

SPS Summer School 2023



« Seeds as a keystone for the transition to agroecology » June 25th - July 1st, 2023 Versailles, France

Participant's guide

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Sponsors and partners









Institut Jean-Pierre Bourgin INRAE Centre Île-de-France Versailles-Saclay Route de St-Cyr (RD 10) 78000 Versailles

Hôtel

Hôtel des Roys 14, Avenue de Paris 78000 Versailles https://www.hotel-roys-versailles.com/

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Important information



Arrival of the participants - Sunday June 25

The meeting point is at the « Ferme Nature et Découvertes », Passage des Etangs Gobert, 78000 Versailles (see map on the page dedicated to the program on June 25).

Please be there at 3 PM. Don't be late!

Attention : During the Summer School, you will stay at the « Hôtel des Roys », 14 Avenue de Paris, 78000 Versailles. The hotel check-in starts at 2 PM. Thus, we advise you to check-in before coming to the « Ferme Nature et Découvertes ».

End of the Summer School - Saturday July 1st

The Summer School will end by the self-guided tour of the Versailles Castle. The visit should start around 10:30AM and after that you will be free to visit the castle at you own pace.

Bus trip from the hotel to the INRAE Center

The bus tickets are not covered by SPS. However, here is some information.

The bus stops closest to the hotel are indicated on the map on page 4. From there, you can take either of the buses 11, 40, 44 or 401, which will all take you to the INRAE stop.

To travel on these buses, you can buy a ticket directly from the driver (2 euros, prepare the exact change) **but as you are a large group, this solution is not recommended.**

You can also buy T+ tickets via the vending machines in the Paris or Versailles stations (https://www. ratp.fr/en/titres-et-tarifs/t-tickets). These tickets are valid for the bus network in the lle-de-France region. These tickets can also be bought and stored on you cell phone (if compatible) through the «Bonjour RATP» app (https://www.ratp.fr/en/achetez-vos-titres-de-transports-par-telephone).

Groups for the practical sessions

> Group 1: Characterization of plant metabolites (coordinated by François Perreau, Stéphanie Boutet and Massimiliano Corso)

> Group 2: Getting the most out of transcriptomes (coordinated by Etienne Delannoy)

> Group 3: Seed treatments and assessment of germination performance (coordinated by Frédéric Chauffour, Omaé Pozza, Corentin Moreau, Shuang Peng and Loïc Rajjou)

Group 1	Group 2	Group 3
Eleonora CAPPELLETTI	Maxime DUPONT	Aldo BORJAS
Kumsal Ecem COLPAN KARISAN	Maryam FOROUGH	Natalia Carolina Moraes EHRHARDT-BROCARDO
Andres HERNANDEZ PRIDYBAILO	Marion GAILLARD	Francesca MESSINA
Alexandra LESKOVA	Laura GONZALEZ-VALENZUELA	Harilala Notahina RASANDIMANANA
Valerio MATTEI	Andrea PAGANO	Carolina THOMAZ DOS SANTOS D'ALMEIDA
Alessandra RENELLA	Tania TRASANTE	Eleni VIKELI

Map of the INRAE Center



Transports in Paris area

https://www.iledefrance-mobilites.fr/en

https://www.ratp.fr/en/

Travel instructions > Versailles

DEPARTURE



!! Warning !! Make sure the train or RER you take stops at the station you need to get off at (stops are indicated on screens or light panels).

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	Sunday June 25	Monday June 26	Tuesday June 27	Wednesday June 28	Thursday June 29	Friday June 30	Saturday July 1st
8 AM					8 AM Bus to IPS2		
9 AM		8:30 AM Session « EU and			8:30M Tour of IPS2		
		international legislation »		8:30 AM Session « Seed	9:30 AM Bus to Campus Agro	8:30 AM Session « Seed tech »	« Hötel des Roys » - Check out
IN AIM		9:30 AM Coffee break	8:30 AM Practical session	defense mechanisms and seed-microbiomes »	9:45 AM Tour of Camous Addo	9:30 AM Coffee break	
11 AM <mark>-</mark>		10:45 AM Coffee break	10:45 AM Coffee break	10 AM Coffee break		10:45 AM Coffee break	10:30 AM - 1 PM
				11:30 AM Coffee break			Tour of the Palace of Versailles
1 PM		12 PM Working Groups				12 PM Working Groups Preparation for restitution	
		1 PM Lunch	1 PM Lunch	1 PM Lunch	1 PM Lunch	1 PM Lunch	1 PM End of the Summer School
2 PM -	« Hôtel des Roys » - Check in	2 PM Presentation and tour (greenhouses) of IJPB		¢ 		2 PM Preparation for the restitution of practical	
	3:15 PM		2 PM Practical session		central and specialized metabolites »	3:15 PM Coffee break	
4 PM	:	3 PM Working Groups	-	••• 2 PM Practical session ••• 4PM Coffee break	2:30 PM Coffee break	3:30 PM	
5 PM	SUCSEED, SPS and IJPB	4PM Coffee break			4:15 PM Coffee break	Restitution of the Practical sessions	
6 PM	Presentation of the 3 practical sessions' topics	•		0 0 0 0 0 0	6 PM Working Groups	and the Working groups	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
7 PM	Flash-talks	Md Oc.2	6:30 PM	6.30 DM	Preparation for restitution		
8 PM	7:30 PM Dinner Versailles	. Pétanque tournament		Social activity / Game Buffet Dinner	7 PM Dinner Saclay		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
9 PM		5			9:30 PM Bus back to the hotel		

Program

Sunday June 25

At 3 PM, please be at the « Ferme Nature et Découvertes », Passage des Etangs Gobert, 78000 Versailles (see map below). Don't be late!

Attention :

During the Summer School, you will stay the « Hôtel des Roys », 14 Avenue de Paris, 78000 Versailles The hotel check-in starts at 2 PM. Thus, we advise you to check-in before coming to the « Ferme Nature et Découvertes ».

3:15 PM – 7:30 PM: Welcome introduction Presentation of SUCSEED, SPS and IJPB Presentation of the the 3 practical session topics Flash-talks of participants' research (2 to 3 Powerpoint slides, 5 min max.)

7:30 PM: Dinner at the « Ferme Nature et Découvertes »



Monday June 26

8:30 AM - 9:30 AM: Session « EU and international legislation and trends in socioeconomics » (Building 7- 1st floor)

Flora Limache (IBMA France)

"Regulations relating to biocontrol" - Biocontrol is a major lever in the agro-ecological transition. Few biocontrol seed treatments are currently on the market. Technical, economic and regulatory obstacles may be encountered. Innovations are expected over the next few years, notably via SUCSEED. However, the regulations will have to be adapted further and in an appropriate manner before these innovative biocontrol solutions designed to protect seeds can be marketed.

9:30 AM - 9:45 AM: Coffee break (Building 7)

9:45 AM - 10:45 AM: Session « EU and international legislation and trends in socioeconomics » (Building 7-1st floor)

Youssef Saadé & Armelle Mazé (Science Action Développement - Activités Produits Territoires - SADAPT, Palaiseau, France) "Imagining the diversity of social and economic organizations of biocontrol and biostimulant solution in the seed sector (Part 1)" - This session will be organized in a participative and interactive mode to imagine the various possible futures of the social and economic organization of biocontrols and biostimulant solutions in the seed sector. Beyond the hegemonic and integrated model of large international firms, the aim of the session is to highlight, using different examples across the world, the diversity of possible alternative models, including innovative one's promoting more inclusive models for farmers and other stakeholders.

10:45 AM - 11 AM: Coffee break (Building 7)

11 AM - 12 PM: Session « EU and international legislationand trends in socioeconomics » (Building 7-1st floor)

Youssef Saadé & Armelle Mazé (Science Action Développement - Activités Produits Territoires - SADAPT, Palaiseau, France) "Imagining the diversity of social and economic organizations of biocontrol and biostimulant solution in the seed sector (Part 2)"

- 12 PM 1 PM: Working Groups (Building 14 Salle TD / TP)
- 1 PM 2 PM: Lunch at the INRAE cafeteria
- 2 PM 3 PM: Presentation of IJPB Tour (greenhouses)
- 3 PM 4 PM: Working Groups (Building 14 Salle TD / TP)
- 4 PM 4:15 PM: Coffee break (Building 14)

4:15 PM – 6:30 PM: Working Groups (Building 14 – Salle TD / TP)

6:30 PM - 9 PM: Buffet dinner and Pétanque tournament (veranda of the INRAE cafeteria)

Tuesday June 27

8:30 AM - 10:45 AM: Practical session

10:45 AM – 11 AM: Coffee break

11 AM – 1 PM: Practical session

1 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM - 4 PM: Practical session

4 PM – 4:15 PM: Coffee break

4:15 PM – 6:30 PM: Practical session

6:30 PM - 8 PM: Poster session (Greenhouse 71)

8 PM: End of the day

FOR THE PARTICIPANTS > Dinner is not included in the Summer School FOR THE INVITED SPEAKERS > 8:30 PM: Dinner at the restaurant « Le Limousin » (1, rue de Satory 78000 Versailles)

Wednesday June 28

8:30 AM - 10 AM: Session « Seed defense mechanisms and seed-microbiomes » (Building 7-1st floor)

Helen North (Jean-Pierre Bourgin Institute - IJPB, Versailles, France)

"Seeds and seed coat anatomy: variability in structure and form" - This introductory talk will review the basics of seed and seed coat anatomy, examine the incredible intra and interspecies structural diversity and highlight how this can contribute to defense strategies in order to protect the embryo and increase its chances of developing into a healthy seedling.

Round table - Chaired by Valérie Geffroy and Jérôme Verdier

"Role of the mother plant in seed defense to pathogens"

Jérôme Verdier (Institut de Recherche en Horticulture et Semences - IRHS, Angers, France)

"Seed defense mechanisms" - The importance of seed-borne pathogens in spreading diseases and initiating epidemics is often overlooked due to the current lack of knowledge on seed defense mechanisms and seed-pathogen interactions, although yield losses due to seed- and soil-borne pathogens have a major economic impact. Here, we will describe an up-to-date view of how seeds can defend themselves against these soil- and seed-borne pathogens.

10 AM - 10:10 AM: Break (Building 7)

10:10 AM - 11:30 AM: Session « Seed defense mechanisms and seed-microbiomes » (Building 7- 1st floor)

Marie Simonin (Institut de Recherche en Horticulture et Semences - IRHS, Angers, France)

"Seed microbiome characterization and engineering for future applications for plant health" - This talk will be an introduction of the current knowledge on seed microbiota across a large diversity of plants. We will explore next how seed-borne taxa can influence germination, plant growth and tolerance to stress. Finally, we will explore different strategies to harness seed microbiota potential using microbiome engineering.

Stéphane Compant (Austrian Institute of Technology, Tulin, Austria)

"The seed microbiota and plant growth and health" - Seed-borne microbes are known as pioneer colonizers of the emerging plant and thus represent the foundation of its microbiota build-up. Seed endophytes might play a minor role in the mature plant microbiota, but as primary plant colonizers they could impact germination, early plant vigor, survival, and have biocontrol properties, directly or indirectly. By dissecting the microbiota associated with seeds from various plants species we highlighted some assemblages of bacterial taxa, their niches, routes of colonization, beneficial properties and how they could stimulate plant growth or reduce pathogenic infection.

11:30 AM - 12 AM: Coffee break (Building 7)

12 AM - 1 PM: Session « Seed defense mechanisms and seed-microbiomes » (Building 7- 1st floor)

Brainstorming - Chaired by Bertrand Dubreucq

"Development of seed defense strategies"

1 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM - 4 PM: Practical session

4 PM – 4:15 PM: Coffee break

4:15 PM – 6:30 PM: Practical session

6:30 PM – 9 PM: Buffet dinner and Social activity - Game (veranda of the INRAE cafeteria)

Thursday June 29

8 AM: Bus trip to IPS2 from the « Hôtel des Roys »

8:30 AM - 9:30 AM: Tour of IPS2 (member of the SPS network)

The IPS2 aims at better understanding the molecular and genetic mechanisms controlling plant growth and their regulation by endogenous and exogenous signals of biotic and abiotic origins. Analysis of these mechanisms is conducted in an integrated manner at cellular, organ and whole plant levels. IPS2 applies multidisciplinary approaches (combining genomics, molecular and cellular biology, bioinformatics, biochemistry, genetics, physiology) and develops tools (including bioinformatics and modelling) required to provide more predictive knowledge and facilitate «translational» research between model species and crops.

9:30 AM: Bus trip to Campus Agro

9:45 AM - 10:45 AM: Tour of Campus Agro

10:45 AM – 1 PM: Working Groups

1 PM - 2 PM: Lunch at the restaurant « Le 19 » (19, Cours Gilbert Simondon, 91120 Palaiseau)

2 PM - 2:30 PM: Session « Seed central and specialized metabolites »

Massimiliano Corso (Jean-Pierre Bourgin Institute - IJPB, Versailles, France)

"Seed central and specialized metabolite diversity and plasticity" - Diversity and plasticity seed central (primary) and secondary metabolism will be introduced and discussed. A particular attention will be given to the genetics and environmental factors influencing metabolites synthesis and decoration.

2:30 PM – 2:45 PM: Coffee break

2:45 PM - 4:15 PM: Session « Seed central and specialized metabolites »

Barbara Ann Halkier (University of Copenhagen, Denmark)

"An UMAMIT-GTR transporter cascade controls glucosinolate seed loading in Arabidopsis" - We are using glucosinolate defense compounds in Arabidopsis as model system to dissect the transport route from biosynthetic source tissue to the seed of a specialized metabolite.

4:15 PM – 4:30 PM: Coffee break

4:30 PM - 5:30 PM: Session « Seed central and specialized metabolites »

Julia Zinsmeister (Jean-Pierre Bourgin Institute - IJPB, Versailles, France)

"Primary and specialized metabolites and their roles in seed quality" - While primary metabolites (PMs) are necessary to plants' survival, specialized metabolites (SMs) play key roles in plant adaptation to their environment. Seeds poduce a high variation of PMs and SMs in relation with seed quality plasticity. We will investigate how metabolites are analyzed in seeds, how their amount and diversity varies in relation with production environment and how their accumulation is related to nutritional and physiological qualities (dormancy, longevity, germination).

6 PM – 7 PM: Working Groups - Preparation for restitution

7:30 PM – 9 PM: Dinner at the restaurant « Brass & Co Paris-Saclay » (24-26 Mail Pierre Potier, Le Moulon - 91190 Gif-sur-Yvette)

9 PM: Bus trip back to the « Hôtel des Roys »

Friday June 30

8:30 AM - 9:30 AM: Session « Seed tech » (Building 14 - Salle TP)

Philippe Rousseau (Independent Consultant & Board Member - Seed and Ag Businesses, France)

"Breeding: a marketing view" - Every breeding programme needs to identify from the beginning what are the targeted products requested by the market. How to do that? How to structure a programme? And why working with marketing is essential for breeders in order to secure success in their programme?

9:30 AM - 9:45 AM: Coffee break (Building 14)

9:45 AM - 10:45 AM: Session « Seed tech » (Building 14 - Salle TP)

Loïc Rajjou (Jean-Pierre Bourgin Institute - IJPB, Versailles, France)

"A Journey into Seed Treatments: Historical Development, Mechanisms of Action, and Future Prospects" - Seed treatments play a pivotal role in modern agriculture by ensuring seed sanitary security and seed health, enhancing germination rates and seedling establishment, and improving overall crop performance. By exploring the historical evolution of seed treatments, mode of action of physical, chemical, and biological technologies, we gain insights into the innovative approaches and advancements that have revolutionized the seed industry.

10:45 AM – 11 AM: Coffee break (Building 14)

11 AM - 12 PM: Session « Seed tech » (Building 14 - Salle TP)

Camille Benetollo (CERIENCE, France)

"Seed Technology development using biosolutions" - With the aim of improving plant installation, we develop applied seed solutions using biosolutions for sustainable and profitable agriculture. We will begin by explaining the different seed treatment techniques, the different crops involved in this technology and the different industrial tools. We will end with the various challenges and levers for the development of biocontrol and biostimulation solutions.

12 PM – 1 PM: Working Groups - Preparation for restitution (Building 14 – Salle TD / TP)

1 PM - 2 PM: Lunch at the INRAE cafeteria

2 PM - 3:15 PM: Practical session - Preparation for restitution (Building 14 – Salle TD / TP)

3:15 PM - 3:30 PM: Coffee break

3:30 PM - 6:30 PM: Restitution of the Practical sessions and the Working Groups (Building 7- 1st floor)

6:30 PM: End of the day

FOR THE PARTICIPANTS > Dinner is not included in the Summer School FOR THE INVITED SPEAKERS > **8 PM**: Dinner at the restaurant « Le Bœuf à la mode », 4, rue au Pain 78000 Versailles

Saturday July 1st

Check-out at the "Hôtel des Roys". Leave your luggage at the hotel.

1030 AM : Tour of the gardens of the Palace of Versailles (self-guided tour)

Please be on time between 10:15AM and 10:30AM at the meeting point indicated on the map below.

Your ticket will allow you to enter the Palace between 10:30AM and 11AM and you will then be able to visit the Palace at your own pace.

You can download the application to benefit from audio tours: https://en.chateauversailles.fr/discover/resources/palace-versailles-application Make sure you bring earphones.

End of the Summer School







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Abstracts

Biopolymers as an alternative to promote the wheat (*Triticum aestivum*) tolerance to drought stress

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Nowadays, biomolecules are associated with a newly form of agricultural practices that could reduce the use of agrochemical products and ensure plant adaptation to environmental stress conditions such as drought and thermal stress. For example, polymers and oligomers are known to enhance plant adaptation in response to different stress. Some of these biopolymers can induce the production of Pathogenesis Related proteins (PR proteins), which stimulate plant defense mechanisms, forewarning against biotic stress and are involved in plant signalization of abiotic stress.

Our project aims to evaluate biopolymers and their potential effect on wheat tolerance under water and thermal stress at different scales. Firstly, molecular, and physiological analyses will be carried out under controlled conditions in order to determine the suitable applications and concentrations of bioactive molecules. Then, using semi-controlled platform dedicated to wheat phenotyping under different water regimes (Pheno3C, UMR GDEC-INRAE Crouël, France), experiments will be performed to validate the effect of the bioformulations under natural field conditions. Finally, some of the most promising bioformulated products will be tested in field using farmers' trials under the supervision of the Agricultural French Cooperative Oxyane. In parallel, total fungi biomass and seed storage proteins of treated plots will be estimated to determine the effect of the bioformulations on the rhizosphere composition and the migrations of nutrients during grain maturation.

Keywords: Biostimulation, Biopolymers, Wheat, Abiotic Stress.

Bio-formulates as a promising control strategy against different *Fusarium* pathogens with intentto reduce food mycotoxins contamination

Eleonora Cappelletti

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Durum wheat is a crucial food crop globally, and Italy is among the primary producers and consumers of this cereal. However, this crop is prone to attacks by microorganisms that cause various diseases, leading to both qualitative and quantitative losses. *Fusarium* Crown Rot (FCR) and *Fusarium* Foot Rot (FFR) are prevalent diseases worldwide, caused by several *Fusarium* species that affect different parts of the plant, leading to the production of harmful mycotoxins. Seed infection can cause reduced germination rates, slow emergence, and post-emergence blight, resulting in a sparser plant population. To combat soil-borne pathogens, new defense strategies are necessary, including the use of organic seed dressing techniques with endophytic bacteria and natural compounds such as essential oils. To identify potential biocontrol agents, the study conducted molecular analysis of the microbiome present in three durum wheat varieties, subsequently testing some of them *in vitro* and *in vivo*. The seed coating technique was also used to test the efficacy of antagonistic bacteria and several essential oils against *Fusarium* strains.

Effects of High Ambient Temperature on Plant Growth and Reproductive Development in Barley

Kumsal Ecem Çolpan Karişan^{1,2}, Maria von Korff Schmising^{1,2}

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The increase in the average ambient temperature threatens crop production worldwide. As one of the most important cereal crops, barley (Hordeum vulgare) is an important target to generate cultivars that are tolerant to high ambient temperatures. Recently, a natural mutation in PHOTOPERIOD RES-PONSE 1 (HvPPD-H1) prevalent in spring barley (ppd-H1) has been reported to cause delayed flowering and impaired reproductive growth under high ambient temperature, while the introgression lines carrying the wild type *Ppd-H1* prevalent in winter barley show accelerated flowering and reproductive growth. Furthermore, the spring genotypes carrying mutated *ppd-H1* showed reduced numbers of grains and florets, while the fertility and spike development were not significantly affected by high ambient temperature in introgression lines. These findings suggest that PPD-H1 is a promising target for the generation of barley cultivars with improved grain set under high ambient temperature. However, genetic control of flowering time as well as reproductive development in response to differences in ambient temperatures in barley remain unclear. *Ppd-H1* is controlled by phytochromes. A natural variation on HvPHYTOCHROME C (HvPHYC) has been shown to interact with Ppd-H1 to accelerate flowering under different photoperiods. However, little is known about their interaction with high ambient temperatures. Therefore, I study the molecular and genetic components involved in the regulation of reproductive development in barley. I analyse the genetic, hormone and metabolite networks in shoot apical meristem (SAM) which control the flowering time, spike development and floret fertility downstream of PPD-H1 and PHYC in response to high ambient temperatures.

Impact of the UPR pathway (Unfolded Protein Response) in wheat on the accumulation and quality of Seed Storage Protein under heat stress

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Wheat is one of the most important cereals consumed worldwide, with 37 million t/year produced. Environmental conditions, such as the temperature increase occurring due to global warming, negatively impact wheat yield by affecting physiological and molecular mechanisms in different cellular compartments. One of these mechanisms is the maturation of the protein, resulting in the accumulation of misfolded protein in the ER (endoplasmic reticulum), a cellular event called ERstress. In that case, during the grain filling, the accumulation and composition of seed storage proteins are modified, leading to a decrease in the grain quality. In response to an ER-stress, a signaling pathway called UPR (Unfolded Protein Response) is induced, aiming at regulating the concentration of unfolded proteins present in the ER.

This project aims to determine the role of the UPR in the accumulation and composition of the SSP in response to heat. In the beginning, the expression of the marker of the UPR induction (TabZIP60s) was characterized in the wheat seedlings under a specific chemical inducer (DTT). Then this pathway will be studied in the grain tissue under heat stress and the SSP composition determined in these seeds. Finally, thanks to the conditions determined before the impact of this pathway on the accumulation, quality and rheological properties will be determined using wheat mutants.

Keywords: Wheat, UPR, Abiotic stress, Grain

Callose in roots of soybean seedlings from seeds with different physiological quality

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Callose is a constitutive component in cell walls and deposited in response to stresses (abiotic and biotic). Considering physiological seed quality as an attribute that determines the rapid and uniform emergence of seedlings under a wide range of environmental conditions, this study aimed to verify the callose accumulation in seedling roots, of soybean cultivars with contrast for physiological seed guality, under water deficit during the germination process. Two cultivars (BS2606 IPRO and NA5909 RG) were used and the physiological seed quality was determined by germination and vigor (accelerated aging) tests. The water deficit simulation was performed by germination test with polyethylene glycol solution (PEG 6000) at the potential of -0.4 MPa, and the control (0.0 MPa) was conducted with distilled water. During different times of seed hydration after radicle protrusion (24, 48, 72 and 96 hours), 2 excised samples of 13 root tips (5 mm), from each treatment, were prepared for callose determination. The results of physiological seed quality showed that the cultivar BS2606 presented higher germination (97%) and vigor (89%) when compared to cultivar NA5909, with 91% and 40%, respectively. The callose content deposited in soybean root tissue cells ranged from 0.018 to 0.356 µg/root tip, and this allows the observation of contrasting patterns of callose accumulation between cultivars in water deficit during the germination process. The analysis of cultivar factor, in water deficit condition, indicated that the cultivar NA5909 showed a 588% increase in callose content when compared to BS2606, 0.172 and 0.025 µg/root tip, respectively, considering all hydration times. This is responsive to its greater susceptibility to stress in order to isolate plant tissue through the deposition of a physical barrier. The callose accumulation can be used as indicative of susceptibility to water stress between soybean cultivars.

Plant hormone-dependent import of proteins

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Photosynthesis that occurs inside the chloroplast provides food for most of the life on the Earth. Chloroplast biogenesis in germinating seeds is a fundamental process for creating active photosynthetic apparatus. During the biogenesis of chloroplasts from undeveloped proplastids, several thousand nuclearencoded proteins are imported from the cytosol into the organelle. These proteins are essential for chloroplast biogenesis in photosynthetic plants. These are encoded in the nucleus as pre-proteins with a targeting sequence and synthesized in the cytoplasm. Pre-proteins are imported through the action of two multi-subunit translocon complexes at the outer membrane (TOC) and inner membrane (TIC). Toc 75 protein acting as a guiding channel and the import receptors GTPases-TOC33 and TOC159, which preferentially recognize and facilitate the import of photosynthetic preproteins. Additional homologs of TOC159, TOC 120 and 132 with TOC34 are involved in preferentially facilitating import importing of nonphotosynthetic proteins. My PhD project analyse the effects of the plant hormone abscisic acid (ABA) on the composition of TOC proteins and accumulation of photosynthetic and non-photosynthetic preproteins, respectively, during early plant development. Preliminary results suggest that, although all composition of TOC proteins is profoundly altered under the conditions tested, they are regulated in different ways including post-translationally and translationally. The final goal of this research is to engineer crops that develop chloroplasts more readily under adverse environmental conditions.

Keywords: chloroplast biogenesis, TOCs, ABA.

Characterization of the Nrf4 homologue gene, a candidate gene to improve apomictic-like maize

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Apomictic-like maize is a maize that reproduces asexually by forming seeds without recombination of the maternal genome and without fertilization. The offspring possesses the same genetic material as the mother plant. While this maize does not exist in the wild, inspiration was taken from wild apomictic plants such as dandelion, hawkweed, or ferns, to mimic apomixis in crops. To achieve this goal, two key phases of the sexual reproduction pathway have to be shut down: meiosis and fertilization. On one hand, a gene implied in fertilization: Babyboom-like is quite well known to induce the development of an embryo without fertilization when ectopically expressed in the egg cell. On the other hand, the knockout of the gene Nrf4 in maize implies a mitotic Diplospory that replaces meiosis. In these mutants, the embryo sac is almost always unreduced and in about 10-30% of the cases, recombination does not occur. In order to obtain embryo sacs that contain the exact same genetic material as the mother plant, recombination must be completely inexistent. The knockout of the Nrf4 gene only is not sufficient to induce apomixis at a reasonable rate, therefore a combination with the knockout of another gene is necessary to produce apomictic maize at a higher frequency.

Is KERBEROS a ROS sensor?

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In plants as in animals, extracellular Reactive Oxygen Species (ROS) play important roles in regulating extracellular matrix properties, and in both autocrine and paracrine signaling at the plasma membrane. ROS signaling involves oxidative modification of intermediary molecules, often through thiol-based modification of target proteins. For example, H2O2 can oxidize cysteine (Cys) in target proteins to form structure-altering disulfide bridges (S-S). In plants the majority of cell wall ROS are produced by plasma-membrane localized Respiratory Burst Oxidase Homologues (RBOHs). ROS are thought to act as important signals in plants, but this activity remains poorly understood. Cys-rich peptides (CRPs) may be involved in ROS perception. A recently discovered CRP named KERBEROS (KRS) is necessary for the formation of a glycoprotein rich structure called the Embryo Sheath (ES), which is deposited on the surface of the developing embryo by the neighbouring endosperm tissues. Consistent with this, KRS is expressed specifically in endosperm cells adjacent to the embryo. Mutating KRS compromises ES formation and the ES-mediated embryo-endosperm separation, impeding embryo growth, and seedling emergence. The KRS Cys-Rich Domain (CRD) alone is sufficient to complement krs mutants, whilst mutating four conserved Cys residues in the CRD abolishes protein function. My proposed project is focused on understanding how ROS signaling in the plant cell wall (apoplast) contributes to seed development and viability. I will test the hypothesis that KRS mediates or modulates the transmission of ROS-dependent signals between seed compartments to ensure proper embryo development.

Maize seed microbiome changes due to cultivation system

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Microbes contained in the seeds may play crucial roles in the seedling adaptation to the challenges following germination. The environment in which the mother plants develop is known to shape the offspring microbiome; nevertheless, how the seed microbiome assembly, functional diversity definition, and its role in promoting growth under stressful conditions remain to be investigated in cereal crops, such as maize. This project aims to characterize the changes in the seed microbiome composition due to the cultivation system in maize and whether, this composition is associated with the seedlings' fitness upon nutritional stress. Fast-Flowering Mini-Maize seeds have been multiplied in hydroponics for three generations in a climate chamber (G3). Then, G3 seeds will be grown for an additional generation in unsterilized soil (G4) and hydroponic conditions. After seed collection and drying, G4 seeds from both cultivation systems and the original seeds (G0, stored at 4°C, 20-30 % RH for three years) will be sown in sterilized soil inside glass jars for two weeks. DNA from the seedlings' roots, rhizosphere, and dry seeds (n=5) will be extracted for sequencing and microbiome analysis. Also, macronutrient starvation experiments will be executed with juvenile plants from different seeds. We hypothesize that cultivation in hydroponic conditions for three generations reduced the seed microbial diversity, that it will be restored upon soil cultivation, and that some endophytic taxa contained in the seed will be transferred to the seedlings root and the rhizosphere to promote growth under nutritionally deficient environments.

Function of manganese transporter NRAMP2 in the seeds

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Manganese (Mn) deficiency is a nutritional disorder that causes substantial yield losses and decreases the quality of edible plant parts. Manganese is transported and redistributed in the plant cells by various transporters. Among them NRAMP2 is located on the trans-Golgi network and pumps Mn into the cytosol. In the plant leaves, NRAMP2-provided Mn cytosolic pools feeds photosystem II, vacuoles to store Mn, and potentially mitochondrial SODs (Alejandro et al. 2017).

NRAMP2 is also expressed in the developing seeds. To understand the function of NRAMP2 in the seeds we determined Mn distribution in this organ. Seed coat ablation, dissection of seeds and XRF analysis showed a misdistribution of Mn between seed coat and embryo. The mutant of *nramp2* is not affected in terms of seed production, and has no germination defects under control conditions, but struggles to germinate in accelerated aging conditions. Such phenotypes mimic mutants, that produce inadequate cell wall components in the seed coat. Indeed, the *nramp2* mutant has a minor defect in pectin accumulation in the mucilage without affecting seed coat ultrastructure. The transporter NRAMP1 teams up with NRAMP2 to provide sufficient Mn for the cytosol and is expressed in the seeds as well. The single and double mutants will be further characterised to understand the contribution of NRAMPs in Mn loading and function in the seeds.

Alejandro S. et al. (2017) Plant Cell, 29: 3068-84.

Streptomyces sp. DEF 39 applied to seed modulate the plant response to biotic and abiotic stresses: deciphering the mechanisms

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Streptomyces spp. belong to the Actinobacterial phylum and are gram-positive bacteria. They are soil-dwelling bacteria which can colonize inner root tissues as endophytes promoting plant growth and/or enhancing plant resistance against both biotic and abiotic stresses once the plant-streptomycete interaction occurs. *Streptomyces* DEF39 was seen to be able to colonize systemically wheat plants, after seed treatment, increasing plant resilience in mild-drought stress growing conditions. Moreover, it was observed to be able to be employed as a biocontrol agent (BCA) instead of chemicals, to counteract *Fusarium graminearum* in *Fusarium* head blight (FHB).

The aim of the project is to use advanced physiological non-destructive methods as well as advanced omics technologies to correlate plant phenotype with molecular data to decipher what is ongoing during the plant-bacteria-fungus tripartite interaction. Overall, the project wants to reach a full comprehension of the crosstalk within the tripartite system, after seed treatment. Moreover, the model aims to analyze the molecules involved within the tripartite system to elucidate which biosynthetic pathways are involved during the biocontrol process.

Assessing the germination performance of lettuce under changing environments

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High-quality seeds represent a priority in the context of sustainable agriculture to fight the dramatic effects of climate change. Seed priming is a workable option to boost the performance of crops in fragile ecosystems. This technology is based on controlled seed rehydration, carried out using water or other priming agents and the treatment is stopped before the occurrence of radicle protrusion. The benefits provided by priming include faster and synchronous germination in the field and optimized harvesting efficiency, even under unfavourable conditions. This allows to save water and reduce the input of fertilizers and pesticides, thus promoting good resource use. Lettuce (*Lactuca sativa* L.) is a crop that grows best with moderate-low temperatures. Exposure of lettuce seeds to high temperatures induces thermodormancy, causing uneven growth and reduced field production. Due to climate change, several lettuce-growing areas in Europe are facing temperatures higher than optimal, resulting into economic losses due to poor stand establishment. Companies are deeply committed to solve these critical issues, using the growing knowledge produced by basic research dedicated to identifying novel and efficient seed priming treatments to improve the germination of lettuce seeds in a range of environmental conditions.

Proteomic profiling of seed priming in *Medicago truncatula*: highlighting novel players in pre-germinative metabolism

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Seed priming represents a resource-effective approach for seed quality improvement. The rehydration-dehydration cycle at the basis of seed priming protocols activates pre-germinative metabolism, thus preparing seeds for a faster germination and an improved stress tolerance. This work investigates the changes occurring in the proteome of *Medicago truncatula* seeds during hydropriming, dry-back and post-priming imbibition. A label-free proteomic approach (LC-MS/MS) allowed the identification and quantitation of 2000-2200 protein entries for each pairwise comparison within the experimental system, highlighting the protein entries that were differentially accumulated with priming progression and/or in comparison with unprimed seeds during re-imbibition, particularly, those implicated in the antioxidant and genotoxic stress response. These results were correlated with the biometrical assessment of priming effectiveness, ROS accumulation, DNA damage profiles, and gene expression analyses. This integrative characterization of the response to priming in *M. truncatula* seeds can be used to identify novel molecular players underlying priming effectiveness in Fabaceae, with applicative perspectives for the optimization of priming protocols adapted to crop legumes.

Biostimulant and bioprotectant seed coating treatments to improve maize seedling growth and plant health

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The objective of this study is to develop an efficient seed coating delivery method to investigate the biostimulant and bioprotectant effect of the technology to improve maize seed germination and seedling growth. The physical properties and thickness of the seed treatment/coating will be also evaluated. Greenhouse and field testing of biostimulant and bioprotectant seed coating formulations will be carried on to maximize the maize seedling vigor. Application of different combination of vermicompost and rice husks ash as source of important plant nutrients, vermicompost juice as a liquid for mixing and soaking grains, chilli and ash serve as biological repellents, clay and soybean or maize flour applied as seed-coating binder in the coating blend will be performed to investigate their potential to enhance germination and germination rate, plant health and plant yield.

Characterization of three Molise Apennine autochthonous lentil (*Lens culinaris* MEDIK.) landraces

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Over the past century, improper human activities have seriously altered natural ecosystems, having a negative impact on the biodiversity of both wild and domesticated plants. In particular, agro-biodiversity and local varieties, characterized by high genetic variability, strong adaptation to environmental stress conditions, distinctive taste, and high nutritional value, are severely threatened by extinction, primarily due to their replacement by commercial varieties. This study aimed to characterize three autochthonous lentil landraces from different villages in the Molise Apennine—Agnone, Capracotta, and Rionero Sannitico-in comparison to other autochthonous populations-one from Umbria (Castelluccio di Norcia, IGP), one from Lazio (Rascino), and one commercial variety (Turca Rossa)-with a multi-integrating approach (morphological, genetic, and metabolomic analysis) to support their conservation. In the first phase, 14 IBPGR morphological descriptors and 8 ISSR molecular markers were used to assess diversity, genetic variability, and phylogenetic relationships among populations. The Principal Component Analysis (PCA) and Clustering Analysis (CA) of morphological descriptors showed that the lentils from Agnone and Castelluccio di Norcia are distinct from all other populations. However, the same analysis (PCA and CA) conducted on the genetic profiles divided populations into two main groups: one including the three autochthonous populations of the Molise region, and another formed by the other three. Further genetic investigation, coupled with metabolomic analysis and in vitro testing of bioactive compounds, will be used to define relationships and peculiar traits of landraces to valorize them from a nutraceutical and health point of view.

Temporal study of phenolic compounds synthesis and protein digestibility in sorghum grains: processing, genotype, and crop impact

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Sorghum is the 5th most produced cereal worldwide. It has agronomic and environmental advantages and is completely safe for coeliac and gluten-sensitive people. Kafirins, sorghum storage proteins, accumulate during grain. They are more hydrophobic and resistant to digestibility by the gastrointestinal proteases when compared with their counterparts in maize and wheat; factors that turned sorghum for animal feed. Currently, its use in human food has been boosted to meet the nutritional needs of current population, but also due to its phenolic compounds (PC) diversity. Although sorghum PC have great bioactive potential, they can complex with kafirins and further reduce its digestibility; however, PC/kafirins synthesis and their interaction are not yet fully known and may vary with genotype, crop and processing. Therefore, this study aims to analyze how genotype and crop influence the synthesis of PC and kafirins during sorghum grains growth (7-40 days after flowering); and its bioaccessibility by *in vitro* digestion after processing (germination, decortication, and cooking). Sensitive techniques such as proteomics and metabolomics were applied to comprehensively characterize sorghum. This work can bring new insights on kafirin assembly in relationship with protein digestibility and contribute to open access PC databases, expanding sorghum consumption and valuing this cereal.

The seed endophytic microbiome to the rescue of maize landraces in Uruguay: conservation of the native microbial diversity that promotes plant growth

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In Uruguay, corn (*Zea mays.L*) is one of our most important socio-economic commodities. Most of the seeds are bought from Argentina, but there are varieties which belong to local landraces that have been conserved *in-situ* – on farm, by farmers in their own establishments, as well as *ex-situ* in germplasm banks since the 1980s. The conservation of local seeds which are adapted to the biotic and abiotic conditions of the territory is necessary to ensure sustainable production and food security for its inhabitants, since it allows greater resilience against environmental changes (pests, climatic conditions, etc.). Some of these varieties have been lost due to genetic contamination with transgenic events, which results in a loss of biodiversity within the national germplasm and its associated ecosystemic functions.

Plants coexist and evolve with microorganisms found both in their environment (rhizosphere, aerial) and within their tissues (endophytes), so it is of special interest to study this co-evolution to achieve sustainable production. The endophytic microorganisms of seeds are particularly relevant for the genetic conservation of crops, since they are pioneer colonizers of the emerging plant. Many possess plant growth promotion (PGP) capabilities such as nitrogen fixation, phosphorus mineralization, and control of phytopathogens, among others. This study aims to be a contribution to the knowledge about the microbial ecology associated with local maize landraces, through the characterization of its endophytic's seed microbiota, and thus promoting the use of native PGP microorganisms to achieve sustainable production of the crop and conservation of national landraces.

Keywords: Microbiome, Seed endophytes, Maize landraces

Preservation of *Pisum* collection and phenotypic characterization: traits and nutrients for seed quality and conservation

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The Germplasm Resources Unit in John Innes Centre is home to the JI broad based Pisum Collection. The Collection comprises of >4000 accessions that include wide range of wild and semi-cultivated material in addition to landraces and modern cultivars from many regions around the world. The collection also includes near-isogenic series for several genes as well as sets of host differentials for all major pea diseases and it incorporates the international collection of mutation stocks on behalf of the Pisum Genetics Association. Recently acquired genotyped mapping populations and induced diversity opened new avenues for *Pisum* diversity research. The collection was genotyped using 42 highly informative molecular markers which enabled us to select a core collection of ~700 lines which were, through international collaboration with the Agricultural Genomics Institute at Shenzhen- re-sequenced to generate high resolution *Pisum* genomic resource. In the last three years, I have been phenotyping the diversity to support association genetics and gene discovery through GWAS. My focus is on nutritional value such as protein and mineral content. I will present the broad phenotypic diversity of the collection and focus specifically on seed morphology and nutritional value. I will show how constructing a marker-based core-collection captures higher genetic diversity for any n accessions and enhances phenotypic diversity, which can be used to construct pre-breeding germplasm panels and to conduct more cost-effective genetic research.

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