin (20.96%) and petunidin (6.52%). The total polyphenols that could be extracted from leaves of green-house grown tobacco plants of the same genotype were about 60-80 mg/g of freeze-dried leaves while anthocyanins were about 0,1 mg/g. On the contrary, pigment yield from tobacco freeze-dried cells was of 25 mg/g, resulting in a 250-fold increase. Gene expression analysis by qRT-PCR were performed on both plant and cell materials, to study the up regulation of key genes of the flavonoid biosynthetic pathway, such as *NtCHS*, *NtDFR* and *NtUFGT* as well as of the proanthocyanidin biosynthetic pathway such as *NtLAR*. The high transactivation activity of *PpMYB10* and *PpbHLH3* transcription factor, the adaptation and further selection of tobacco transgenic cells and the following purification steps allowed a small-scale production of valuable polyphenolic molecules from tobacco cells. Further scale up optimization will allow industrial production.

### References

- [1] Zhang P, Zhu H. *Molecules*. (2023) 28:866.
- [2] Appelhagen I, et al. *Metab Eng*. (2018) 48:218–32.
- [3] Chaves-Silva S et al. *Phytochemistry*. (2018) 153:11–27.
- [4] Butelli E et al. *Nat Biotechnol*. (2008) 26:1301–8.
- [5] Rahim MA, Busatto N, Trainotti L. *Planta*. (2014) 240:913–29.
- [6] Rahim MA, Resentini F, Dalla Vecchia F, Trainotti L. *Front Plant Sci*. (2019) 10:1143.