

**IPPS 2022 Conference Book**

**7<sup>th</sup> International  
Plant Phenotyping Symposium**

**'Plant Phenotyping for a  
Sustainable Future'**

**Wageningen, the Netherlands  
September 26-30, 2022**

**[www.ipps7.org](http://www.ipps7.org)**

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The International Plant Phenotyping Symposium is a conference jointly organized by:

The International Plant Phenotyping Network (IPPN) e. V.  
Wageningen University & Research (WUR)  
The Netherlands Plant Eco-phenotyping Centre (NPEC)

Welcome

## Welcome

### **Plant phenotyping for a sustainable future**

Food security, managing population growth and improving crop yields in the face of climate change are some of the greatest challenges facing our global society. We will need to produce food for 9.7 billion people in a sustainable way by 2050, whilst transitioning from a fossil-based economy towards a bioeconomy to mitigate the effects of global climate change. This will require a fast increase in global crop productivity to produce enough plant biomass to achieve both food and nutrition security, as well as to meet the demands of a future bioeconomy.

Crop production must be increased whilst maintaining crop quality. Society will require crops that combine sustainability, efficient use of scarce resources, e.g., water and mineral fertilizer, and are suitable for cultivation schemes and practices that preserve the Earth's biodiversity. Current intensive cropping systems will need to become more sustainable, and more crop varieties must also meet the needs of the agroecology, organic and urban/vertical farming sectors. "Future-proofed" crops must have good yield stability with a high resilience to adverse climate, volatile weather conditions and biotic stresses.

To meet these aspirations, both current and new crop plants need to be optimized and adapted which will require an ultimate effort both from the plant sciences as from the private sector. And in this process, the availability of versatile, high-throughput plant phenotyping methodology and infrastructure will be an absolute requirement for success.

For this reason, we are very excited that over 400 scientists and members of the private sector have come to Wageningen to the *7<sup>th</sup> International Plant Phenotyping Symposium, IPPS2022*, to present and discuss their latest results and developments in the field of plant phenotyping and to advance the field further towards a sustainable future.

As a community, we are together again for the first time in a couple of years, and we have never been with so many experts at one single location. This offers a great opportunity for all of you to interact, launch ideas, make friends, and explore new collaborations!

We wish you all a very inspiring and fruitful symposium,

The Organizing Committee,

Rick van de Zedde, Philippe von Gillhausen, Judith van Veen-Borsboom and René Klein Lankhorst

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We would like to thank the sponsors of our conference book (in alphabetical order):

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WPS



## Local Organizing Committee



Rick van de Zedde  
Wageningen University & Research, The Netherlands



René Klein Lankhorst  
Wageningen University & Research, The Netherlands



Judith van Veen-Borsboom  
Wageningen University & Research, The Netherlands



Philipp van Gillhaussen  
IPPN, Germany

## Local Scientific Committee

All members of the local scientific committee are affiliated to Wageningen University & Research or to Utrecht University.

Fred van Eeuwijk (Mathematical and Statistical Methods)

Leo Marcelis (Horticulture & Product Physiology)

Luisa Trindade (Bioresources Breeding and Genetics)

Christa Testerink (Plant Physiology)

Paul Struik (Modelling)

Eldert van Henten (Farm Technology)

Corné Pieterse (Plant-Microbe Interactions)

George Kowalchuk (Ecology and Biodiversity)

Mark Aarts (Genetics)

Jeremy Harbinson (Photosynthesis)

Rick van de Zedde (Phenomics & Automation)

Elias Kaiser (Plant Phenotyping)

Gerrit Kootstra (Deep Learning)

Rumyana Karlova (Root phenotyping)

## International Scientific Committee

Stefan Gerth, Fraunhofer (IIS), Germany

Robert Koller, Forschungszentrum Juelich (IBG-2), Germany

Mark Müller Linow, IBG-2, Forschungszentrum Jülich, Germany

David Rousseau, University of Angers, France

Thomas Altmann, IPK Gatersleben, Germany

April Agee Carroll, Purdue Univ., USA

Maxime Bombrun, Scion, New Zealand

Lin Cao, Nanjing Forestry University, China

David Pont, Scion, New Zealand

Tony Pridmore, University of Nottingham, United Kingdom

Hanno Scharr, IAS-8, Forschungszentrum Jülich, Germany

Scott Chapman, University of Queensland, Australia

Michelle Watt, University of Melbourne, Australia

Malcolm Bennett, University of Nottingham, United Kingdom

Kioumars Ghamkhar, AGResearch, New Zealand

Heiner Kuhlmann, University Bonn, Germany

Gregoire Hummel, Phenospex b.v., Netherlands

## General Information

### About Wageningen

Wageningen is a city and municipality in the Dutch province of Gelderland, region Gelderse Vallei. The city has ca. 40.000 inhabitants, many thousands of whom are students.

In 1876 the National Agricultural School was founded in Wageningen. In 1918 this became the Agricultural College, in 1986 the Agricultural University and in 2016 Wageningen University & Research (WUR). This was the beginning of a special development from a small, fortified town into a special university town: the City of Life Sciences.

Wageningen is also 'The city of Liberation'. At the end of the Second World War, the Allies led by General Foulkes and the German occupier negotiated the capitulation in Wageningen. The talks started on 4 May 1945 and continued on 5 May in Hotel De Wereld in Wageningen. The signed document, which has become known as the Dutch Capitulation Act, can be regarded as an elaboration of the general capitulation by the German armed forces for Northwestern Europe on 4 May. The deed itself, present in the Municipal Archives of Wageningen, is dated Wageningen, May 5, 1945. The celebration of Liberation Day in the Netherlands on May 5 is based on this. The capitulation is still commemorated annually in Wageningen.

Wageningen is a small city surrounded by nature. From the center, with the atmospheric Torckpark, you can easily walk to the beautiful botanical gardens with a unique view. When you leave the city, you will fall immediately in love with the landscape. You can really go in all directions: through the woods and moraines on the Veluwe and the Utrechtse Heuvelrug, along the river Rhine with its rugged floodplains or to the Betuwe with its fruit trees for a blossom tour.

In the city center you can find many different establishments; from small coffee bar to luxury restaurants, from a quick bite to a local beer on a terrace.



## About Wageningen University & Research

'To explore the potential of nature to improve the quality of life'

This is the mission of Wageningen University & Research (WUR) which is a collaboration between Wageningen University and the Wageningen Research foundation.

Over 7,200 employees, 13,200 students and over 150.000 participants to WUR's Life Long Learning from more than hundred countries work everywhere around the world in the domain of healthy food and living environment for governments and the business community-at-large.

The strength of Wageningen University & Research lies in its ability to join the forces of specialised research institutes and the university. It also lies in the combined efforts of the various fields of natural and social sciences. This union of expertise leads to scientific breakthroughs that can quickly be put into practice and be incorporated into education. Collaboration with other parties such as government, business and NGOs is indispensable.

The scientific quality of Wageningen University & Research is affirmed by the prominent position we occupy yearly in international rankings and citation indexes.

## Wageningen Campus

Wageningen Campus is a place for exchanges and connections between knowledge organisations, the business sector and start-ups. On Wageningen Campus we understand that sharing knowledge is just as important as developing knowledge, which is why the campus offers numerous opportunities for meetings, collaboration, inspiration and reflection.

## Visiting the campus

Wageningen Campus is centrally located in the Netherlands, between Utrecht, Arnhem and Nijmegen. It is easily accessible from both the Randstad with Schiphol Airport and from the Ruhr region in Germany with its international airport in Düsseldorf. The campus has ample parking, usually only a few minutes' walk from your final destination.



## **Netherlands Plant Eco-phenotyping Centre (NPEC)**

Our NPEC facilities offer high-throughput and high-resolution data from plants both above and below ground. Automatic phenotyping will allow for a dramatic increase in the speed of plant breeding, allowing for a very short time to market for novel crop varieties.

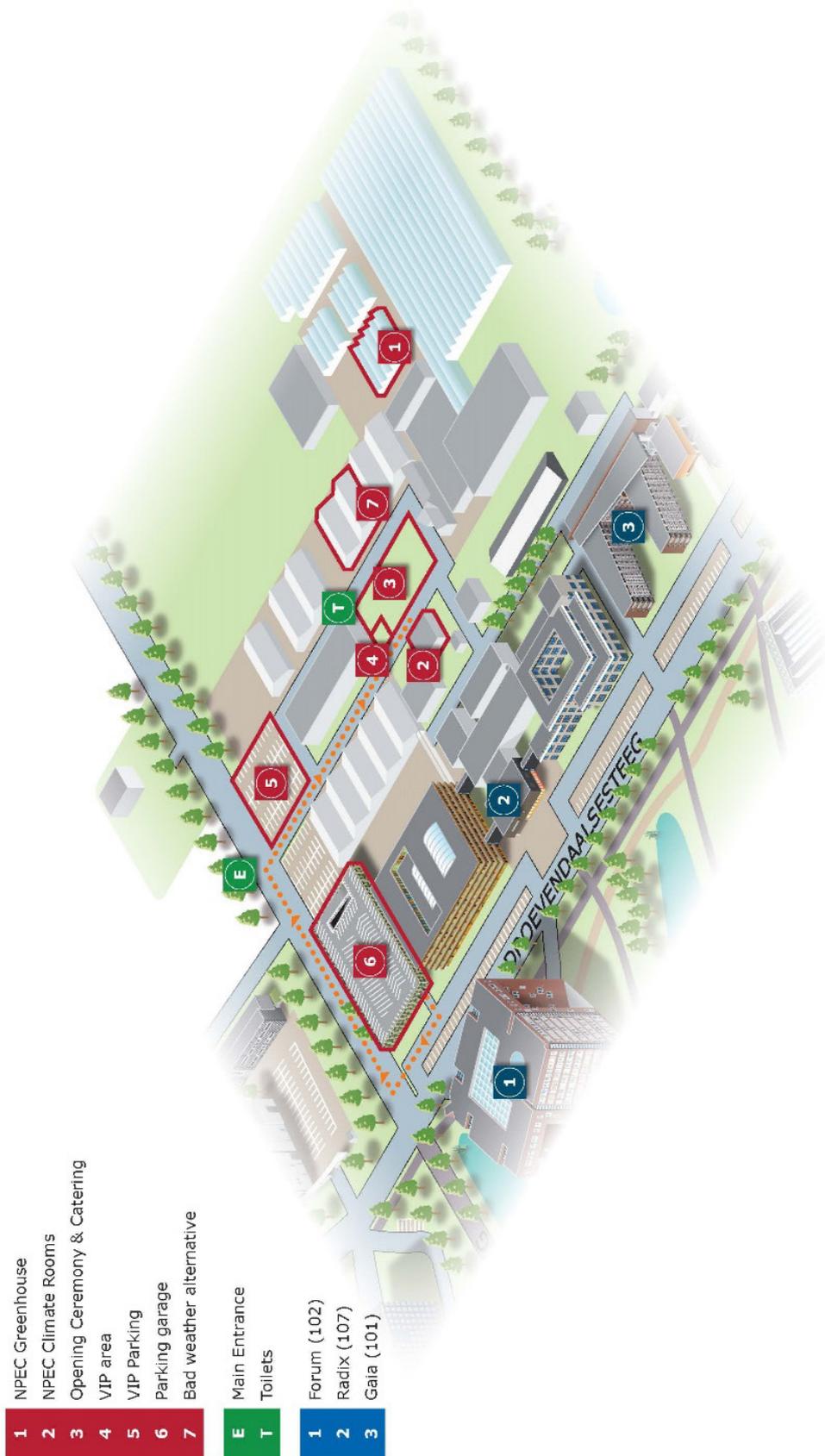
With the NPEC facilities we make accurate, high-throughput studies of plant performance possible in relation to relevant biotic (microbiome interactions, competition, disease) and abiotic (light quantity and quality, nutrients, temperature, moisture, soil pH and atmospheric CO<sub>2</sub> level) factors across a range of scales.

NPEC is a joint initiative of Wageningen University & Research and Utrecht University. This integrated, national research facility is housed by Wageningen University & Research and Utrecht University and is co-funded by The Netherlands Organisation for Scientific Research (NWO).





## Site Map NPEC opening area



## Venue Information IPPS 2022

The IPPS 2022 Conference takes place in Wageningen, the Netherlands. The conference location is the Hotel WICC (Wageningen International Conference Centre).



Hotel WICC is a family hotel under current management since December 2017. The hotel exists since 1972 and major renovations took place since the takeover. All innovations root in the fact that the WICC dares to change and inspire guests every day by creating a 'home feeling'.

With 9 boardrooms, 15 halls and 2 large conference rooms, the WICC has spacious capacity for the IPPS conference.

Due to the central location in the middle of the Netherlands, on the edge of the centre of Wageningen, it is very easily accessible for all attendants.

There is free use of the open WiFi network.

Address:

Lawickse Allee 9, 6701 AN Wageningen

+31 (0) 317 490 133

[www.wicc.nl](http://www.wicc.nl)

The hotel has three restaurants. Please check the website for availability and opening hours.

## **How to get to the WICC**

- From Ede-Wageningen train station you can take a bus to Wageningen bus station. This will take you approx. 20 minutes. From here it is a 5 minute walk to the WICC.
- From Arnhem Centraal train station you can take a bus to Wageningen bus station. This will take you approx. 40 minutes. From here it is a 5 minute walk to the WICC.
- From Hotel Reehorst in Ede you can take the bus (which stops in front of the hotel) to Wageningen bus station. This will take you approx. 20 minutes. From here it is a 5 minute walk to the WICC.
- From Hotel de Wageningsche Berg you can walk the 2,5 km to the WICC. On your way you will pass beautiful Botanical Gardens, Arboreta De Dreijen and Hinkelooors and the Sculpture Gallery.  
The alternative is to go by bus. Walk for 5 minutes to the bus stop 'Wageningsche Berg'. Take the bus to Wageningen bus station, this will take you approx. 10 minutes. From here it is a 5 minute walk to the WICC.
- From Hotel De Wereld it is only an 8 minute walk through the city centre of Wageningen.

It is also possible to book a taxi via the hotel reception.

## Hotel Information

We have pre-selected four hotels in the vicinity of the conference venue for our participants.

### *Hotel WICC (conference venue)*

See venue information

### *Fletcher Hotel De Wageningsche Berg*

This hotel is buried in the centre of the woods between the Utrechtse Heuvelrug and the Lower Rhine. It is located 2,8 km from the conference venue which can be reached by taxi, bike or even by foot. This contemporary 4-star hotel offers an à la carte restaurant, a brasserie, a panoramic terrace with stunning views and a gym.

The Fletcher Hotel De Wageningsche Berg is located on the edge of National Park De Hoge Veluwe and close to the beautiful Botanical Gardens and Arboretum at The Arboretum Belmonte Dreijen.

### *Hotel De Reehorst*

ReeHorst in Ede is centrally located in the Netherlands and at the heart of the stunning Veluwe region, an area of breathtaking natural beauty.

This hotel is located 8,6 km from the conference venue which can be reached by taxi or public transport. For our sportive guests even by bike. This 4-star hotel offers Live Cooking Restaurant Valentino, Brasserie and Terrace.

The hotel is easy accessible from the motorway (A12 and A30/5 min) and by train (Ede-Wageningen station/3 min). With 700 own parking spaces out front.

Address:

Bennekomseweg 24  
6717 LM EDE  
+31 (0)318-750300

### *Hotel De Wereld (Keynote speakers)*

Boutique Hotel de Wereld is perhaps the most famous 4-star hotel in the Netherlands renown for the peace negotiations of 1945. The characteristic, monumental hotel is located in the center of the city of Wageningen on the southern edge of the Veluwe. The hotel is an attractive and contemporary hotel with a restaurant, lounge bar and terrace. Hotel de Wereld is the hotel where freedom is our guest!

Address:

5 Mei Plein 1  
6703 CD Wageningen  
+31 (0)317 - 460 444  
[www.hoteldewereld.nl](http://www.hoteldewereld.nl)

## Opening Reception

Monday September 26, 2022

The Opening Reception will take place at the NPEC facilities at Wageningen Campus (see Site Map NPEC opening area).

The IPPS2022 Opening Reception is part of the Official Opening of NPEC which will commence earlier this day. You will be given the opportunity to visit our brand new plant phenotyping facilities. Also, you can meet and greet our partners from industry who helped us realize NPEC.

At the opening reception we will serve drinks and snacks, as well as a buffet-style dinner. If weather allows, the opening reception will be an outdoor event, so you are advised to dress to the occasion. In case of rain, we will have an indoor alternative.

Busses will leave in front of the WICC at 18.15

Busses will return at 22.00



## **Conference Dinner**

Thursday September 29, 2022

The IPPS 2022 Conference Dinner will be held at Burgers Zoo in Arnhem. This world-famous zoo was founded already in 1918.

We will start our evening with a tour along the several eco-displays of Burgers Zoo. Unlike traditional zoos, Burgers' Zoo has eco-displays, where large scale natural habitats are recreated in which animals, nature and visitor roam together. We will step inside and experience the humidity, dense vegetation, smells and sounds of a real jungle in the one and a half hectares of tropical rainforest (the Bush). We will continue our journey through the coral reef in the Indian Pacific Ocean (the Ocean) and the largest indoor mangrove in the world, inspired by the nature area in Belize. In the mangrove, you can discover butterflies, manatees and many different species of crabs.

We will continue this evening with a wonderful dinner followed by music and dancing.

Buses will leave in front of the WICC at 5.15 PM

Bussed will return to Wageningen at 23.00 PM



## Excursions

All excursions will take place on Friday, September 30, 2022

The buses will depart in front of the WICC at 12.30 pm. Lunchboxes will be provided

As part of the side program of IPPS2022, we offer our attendees the chance to experience Dutch culture as well as a taste of the world renowned Netherlands expertise in plant cultivation & production.

### EXCURSION 1: Dutch Open Air Museum

The Dutch Open Air Museum in Arnhem captures the history of Dutch everyday lives. In authentic buildings, objects and true stories they take you on a journey through time. From two centuries ago to very recently. The people of the museum seem to have stepped out of the past. Proud of their heritage, they will inspire you with their stories and are curious about yours.

#### *Authentic buildings from across the Netherlands*

The Dutch Open Air Museum covers an area of around 44 hectares and is full of all kinds of authentic, unusual buildings. Each of these has its own fascinating history and an interesting story to tell. In the museum park you will find around 100 historic buildings, farmhouses and mills. Not only can these be viewed from the outside, but you can also step inside for a look around! This brings you closer to history and gives you the chance to experience the past again.



#### *The Canon exhibition*

Based around 50 windows to the past, the Canon of Dutch History leads you through the most important events, people and objects that have helped shape the Netherlands we live in today. Spectacular 'film sets', flowing one into another, tell stories from the Canon, spread across 10 successive periods of history. In the exhibition you will come face to face with historic figures from each era. You will get to know them and their world through games, animations and films. And, of course, through objects – evidence of their stories that has survived the passage of time. In the film room you can learn about the origins of the Netherlands and its population, climate and geography, while, in the final room, there is a wealth of information about the entire Canon to explore on an 18-metre-wide touchscreen.

### *Guided Tour*

There is much to see and do in the Open Air Museum! It is almost impossible to see and experience everything. That is why the guided tour is a godsend. An inspired guide will take you back in time and tell stories that tie in with the highlights of the museum.

## **Program**

### **Time**

<b>12.30</b> pm	Departure from WICC Wageningen
<b>1:00</b> pm – <b>1:30</b> pm	Arrival Service point Open Air Museum Arnhem
<b>1:30</b> pm – <b>3:00</b> pm	Guided Tour English
<b>3:00</b> pm – <b>5:00</b> pm	Museum visit on your own
<b>5:15</b> pm – <b>5:30</b> pm	Departure to Wageningen
<b>6:00</b> pm	Arrival WICC Wageningen

## **EXCURSION 2: Hoge Veluwe National Park and Kröller-Müller Museum**

At the beginning of the last century, in 1909, the wealthy couple Kröller-Müller bought a large piece of land in the Veluwe. The family, who lived in the west of the country, spent their holidays here. This former estate and private hunting ground is the current 'National Park De Hoge Veluwe'.

### *The Park*

The park covers 5,500 hectares of woodland, heath, grasslands and shifting sands, and is the natural habitat for deer, moeflon, wolf and wild boar. On foot or on one of the free White Bicycles, you are free to roam around in nature. The free White Bicycles (1,800 in total) are stationed at the three entrances to the park, at the visitors' centre and at the museum.

In the visitors' centre you can learn more about the nature, landscape, animals, culture and history of The Hoge Veluwe. Nature films are shown in the auditorium, and in the Museonder you get a surprising look at everything that lives and has lived under the earth surface!

At the Kröller-Müller Museum, you will find the world's finest Vincent van Gogh collection and enjoy masterpieces by modern masters such as Claude Monet, Georges Seurat, Pablo Picasso and Piet Mondriaan. The museum is a treasure trove of De Stijl and futurism, and will surprise you with presentations of contemporary artists. Jachthuis Sint Hubertus is the former country house of Mr and Mrs Kröller-Müller. This 'total artwork' by architect H.P. Berlage is one of the Netherlands' most important monuments.

In the sculpture garden, you will stroll past works by Barbara Hepworth, Jean Dubuffet, Marta Pan and others.



## Program

### Time

<b>12:30</b> pm	Departure from WICC Wageningen
<b>1:30</b> pm	Arrival Kröller-Müller Museum
<b>1:45</b> pm – <b>2:45</b> pm	Guided Tour English
<b>2:45</b> pm – <b>5:00</b> pm	Visit Hoge Veluwe National Park on your own
<b>5:15</b> pm	Departure to Wageningen
<b>6:15</b> pm	Arrival WICC Wageningen

## EXCURSION 3: Ter Laak Orchids & Tomatoworld

This excursion consists of two visits to horticulture companies.

### *Ter Laak Orchids*

Ter Laak Orchids was founded in 1954 and is currently run by the brothers Eduard and Richard ter Laak. In the two nurseries in Wateringen, they grow around 6 million Phalaenopsis plants annually for garden centres, florists and retailers throughout Europe. At the moment at any rate; once the new Daylight Greenhouse is ready, they can produce 8 million plants per year on a total surface area of 17.5 ha. This greenhouse showcases one of most advanced and automated greenhouse productions system of the Netherlands.

Ter Laak Orchids wants to make a positive contribution to a sustainable world and minimise the environmental impact. They invest in sustainable solutions that help reduce energy consumption and CO<sub>2</sub> emissions and stop wasting water. The most recent developments are the building of a energy-efficient Daylight Greenhouse and underground water storage. Ter Laak makes optimal use of the surface area by having a second cultivation layer, uses LED lighting and grows as organically as possible.

During the excursion you will get a guided tour through the technical and production area, followed by a tour through the inspiration center.

### *Tomatoworld*

TOMATOWORLD is an information and education centre in Westland that expresses the great value of the Dutch greenhouse horticulture sector and its contribution to the global food challenge.

The greenhouse of Tomatoworld is the ultimate data driven greenhouse (DDG), the future of growing! The concept of DDG is, as the name implies, based on the use and analysis of data to optimize crop-health and production. Data is collected 24/7 from the climate computer, sensors and cameras in the greenhouse. The advanced IIVO computer controls the cultivation.

During the excursion you will get a personalized tour highlighting sustainable cultivation methods, innovative techniques and knowledge of the Dutch horticultural cluster.



## **Program - Bus 1 (maximum 40 people)**

### **Time**

<b>12:30</b> pm	Departure from WICC Wageningen
<b>2:15</b> pm	Arrival Ter Laak Orchids
<b>2:30</b> pm – <b>3:00</b> pm	Group 1 technical and production area Group 2 inspiration center
<b>3:00</b> pm – <b>3.30</b> pm	Group 1 inspiration center Group 2 technical and production area
<b>3:45</b> pm	Departure to Tomatoworld
<b>4:00</b> pm - <b>5:00</b> pm	Group tour
<b>5:15</b> pm	Departure to Wageningen
<b>7:00</b> pm	Arrival WICC Wageningen

## **Program - Bus 2 (maximum 40 people)**

### **Time**

<b>12:30</b> pm	Departure from WICC Wageningen
<b>2:15</b> pm	Arrival Tomatoworld
<b>2:30</b> pm – <b>3:30</b> pm	Group tour
<b>3:45</b> pm	Departure to Ter Laak Orchids
<b>4:00</b> pm – <b>4:30</b> pm	Group 1 technical and production area Group 2 inspiration center
<b>4:30</b> pm – <b>5:00</b> pm	Group 1 inspiration center Group 2 technical and production area
<b>5:15</b> pm	Departure to Wageningen
<b>7:00</b> pm	Arrival WICC Wageningen

## **Weather information: September in the Netherlands**

September often provides beautiful late summer weather. However, it can also rain heavily. Quiet days with fog in the morning are also possible. During the increasingly longer nights, the temperature on the ground can drop close to or even below freezing. The average temperature in September is 14.7 degrees. During the day it is on average 19.5 degrees and at night 10 degrees.

It is advised to bring an extra layer of clothing to outdoor and evening events, such as the Opening Reception and Conference Dinner.

An umbrella standby could be useful.

## **Current Coronavirus (Covid-19) measures in the Netherlands**

All coronavirus measures have been lifted. Following the advice below can help you and others avoid getting infected. And you'll be helping to protect people with health concerns:

- wash your hands
- cough and sneeze into your elbow
- stay home or in your hotel and do a Covid-19 self-test if you have symptoms
- ensure a good flow of fresh air indoors
- get vaccinated and boosted.



# Program

## Program

**IPPS 2022 Wageningen, September 26-30, 2022****Monday September 26**

<b>Time</b>	<b>Activity</b>	<b>Location</b>
13.30 – 17.00	Conference Registration open	WICC, Foyer
08:30 – 16:30	Exhibitors set up	WICC, W-Invite
09.00 – 16.30	Satellite Sessions and workshops	Wageningen Campus
09.00 – 13.00	Phenomix for genebanks – future prospects	Room: Atlas 2 Building: Atlas 104
09.00 – 15.00	EMPHASIS IGA meeting (ministry representatives and academic from Europe)	Room: Atlas 1 building: Atlas 104
12.00 – 14.00	Advancements in Forest & Tree Phenotyping	Room: Speakers Corner Building: Impulse 115
12.00 – 16.00	Advances in plant phenotyping data management standard: the MIAPPE adoption status and perspectives	Room: Ncountr Building: Impulse 115
12.30 – 15.00	Affordable Plant Phenotyping Working Group Assembly	Room: W01 & W02 Building: Radix 107
12.30 – 15.30	Measuring photosynthesis: bridging the gap between high and low throughput (WUR Photosynthesis Group - Workshop)	Room: B0521 & B0525 Building: Forum 102
12.45 – 16.00	Photonics for plant phenotyping (Agrofood Robotics - Workshop)	Room: Podium Building: Omnia 105
13.00 – 16.00	Recent advances in controlled environment phenotyping and lab-to-field translation	Room: Gaia 1 Building: Gaia 101
15.00 – 16.00	Advanced Sensor Applications working group assembly	Room: Atlas 2 Building: Atlas 104
18.15 – 22.00	Opening Reception	
18.15	Busses from WICC to Unifarm	
18.30 – 22.00	Opening Reception IPPS2022 and celebration of launching NPEC (Buffet style dinner provided)	Wageningen Campus, Unifarm building 109
22.00	Busses back to hotels	

## Tuesday September 27

Time	Activity	Location
08.00 – 12.00	Conference Registration open	WICC, Foyer
09.00 – 09.15	Official opening IPPS2022	WICC, HUGOtech
09.15 – 12.15	Session 1: Climate change & Photosynthesis Session chair: Mark Aarts	WICC, HUGOtech
09.15 – 10.00	Keynote; Stephen P. Long	
10.00 – 10.30	Coffee break and vendor exhibit	WICC, W-Invite
10.30 – 11.00	The Sound of Photosynthesis; Martin Tritilek	
11.00 – 11.30	Forced Oscillating Light - A Method to Study Regulation of Photosynthesis in Dynamic Environments; Yuxi Niu	
11.30 – 12.00	The happy marriage between phenotyping and genetics: the key to unravel natural variation for photosynthesis; Tom Theeuwens	
12.00 – 12.15	Poster flashes 1	
12.15 – 14.00	Lunch and vendor exhibit	WICC, W-Invite
14.00 – 17.00	Session 2: Plant Development & Allocation Session chair: Xavier Draijje	WICC, HUGOtech
14.00 – 14.45	Keynote; Tracy Lawson	
14.45 – 15.15	An automatic phenotyping pipeline to track maize organs over time; Benoit Daviet	
15.15 – 15.45	Coffee break and vendor exhibit	WICC, W-Invite
15.45 – 16.15	Exploring the potential of using UAV phenotyping platforms to support breeding for improved root systems; Yichen Kang	
16.15 – 16.45	Automated analysis of root growth development using x-ray technology; Joelle Claussen	
16.45 – 17.00	Poster flashes 2	
17.00 – 19.00	Poster session 1 (odd numbers) and drinks	WICC, Expert Street 2+3/6+7 and 8+9
17.30 – 19.30	IPPN General Assembly (members only)	WICC, HUGOtech

## Wednesday September 28

Time	Activity	Location
09.00 – 12.00	Session 3: Microbiome, ecology & biotic interactions Session chair: Corné Pieterse	WICC, HUGOtech
09.00 – 09.45	Keynote; Gert Kema	
09.45 – 10.15	Combinatorial study of the impact of drought on barley resistance against fungal pathogens by high-throughput multispectral, microscopic and physiological phenotyping; Chandana Pandey	
10.15 – 10.45	Coffee break and vendor exhibit	WICC, W-Invite
10.45 – 11.15	At the crime scene of a cereal killer: How multispectral imaging can advance insights in the fusarium head blight disease complex in wheat; Noémie De Zutter	
11.15 – 11.45	Microbiome-based prediction of potato phenotype in the field; Yang Song	
11.45 – 12.00	Poster flashes 3	
12.00 – 14.00	Lunch and vendor exhibit	WICC, W-Invite
14.00 – 17.00	Session 4: Modelling of physiological & ecological processes Session chair: David Rousseau	WICC, HUGOtech
14.00 – 14.45	Keynote: Towards developing a digital twin of perennial horticulture production and supply chain systems; Junqi Zhu	
14.45 – 15.15	Effects of Free-Air Carbon Dioxide Enrichment (FACE) on Photosynthesis, Phenology and Yield of Winter Wheat; Oliver Michael Knopf	
15.15 – 15.45	Coffee break and vendor exhibit	WICC, W-Invite
15.45 – 16.15	"I spy with my little eye ..."- Using phenotyping tools and machine learning to improve robustness in plant based recombinant expression systems; Frederike Stock	
16.15 – 16.45	Mechanistic insights on chlorophyll a fluorescence advance the quest for the Holy Grail in phenotyping; Carmela Rosaria Guadagno	
16.45 – 17.00	Poster flashes 4	
17.00 – 19.00	Poster session 2 (even numbers) and drinks	WICC, Expert Street 2+3/ 6+7 and 8+9
18.00 – 19.00	EMPHASIS Phenotyping Networks Sessions (representatives of national networks only)	WICC, HUGOtech

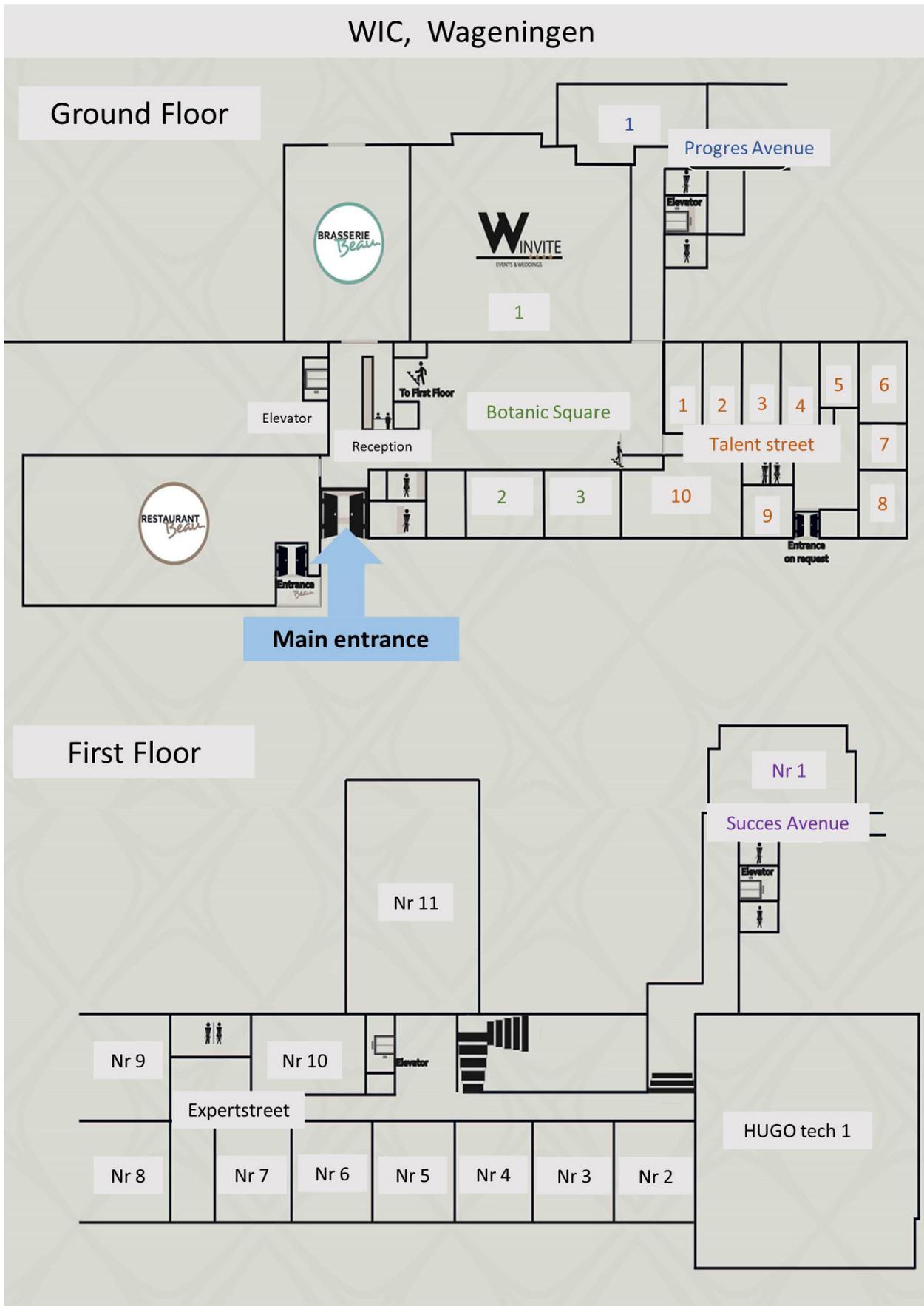
## Thursday September 29

Time	Activity	Location
09.00 – 12.00	Session 5: Food & Bio-economy Session chair: Ulrich Schurr	WICC, HUGOtech
09.00 – 09.45	Keynote; Lee Hickey	
09.45 – 10.15	Morphological evolution during wheat domestication and the potential for further yield gains; Yixiang Shan	
10.15 – 10.45	Coffee break and vendor exhibit	WICC, W-Invite
10.45 – 11.15	Understanding the genetics of canopy dynamics using longitudinal UAV data in mungbean; Shanice Van Haeften	
11.15 – 11.45	EPPN2020/FloweringUnderStress – Sensor and deep physiological phenotyping of terminal drought and heat-effects on chickpea flowering, seed yield and quality; Thomas Roitsch	
11.45 – 12.00	Poster flashes 5	
12.00 – 14.00	Lunch and vendor exhibit	WICC, W-Invite
14.00 – 17.00	Session 6: Sensors, Robotics & Automation Session chair: Tony Pridmore	WICC, HUGOtech
14.00 – 14.45	Keynote; Cyrill Stachniss	
14.45 – 15.15	Assessment of kernel presence in winter wheat ears at spikelet scale using Near-Infrared Hyperspectral Imaging; Damien Vincke	
15.15 – 15.45	Coffee break and vendor exhibit	WICC, W-Invite
15.45 – 16.15	Leaf shape phenotyping method by instance segmentation and curve-based 3D reconstruction; Hidekazu Murata	
16.15 – 16.45	Recent development and potential usage of MultiSpectral LIDAR; Antoine Fournier	
16.45 – 17.00	Poster flashes 6	
17.15 – 23.00	Conference Diner and Party	Burgers' Zoo
17.15 – 18.00	Busses from WICC to Burgers' Zoo, Arnhem	
18.00 – 23.00	Diner and Entertainment	
23.00	Busses to the hotels	

## Friday September 30

Time	Activity	Location
09.00 – 12.00	Session 7: Informatics, Data & Analytics Session chair: Jennifer Clarke	WICC, HUGOtech
09.00 – 09.45	Keynote; Emilie Millet	
09.45 – 10.15	Weakly Supervised Semantic Segmentation of Multi-Species Canopies using Active Learning and Fully Convolutional Neural Networks; Charles Rongione	
10.15 – 10.45	Coffee break and vendor exhibit	WICC, W-Invite
10.45 – 11.15	Channel Attention-GAN based Synthetic Weed Generation for precise Weed identification; Tang Li	
11.15 – 11.45	Using Deep Learning (DL) to Improve Segmentation from RGB and Hyperspectral Imaging Data; Jason Walsh	
11.45 – 12.00	Closing of IPPS2022 and IPPN poster Awards	
12.00 – 12.30	Handing-out of lunchboxes	WICC, W-Invite
12.30 – 18.30	Excursions (field trips and cultural visits)	
	Excursion 1: Dutch Open Air Museum	
	Excursion 2: Hoge Veluwe National Park and Kröller-Müller Museum	
	Excursion 3: Ter Laak Orchids & Tomatoworld	

# Site Map WICC





# Oral presentations



# Climate Change & Photosynthesis

## **KEYNOTE LECTURE 1**

**PROF. DR. STEPHEN P. LONG**

Department of Crop Sciences and Department of Plant Biology, University of Illinois, USA

Steve Long FRS holds the Ikenberry Chair of Crop Sciences. His research concerns bioengineering of photosynthesis toward gaining sustainable increases in crop yield potential and adaptation to global change. He is Director of the multinational Bill & Melinda Gates Foundation RIPE Project which has developed and is developing these technologies for increased photosynthetic efficiency in crops for sustainable yield increases, under climate change. His mathematically guided engineering of photosynthesis led in November 2016 to a demonstrated on farm 20% increase in crop productivity. This year his lab also demonstrated the first single gene manipulation that resulted in a 15% increase in crop water use efficiency in the field. He is Founding and Chief Editor of the journals *Global Change Biology*, *GCB Bioenergy*, and *in silico Plants*. He has been listed by Thomson-Reuters/Clarivate as one of the most highly cited authors on Plant & Animal Biology in every year since 2006, elected Fellow of the Royal Society (2013) and elected Member of the National Academy of Sciences USA (2019).

# **THE SOUND OF PHOTOSYNTHESIS**

**MARTIN TRTILEK**

PSI (Photon Systems Instruments) spol. s r. o.

All living organisms are exposed to changing environmental conditions. These changing conditions help to develop various protection mechanisms, powerful regulatory pathways and feedbacks. The evolution of photosynthesis is, among other constraints, driven by selection of genes for photochemical energy conversion that is robust and, yet effective, in a fluctuating light environment. Variable cloud cover, moving canopy and ocean waves are among the dynamic factors that have been shaping the photosynthetic regulation since the first ancestors of modern plants emerged. The regulatory systems contain nonlinearly negative and positive feedback loops. This work present time frequency analysis of the chlorophyll fluorescence signal, like the response to harmonically modulated irradiance. We can tune different frequency of oscillating actinic light, which allows us to selectively play with individual feedback loop. This enables us to distinguish among mutations on the basis of patterns given by Fourier's analysis.

# FORCED OSCILLATING LIGHT - A METHOD TO STUDY REGULATION OF PHOTOSYNTHESIS IN DYNAMIC ENVIRONMENTS

**YUXI NIU PHD<sup>1</sup>**; PROF. DR. DUŠAN LAZÁR<sup>2</sup>; DR. SILVIA D. SCHREY<sup>1</sup>;  
DR. FABIO FIORANI PHD<sup>1</sup>; DR. SHIZUE MATSUBARA<sup>1</sup>; DR. LADISLAV NEDBAL<sup>1</sup>

<sup>1</sup> Forchungszentrum Juelich;

<sup>2</sup> Palacký University in Olomouc

Dynamics of photosynthesis regulation is mostly studied by exposing dark-acclimated plants to constant light or to light flashes. This approach requires first acclimating plants to darkness and is based the assumption that rate constants of reactions do not change between dark- and light-acclimated conditions. We conducted a comparative study using a forced oscillating light method (no dark acclimation required) and the conventional dark-light method. In addition, to interpret the dynamic optical signals of photosystems and redox status of electron carriers, saturating light pulses were iteratively applied at various phases of oscillating light by DUAL-KLAS-NIR spectrometer. To characterize different regulatory pathways of photosynthetic electron transport, *Arabidopsis thaliana* wild type and mutants with various photosynthetic regulation defects were analyzed. The NDH-like complex mutant (*crr2-2*) deficient in one pathway of cyclic electron transport (CET) showed similar signals with wild type under dark-light transition, while it exhibited substantially different dynamics of all optical proxies under oscillating light. This was in contrast to another mutant (*pgrl1ab*) deficient in PGR5/PGRL1-dependent CET, which differed from wild type in both conventional and oscillation measurements. Interestingly, the distinctive phenotype of the *crr2-2* mutant emerged only at high oscillation amplitude, whereas the *pgrl1ab* mutant constitutively showed clear contrasts with wild type at all oscillation amplitudes. The analysis of saturating pulses applied under oscillating light could only partially explain the dynamic signals observed, possibly due to invasive nature of the intense light pulses affecting the redox state of the electron carriers. Our findings support the application of forced oscillating light method as a potential complement to the conventional method for optically monitoring dynamic response and regulation of photosynthesis under natural fluctuating environments.

# **THE HAPPY MARRIAGE BETWEEN PHENOTYPING AND GENETICS: THE KEY TO UNRAVEL NATURAL VARIATION FOR PHOTOSYNTHESIS**

**TOM THEEUWEN**; DR. JEREMY HARBINSON; PROF. DR. MARK AARTS

Wageningen University & Research, The Netherlands

Natural variation in photosynthesis offers a largely unexplored resource to both better understand photosynthetic functioning and in the use for breeding crops with improved photosynthesis. This source of variation remains largely unexplored because the variation likely involves thousands of genes, each contributing only little to photosynthesis, making them hard to identify without the proper phenotyping and genetic tools. To quantify photosynthesis in dynamic environments high-throughput chlorophyll fluorescence imaging systems capable of phenotyping hundreds of plants in a short time period, or even instantaneously, are essential. To reveal the number of genes and mechanisms responsible for photosynthetic variation, especially in dynamic environments, a large doubled-haploid population between (just) two genotypes of *Arabidopsis thaliana* was produced. Phenotyping this population in highly dynamic environments revealed a dozen QTLs, some of which are caused by allelic variation in genes for which the exact role in photosynthesis had been unknown so far. Moreover, in these efforts the impact of variation in chloroplast and mitochondrial genomes are largely ignored due to the difficulty in separating their effects from nuclear-derived variation. Using haploid-inducer lines we developed an efficient system to generate new organelle-nuclear combinations, with the resulting genotypes referred to as cybrids. A large cybrid panel representing species wide organelle variation in *A. thaliana* reveals variation for a large range of photosynthetic traits. We have revealed that one of these phenotypic differences is caused by a mechanism that was previously thought to hardly play a significant role in photosynthesis. All together we show that when combining high-throughput phenotyping in dynamic environments with state-of-the art genetic tools, nature's often elegant solutions to problems can be explored and learned from.



# Plant Development and Allocation

## **KEYNOTE LECTURE 2**

### **PROF. DR. TRACY LAWSON**

School of Biological Sciences, University of Essex, UK

Professor Tracy Lawson is a professor in the Plant Productivity group and Director of Plant Phenotyping at Essex, with over 25 years' experience in photosynthesis research. She obtained her first degree in Applied Biology in 1993 from Liverpool and PhD from Dundee in 1997. Her research focuses on the stomatal control of atmospheric gas entry into the leaf, associated water loss and the mechanisms that regulate this process. Recent research has paid particular attention to stomatal kinetics and the impact of dynamic environments on both photosynthesis and stomatal behaviour. Tracy's work also concentrates on phenotyping including chlorophyll fluorescence techniques (for quantifying light use and photosynthetic efficiency) and thermal imaging (for measuring stomatal responses and kinetics). Lawson's lab developed the first imaging system for screening plant water-use-efficiency (McAusland et al., 2013).

# AN AUTOMATIC PHENOTYPING PIPELINE TO TRACK MAIZE ORGANS OVER TIME

**BENOIT DAVIET**<sup>1</sup>; ROMAIN FERNANDEZ<sup>2</sup>; LLORENÇ CABRERA-BOSQUET<sup>1</sup>;  
CHRISTOPHE PRADAL<sup>2</sup>; CHRISTIAN FOURNIER<sup>1</sup>

<sup>1</sup> INRAE;

<sup>2</sup> CIRAD

High-throughput phenotyping platforms allow to study the function and form of a large number of genotypes subjected to different growing conditions (GxE). A number of image acquisition and processing pipelines have been developed to automate this process for various plant species. However, complex architecture and developmental shoot traits require extracting both a 3D reconstruction of the plant, and a temporal tracking of each organ, from images. Currently, no such method has been validated for Poaceae with complex experimental conditions (thousands of plants, complete development cycle follow-up). Here we propose a new pipeline to extract a 3D+t reconstruction of maize at organ level from images, which allows studying plant architecture and individual organ development over time during the entire growth cycle. The Phenomenal pipeline [Artzet et al. 2019] is used to segment stem, ligulated leaves, and growing leaves in 3D from RGB images for each date, with an improved stem detection based on deep-learning. Sequence alignment is used as an original way to perform the temporal tracking of ligulated leaves, by exploiting both their consistent shape over time, and unambiguous topology along the stem axis. Growing leaves are tracked afterwards with a distance-based approach. This pipeline was validated on a dataset of 60 maize hybrids imaged daily from emergence to maturity in the PHENOARCH platform. Stem tip was precisely detected (RMSE < 2.1cm) over time. 97.7% and 85.3% of ligulated and growing leaves respectively were assigned to the correct rank after tracking, on 30 plants x 55 dates. The pipeline allowed to extract various development and architecture traits at organ level, with good correlation to manual observations overall, on random subsets of 10 to 355 plants.

These results show the applicability of sequence alignment to the field of phenotyping and temporal tracking, and the usability of this pipeline for large maize GxE analysis.

# EXPLORING THE POTENTIAL OF USING UAV PHENOTYPING PLATFORMS TO SUPPORT BREEDING FOR IMPROVED ROOT SYSTEMS

**YICHEN KANG**<sup>1</sup>; DR. SAMIR ALAHMAD PHD<sup>1</sup>; DANIEL SMITH<sup>2</sup>; SHANICE VAN HAEFTEN<sup>1</sup>;  
CHARLOTTE RAMBLA PHD<sup>1</sup>; SARAH V. MEER<sup>1</sup>; DR. MILLICENT SMITH PHD<sup>1</sup>;  
JACK CHRISTOPHER PHD<sup>1</sup>; DR. KARINE CHENU PHD<sup>1</sup>; JASON A. ABLE<sup>3</sup>; KAI P. VOSS-FELS<sup>1</sup>;  
DAVID R. JORDAN<sup>1</sup>; PROF. ANDREW BOR

<sup>1</sup> Queensland Alliance for Agriculture and Food Innovation, The University of Queensland;

<sup>2</sup> School of Agriculture and Food Sciences, The University of Queensland;

<sup>3</sup> School of Agriculture, Food & Wine, Waite Research Institute, The University of Adelaide;

Root growth angle influences the way roots anchor to different soil layers, thereby affecting the capacity of a root network to capture soil resources. In this durum wheat exemplar, we explore the potential to integrate multiple phenotyping platforms, including field-based root coring and UAV-based remote sensing, to support the selection of improved root systems. Recently, we identified a major genetic locus for seminal root angle in durum wheat on chromosome 6A (i.e. qSRA-6A). To evaluate the potential of qSRA-6A to support grain yield, we developed a set of lines by introgressing the narrow-angle allele into the durum variety DBA Aurora. These lines were evaluated under field conditions. Our results showed the great influence of qSRA-6A on the root distribution of mature plants. Notably, the difference in root distribution of DBA Aurora lines carrying the wide- or narrow-angle allele could be linked to UAV-derived canopy temperature. This highlights the potential to target UAV-derived traits as 'proxies' to indirectly select for root traits in the field. Moreover, differences in root distribution influence the uptake of soil water and nutrients through the season, which can impact above-ground growth and grain yield. To explore root-shoot dynamics in the field, we performed weekly UAV flights to develop crop growth curves. Use of the UAV platform enables the easy acquisition of vegetation index data to characterise the canopy growth dynamics throughout the growing season. Pairing of above- and below-ground datasets provided new insight of the pleiotropic nature of qSRA-6A to support yield in a range of environments. Overall, our study showed the promise of UAV-based remote sensing technology as a powerful tool for high-throughput phenotyping of above- and below-ground traits. We highlight the potential for plant breeders to use UAV-derived traits as a selection tool to accelerate the development of new crop varieties with optimal root and shoot traits.

# **AUTOMATED ANALYSIS OF ROOT GROWTH DEVELOPMENT USING X-RAY TECHNOLOGY**

**JOELLE CLAUSSEN**; MAREIKE WEULE; EVA HUFNAGEL; MICHAEL WAININGER;  
DR. STEFAN GERTH PHD

Fraunhofer IIS

During the last years, X-ray technology has been applied for the non-destructive visualization of optical inaccessible structures in plants intensively. Formerly, this technology was only used for medical imaging. Nowadays, it is used as a standard tool in industrial applications for material analysis. With X-ray computed tomography (CT) the 3D volume information of objects can be reconstructed using X-ray projections of the object from different points of view. This sensor technology allows the non-destructive analysis of hidden structures like root systems in a pot or internal structures within seed material. However, the scanning time of several minutes per plant demand for an automated system. Thus, running these sensor systems automatically, will a gain in a higher throughput. With our new integrated CT system in a controlled environment the plants can be scanned 24/7, which enables us to scan more plants in total. There is no user interaction needed for conducting the measurement and watering the plants in a controlled environment. The system can measure the root growth, but also scan the above ground part of the plants and enable the user to analyze the time-resolved effect of abiotic stress factors. In this presentation we will demonstrate the system and its possibilities in root growth analysis. As example, we scanned different maize genotypes with variation in root architecture. We used 8 genotypes with 5 replicates to analyze the early root growth. For the image analysis, we used the automated imaging pipeline CTProcessing.net. Thus, we are able to monitor and control all parameters starting from the 2D imaging, corrections, normalizations, 3D reconstruction and segmentation. The automation of the CT measurements and all individual parameters within the data processing in the background allows bridging the gap between data and information in the future.



# Microbiome, Ecology & Biotic Interactions

## KEYNOTE LECTURE 3

**PROF.DR.IR. GERT KEMA**

Wageningen University, Laboratory of Phytopathology, The Netherlands

Gerrit H.J. Kema (21.12.1957) is professor of (tropical) phytopathology and head of the Laboratory of Phytopathology of Wageningen University, The Netherlands. He obtained a BSc degree in agronomy, a MSc in plant breeding and PhD in phytopathology. He has 40 years of experience in plant pathology and his research focus has been on host and pathogen genetics and genomics, currently specializing on foliar and vascular diseases of tropical perennial crops, primarily banana. He published over 120 peer reviewed scientific articles, advised numerous BSc, MSc and PhD students and coordinated major international research projects funded by The Royal Netherlands Society of Arts and Sciences with Indonesia, the Dutch Facility for Sustainable Entrepreneurship and Food Security with the Philippines, the Wageningen INREF program with partners in Uganda, Colombia, Ecuador, the Philippines, Costa Rica. He is one of the initiators of the Global Alliance Against TR4, <https://iica.int/en/global-alliance> and a member of the World Banana Forum, <http://www.fao.org/world-banana-forum/about-the-forum/en/>. He is currently involved in the ABBB project, <https://breedingbetterbananas.org>, funded by the Bill & Melinda Gates Foundation in Tanzania on banana breeding. He is a serial entrepreneur and co-founder of several companies in the field of bioprocessing and production, protection and genetic improvement of banana. Together with Prof. André Drenth, Queensland University, he is an editor of a book series on the sustainable cultivation of banana, <https://shop.bdspublishing.com/store/bds>

# COMBINATORIAL STUDY OF THE IMPACT OF DROUGHT ON BARLEY RESISTANCE AGAINST FUNGAL PATHOGENS BY HIGH-THROUGHPUT MULTISPECTRAL, MICROSCOPIC AND PHYSIOLOGICAL PHENOTYPING

DR. CHANDANA PANDEY PHD<sup>1</sup>; ŽIVA RAMŠAK<sup>2</sup>; MAJA ZAGORŠČAK<sup>2</sup>; KRISTINA GRUDEN<sup>2</sup>;  
PROF. ALEXANDER SCHULZ<sup>1</sup>; PROF. DR. THOMAS ROITSCH<sup>1</sup>

<sup>1</sup> University of Copenhagen, Denmark;

<sup>2</sup> National Institute of Biology, Slovenia

The external phenotype of a plant is determined by the sum of interactions of metabolism and regulation that mirror the internal, biochemical and molecular phenotype. For a holistic understanding of pathogen resistance, these various scales of interactions should be considered, and genotyping and image-based phenotyping must be linked to the physiology at the cellular and tissue level (1). The pathogen response of barley genotypes differing in their resistance to two fungal pathogens, the obligate biotroph *Blumeria graminis* and the hemi-biotroph *Pyrenophora teres* (2), causal agents of powdery mildew and net blotch, were characterized by macro- and microscopic phenotyping using high-throughput multispectral imaging in the automated phenotyping facility PhenoLab and confocal microscopy. Through analysis of reflectance and fluorescence patterns it was possible to relate spatial and temporal dynamics at certain wavelength bands to specific resistance types and compare whole leaf changes with those at the cellular level. The non-invasive phenotyping was complemented with molecular and metabolic fingerprinting via the determination of activity signatures of key enzymes of the carbohydrate and antioxidative metabolism, phytohormone profiles and transcriptomics. Indeed, different resistance mechanisms could be related to distinct temporal dynamics of physiological and molecular responses. By combining drought and pathogen infection we identified the impact of abiotic stress on the immune signaling network. This is a proof-of-concept study to show the potential of a holistic functional phenomics approach by complementing non-invasive imaging with deep physiological and molecular phenotyping (2). Thus the identification of predictive bio-signatures for barley pathogen resistance based on spectral imaging is combined with a mechanistic verification at the cellular level.

- (1) Großkinsky et al., 2015. *J. Expt. Bot.* 66: 5429
- (2) Pandey et al., 2021. *Plant Science*, 313, p.11107

# AT THE CRIME SCENE OF A CEREAL KILLER: HOW MULTISPECTRAL IMAGING CAN ADVANCE INSIGHTS IN THE FUSARIUM HEAD BLIGHT DISEASE COMPLEX IN WHEAT

DR. JIANG TAN<sup>1</sup>; **NOÉMIE DE ZUTTER**<sup>1</sup>; PROF. DR. JAN VERWAEREN<sup>1</sup>;  
DR. MAARTEN AMEYE<sup>2</sup>; PROF. DR. SARAH DE SAEGER<sup>1</sup>; DR. MARTHE DE BOEVRE<sup>1</sup>;  
LARISSA DE TROYER<sup>1</sup>; DR. THEO VAN DER LEE<sup>3</sup>; DR. CEES WAALWIJK<sup>3</sup>;  
PROF. DR. KRIS AUDENAERT<sup>1</sup>

<sup>1</sup> Ghent University;

<sup>2</sup> Inagro;

<sup>3</sup> Wageningen University & Research

In plant pathology, the response of a plant upon pathogenic infection is often studied as a bilateral interaction between one pathogenic strain and a host. However, in an agroecosystem, disease development is the result of inter-species, inter-genera and even inter-kingdom interactions. This type of multipartite interactions make an interesting 'crime scene' for research on plants coping with multiple invaders at a time. Fusarium head blight (FHB) is one of the most important fungal diseases on wheat. In Europe, *F. graminearum* (Fg) and *F. poae* (Fp) are the predominant species associated with the disease. Despite their presence in diseased kernels, only Fg is considered pathogenic while Fp is not virulent. The role in disease development of such weakly pathogenic species is enigmatic. By using several multispectral imaging tools, we investigated the interaction between Fg and Fp on the one hand, and evaluated the effectiveness of biocontrol agents hereon on the other hand. Using a GFP-tagged Fg and an RFP tagged Fp, we first studied the effect of their single- and co-inoculation by assessing fungal progression and symptom development through high resolution multispectral imaging. Next, the impact of several potential biocontrol bacteria against Fg and Fp was investigated using the same multispectral tools. Downstream processing of the multispectral indices was done in a bipartite manner, using a mean-based approach on the one hand, and the distribution of spectral indices on the other hand. These whole plant distributions were evaluated using the Earth Mover's Distance as a dissimilarity measurement and were visualized by means of multidimensional scaling. Hence, we were able to detect disease symptoms and biocontrol effects 24h earlier as compared to the mean-based approach. Lastly, using the anthocyanin reflectance index, we were able to identify the hypersensitive response as a mode-of-action involved in biocontrol.

## **MICROBIOME-BASED PREDICTION OF POTATO PHENOTYPE IN THE FIELD**

**DR. YANG SONG POSTDOC**; DR. PETER A.H.M. BAKKER; DR. RONNIE DE JONGE;  
PROF. DR. CORNÉ M.J. PIETERSE; DR. ROELAND L. BERENDSEN

Utrecht University, The Netherlands

Potato is one of the world's most important food crops, but potato production also requires high quantities of agrochemicals. The microbial communities that pervade and surround plants are known to carry out important functions for plant health and performance. The plant microbiome therefore has generated great interest as an integral part of plant biology with great new opportunities for microbiome-assisted agriculture as a biological alternative for chemical fertilizers and pesticides. In this study, we combined high-throughput microbial sequencing with drone imaging data to generate a novel microbiome-based prediction model for the performance of seed potatoes. We investigated the microbiome of seed potatoes derived from more than 240 Dutch fields and subsequently assessed the performance of the emerging potato plants in trial field in the subsequent growing season. We observed a strong influence of the field of production of the seed potato tubers on potato vitality. This indicates that potato vitality is a trait that is imprinted in the seed potato tubers by local biotic and abiotic conditions in the production field and is transferrable from one potato generation to the next. Using advanced bioinformatics and machine learning approaches, we show that sequence-based potato analysis of microbiome of seed potato tubers can be used to predict the growth of the potato crop that emerges from them. Furthermore, we were able to identify microbial signatures that are predictive for the vitality of the seed potato tuber and can thus be developed as potential biomarkers for vitality by analysing microbiome fingerprints of seed potato tubers.



# Modelling of Physiological & Ecological Processes

## KEYNOTE LECTURE 4

# TOWARDS DEVELOPING A DIGITAL TWIN OF PERENNIAL HORTICULTURE PRODUCTION AND SUPPLY CHAIN SYSTEMS

**J. ZHU**<sup>1</sup>, C.J. STANLEY<sup>2</sup>, F. ROJO<sup>3</sup>, H. BROWN<sup>4</sup>, B. VAN HOOIJDONK<sup>3</sup>, T. KANEKO<sup>3</sup>, K. BREEN<sup>3</sup>, E. TEIXEIRA<sup>4</sup>, A. FRIEND<sup>5</sup>, N. NIEMANN<sup>3</sup>, X. YANG<sup>4</sup>, C. SCOFIELD<sup>2</sup>, A.J. MAWSON<sup>6</sup>

The New Zealand Institute for Plant and Food Research Ltd;

<sup>1</sup> Blenheim, <sup>2</sup> Alexandra, <sup>3</sup> Hawke's Bay, <sup>4</sup> Lincoln, <sup>5</sup> Motueka, <sup>6</sup> Hamilton, New Zealand

Digital technology offers great opportunities for rapidly characterizing plant responses to environmental challenges for both controlled and field experimentation. However, many current agritech products and services address only single needs and do not explicitly draw on the richness of our knowledge in plant and ecological sciences. These limitations can be addressed through digital twins, which are increasingly being adopted across diverse fields to monitor, analyse, simulate and control complex systems.

At Plant & Food Research, we are developing digital twins of perennial horticulture systems to dynamically couple physical components of production and supply chain systems with their virtual analogues. As the first step in developing the digital twin of horticultural systems, we are using apple as the exemplar species and the narrow-row planar cordon system as the exemplar system.

Previous study has shown that a narrow-row planar cordon system can double the productivity compared with conventional systems, and reduce variability in fruit quality. However, there are many gaps in our understanding of the complex biological interactions that increase crop performance, and of the implications for pre- and post-harvest fruit quality under different growing and storage Modelling.

Modelling will facilitate the design of the production and supply chain system by rapidly identifying and testing alternatives, querying 'what if' scenarios, improving our predictive capacity, and ultimately speeding up our progress towards data-informed precision management. We are currently exploring the underlying physiological mechanisms that improve crop performance:

- 1) Tree structure and function, characterizing leaf, shoot and bud development and allowing understanding of their responses to internal and external conditions;
- 2) Energy capture, distribution and utilization, characterizing and allowing understanding of within-tree variations in light intensity and quality, leaf traits, rate of carbohydrate assimilation, and carbohydrate allocation;
- 3) Root architecture and function, characterizing and allowing understanding of the root phenology and growth of different rootstocks, and their interactions with scion and soil water and nitrogen conditions;

4) Fruit development, quality and storage, linking orchard factors and local plant carbohydrate and water potential with fruit texture and carbohydrate development and storage responses.

Together with the physiological studies, we are rapidly developing a new functional-structural plant model framework to integrate the experimental findings. The new model framework will also enable users to add or remove different functionalities in the interface. Along with other programmes, we aim to integrate 3D imaging, data science, plant modelling, tree physiology, and ecology to develop a real-time, feedback-driven virtual orchard and supply chain, which will be connected to a model that predicts fruit texture after cold storage.

In the future, scientists will be able to test their theories within the model, determine what modifications to the growing system would improve its functionality, and how resilient the system would be to different environmental scenarios. Ultimately, this will move perennial horticulture beyond simplistic rule-based design and management using unconnected tools, to situation-specific and fully coordinated operations grounded in the in-depth knowledge of the system.

# EFFECTS OF FREE-AIR CARBON DIOXIDE ENRICHMENT (FACE) ON PHOTOSYNTHESIS, PHENOLOGY AND YIELD OF WINTER WHEAT

**OLIVER MICHAEL KNOPF**<sup>1</sup>; ANTONY OSWALDO CASTRO<sup>1</sup>; DAVID LENZEN<sup>1</sup>; JUAN QUIROS<sup>1</sup>; SANDRA MARKWITZ<sup>1</sup>; DR. EINHARD KLEIST<sup>1</sup>; MICHAEL QUARTEN<sup>1</sup>; ANGELINA STEIER<sup>1</sup>; DR. MANUEL GEYER<sup>2</sup>; DR. LORENZ HARTL<sup>2</sup>; CHRISTINE KÄMPER<sup>3</sup>; PROF. DR. KATHARINA SCHERF<sup>3</sup>; PROF. DR. RALF PUD<sup>4</sup>

<sup>1</sup> IBG-2: Pflanzenwissenschaften, Forschungszentrum Jülich, Jülich, 52425, Germany;

<sup>2</sup> Bavarian State Research Center for Agriculture (LfL);

<sup>3</sup> KIT - Karlsruhe Institute of Technology;

<sup>4</sup> University of Bonn

Climate change and the associated continuous rise of the atmospheric carbon dioxide concentration [CO<sub>2</sub>] pose a major challenge for future sustainable wheat production. Alterations in the [CO<sub>2</sub>] can affect photosynthesis and thus have considerable impacts on crops, e.g. growth, phenology and quality. A free-air CO<sub>2</sub> enrichment (FACE) facility was combined with an automated, mobile phenotyping system within the transdisciplinary 'BigBaking' project to get a better understanding of the complex plant response in wheat. Ten different winter wheat (*Triticum aestivum*) cultivars were grown in 2 x 3 m plots in the BreedFACE at ambient (~400 ppm) and elevated [CO<sub>2</sub>] (~600 ppm). In this study, we aimed to monitor plants throughout the vegetation period with the help of high-throughput photosynthesis measurements. A light-induced fluorescence transient (LIFT) sensor enabled us to observe the seasonal development of relevant traits such as the operating efficiency of photosystem II ( $F_q'/F_m'$ ) at unprecedented speed and under contrasting weather conditions. In addition, RGB data were collected by two PhenoCams continuously monitoring the plots. Winter wheat grown under elevated [CO<sub>2</sub>] tends to have a higher  $F_q'/F_m'$  during vegetative growth at the beginning of the growing period. During the grain-filling phase, plants showed a more cultivar-specific response with a larger variation in  $F_q'/F_m'$ . PhenoCam data indicate a delay in the onset of senescence under elevated [CO<sub>2</sub>] but, in turn, reveal a more pronounced senescence intensity. Furthermore, plants grown under elevated [CO<sub>2</sub>] accumulated significantly more straw biomass, yield, and increased plant height, suggesting a different resource allocation within winter wheat. Our results indicate that the rise in atmospheric [CO<sub>2</sub>] has wide-ranging effects on the cultivation of winter wheat and that high-throughput phenotyping can contribute to a better understanding of complex plant adaptation strategies in a highly dynamic environment.

# **“I SPY WITH MY LITTLE EYE ...”- USING PHENOTYPING TOOLS AND MACHINE LEARNING TO IMPROVE ROBUSTNESS IN PLANT BASED RECOMBINANT EXPRESSION SYSTEMS**

**DR. FREDERIKE STOCK**; DR. RICHARD POIRE; DR. TIM BROWN; DR. LIANG ZHENG;  
PROF. HONGDONG LI; PROF. OWEN ATKIN

Australian National University

Plant-based systems for recombinant protein production offer several advantages over other production platforms such as ease of scale-up, the ability to glycosylate proteins, low risk of contamination and low cost of production. However, to date the lion's share of recombinant proteins is still produced in microbes and animal cells because of more consistent protein yields. Therefore, tools are needed to improve process control and develop a deeper knowledge of recombinant protein production in plants.

To evaluate the potential of combining plant phenotyping and machine learning for continuous monitoring of plant production, a multidisciplinary team of plant biologists and computer vision scientists at the Australian National University was formed to develop state-of-the-art imaging tools for plant phenotyping. Using 3D- and hyperspectral imaging together with machine learning the team aims to develop a system that can i) extract custom plant traits from 3D plant models non-invasively and ii) identify changes in growth conditions in plants to enable growers to take corrective action, if needed.

Until now the team has successfully developed a pilot imaging platform that produces high-resolution 3D plant models which are used to extract custom plant traits. In addition, a library of plant morphological, functional and spectral signatures has been created by phenotyping plants grown under different environmental conditions. As the library continues to grow, machine learning models are being trained to recognize plant signatures that correspond to a set of pre-defined growth conditions.

In this multi-year project, the team aims to build a toolset that will assist growers with their decision-making in large scale plant production. Ultimately this work will support efforts to further improve plant-based protein production of recombinant proteins.

# **MECHANISTIC INSIGHTS ON CHLOROPHYLL A FLUORESCENCE ADVANCE THE QUEST FOR THE HOLY GRAIL IN PHENOTYPING**

**DR. CARMELA ROSARIA GUADAGNO PHD; PROF. DR. BRENT E. EWERS**

University of Wyoming

One of the grand challenges in phenomics is identifying a few meaningful traits to explain and predict plant response under a changing environment and across genotypic variation. Since the early 2000s, chlorophyll a fluorescence (ChlF) has been considered the possible 'Holy Grail' in phenotyping, thanks to data-rich results, high-throughput measurements, and its process insights all for a reasonable price. To be phenotypically relevant, a trait must hold a high mechanistic value, i.e., its capacity to quantitatively inform on key plant processes across scales. Although ChlF is a powerful tool for studying spatial and temporal heterogeneity of photosynthetic performance, our predictive understanding is limited by the need for additional mechanistic insights into the causality of ChlF dynamics from photosystem II (PSII) in different environments. While waters as the substrate of PSII and the electron flow leading to the onset of ChlF is well understood, thousands of waters embedded in the PSII structure still lack known functions. We describe a conceptual model where the variations in hydration directly influence chlorophyll behavior leading to changes in energy partitioning and ultimately ChlF from PSII. To test our hypothesized model, we correlated leaf ChlF to various proxies of water content. We found a tight relationship ( $R = 0.85$ ) between ChlF and leaf water potential across disparate species, from *Brassica rapa* to *Ponderosa* pine, and over a full range of hydration, from mild to severe drought. We further test how well this relationship holds across time and space by quantifying the response of both entire leaves and disks of *Gossypium hirsutum* over the time of drought using imaging and hand-held fluorometry. If our first principles approach between PSII hydration and ChlF holds, it will allow for verifying the potential heritability of ChlF as a phenotyping tool with far-reaching impacts on phenomics and predictive understanding of plant-environment interaction.

# Food & Bio-economy

## **KEYNOTE LECTURE 5**

### **DR. LEE HICKEY**

Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Australia

Associate Professor Lee Hickey is a crop geneticist within the Queensland Alliance for Agriculture and Food Innovation at The University of Queensland, Australia. He leads a diverse research team that specialises in plant breeding innovation with a focus on understanding the genetics of above- and below-ground traits that underpin drought adaptation. The Hickey Lab research program spans from lab to field, where designer plant materials are created through speed breeding or engineered in the lab using genome editing, and then evaluated under controlled and field environments using state-of-the-art UAV phenotyping technology. Lee is passionate about training the next generation of crop scientists and currently mentors 13 PhD students, while 12 of his previous graduates now work for leading plant breeding companies and high-profile research institutes around the globe.

# **MORPHOLOGICAL EVOLUTION DURING WHEAT DOMESTICATION AND THE POTENTIAL FOR FURTHER YIELD GAINS**

**YIXIANG SHAN PHD; PROF. COLIN OSBORNE<sup>1</sup>**

<sup>1</sup> The University of Sheffield

The development and morphology of crop plants have been profoundly altered by evolution under cultivation. Domestication of the earliest crops is thought to have occurred without foresight by early farmers, through natural selection arising from cultivation, management and harvesting. However, subsequent evolution has involved deliberate selection for particular phenotypes, alongside continuing natural selection within agricultural environments. Wild wheats are an important potential source of variation for breeders, however, the precise timing of morphological changes during domestication is not fully resolved. Here, we grew and measured 160 wheat accessions representing different stages in wheat evolution, defining the morphospace of these groups to evaluate size and allocation. We also applied morphological data in functional-structural plant (FSP) models, combining values within the observed range to test whether higher yield is possible within the morphospace that already exists across species. The results show that wild and domesticated wheats have overlapping morphospaces, but each also occupies a distinct area of morphospace from one another. Domestication produces larger shoots, leaves, inflorescences and seeds, but on a relative basis the total allocation to inflorescences decreases up until the Green Revolution. The tendency for stems to become thicker and architecture to become more compact has continued throughout crop evolution. Decreasing tillering strength happened before and after domestication, causing fewer spikes in modern wheats. However, this does not lead to yield loss, due to improvements in the number and weight of grains from individual spikes. The FSP simulations show the potential for further improvements by maximizing leaf and flower biomass, while minimizing the shoot size. The finding suggests that knowledge of wheat morphological variation and diversity could further benefit breeding work by using existing germplasm resources.

## UNDERSTANDING THE GENETICS OF CANOPY DYNAMICS USING LONGITUDINAL UAV DATA IN MUNGBEAN

**SHANICE VAN HAEFTEN**<sup>1</sup>; YICHEN KANG<sup>1</sup>; CAITLIN DUDLEY<sup>1</sup>; COLIN DOUGLAS<sup>2</sup>;  
DR. MILLICENT SMITH PHD<sup>3</sup>; DR. HANNAH ROBINSON PHD<sup>1</sup>;  
ASSOCIATE PROF. ANDRIES POTGIETER PHD<sup>1</sup>; DR. LEE T. HICKEY PHD<sup>1</sup>

<sup>1</sup> Queensland Alliance for Agriculture and Food Innovation, The University of Queensland;

<sup>2</sup> Department of Agriculture and Fisheries;

<sup>3</sup> School of Agriculture and Food Sciences, University of Queensland

Mungbean is an important pulse crop that is a high-quality plant protein source and a major high-value export crop for many growers globally. While substantial yield improvements have been made, the rate of genetic improvement in mungbean is limited and could be further advanced by overcoming the phenotyping bottleneck associated with ground-based methods. Traditional visual phenotyping approach limits a breeder's capacity to identify complex traits that could provide a yield benefit in different environments and agricultural systems. Unmanned aerial vehicles (UAV) offer a powerful tool to support efficient, non-invasive, field-based phenotyping, of canopy traits across the growing season. This technology has been successfully applied to major cereal crops, however their potential to advance the breeding of many pulse crops, such as mungbean, is yet to be explored. This study investigated the utility of UAV-based imaging to identify and dissect the genetics of promising canopy traits that underpin yield. A diverse nested association mapping (NAM) population was subjected to weekly UAV flights using a multi-spectral camera. Several vegetative indices (VIs) (i.e. OSAVI, NDRE and thermal) were associated with important canopy traits at specific time-points, such as vigour and canopy cover. Spline curve fitting was used to integrate the extracted VIs from single flights into a continuous time course. A range of canopy parameters were then examined across genotypes, including canopy growth rates and senescence, as well as the identification of key growth stages when these UAV-derived traits are most predictive of yield. Finally, a genome-wide association study identified key genomic regions that could be targeted in breeding programs to modulate canopy development. This study highlights the potential for mungbean improvement programs to scale up phenotyping of canopy traits using UAVs, that could be integrated with genomic selection strategies to accelerate genetic gain.

# **EPPN2020/FLOWERING UNDER STRESS – SENSOR AND DEEP PHYSIOLOGICAL PHENOTYPING OF TERMINAL DROUGHT AND HEAT-EFFECTS ON CHICKPEA FLOWERING, SEED YIELD AND QUALITY**

PROF. DR. CARLA PINHEIRO PHD<sup>1</sup>; DR. LEONOR GUERRA-GUIMARÃES PHD<sup>2</sup>;  
DR. JOSE MELO<sup>1</sup>; DR. ISABEL DUARTE<sup>3</sup>; **PROF. DR. THOMAS ROITSCH**<sup>4</sup>

<sup>1</sup> Universidade NOVA de Lisboa;

<sup>2</sup> Universidade de Lisboa;

<sup>3</sup> Instituto Nacional de Investigação Agrária e Veterinária I.P.;

<sup>4</sup> University of Copenhagen

Chickpeas (*Cicer arietinum* L.) are included in the Zero Hunger program, are the world's second most cultivated food legume, and have one of the best nutritional compositions among the grain legumes. Drought typically reduces grain yield, namely when occurring during pod set and seed filling, which is further reduced in combination with elevated temperatures. With this EPPN2020 project, we focused the phenotyping during the critical flowering and seed filling stages. Phenological responses to terminal drought and heat were evaluated by non-invasive thermo-, multireflectance- and multifluorescence imaging in the Phenolab at the University of Copenhagen (DK) to relate physiological responses at plant level with the activity signatures of key enzymes of carbohydrate and antioxidant metabolism and its final impact on seed yield and quality. Two chickpea genotypes with high production potential were evaluated: Elvar, adapted to dry conditions; Electra, selected due to large seed size. The impact of high temperature was combined with a 40% or 10% watering regime and compared with plants kept at 40% watering regime and ambient temperature. The sensor based phenotyping in the Phenolab was complemented with seed yield and quality analysis. Both seed weight and seed number were negatively affected by high temperatures irrespectively of the watering regime. Seeds from both genotypes obtained under high temperature conditions showed increased protein content and lower starch. Protein content was further affected by the watering regime. Alterations in protein-to-starch indicates modifications in sink-source relationships. The differential effects were mirrored in distinct sensor based and biochemical biosignatures. This study contributes to the mechanistic knowledge on how combined effects of severe terminal drought and high temperature modulate sink capacity and chickpea productivity (yield and quality) and identifies predictive biomarkers for the breeding.



# Sensors, Robotics & Automation

## KEYNOTE LECTURE 6

**PROF. DR. CYRILL STACHNISS**

Lab for Photogrammetry and Robotics, University Bonn, Germany

Crop farming plays an essential role in our society, providing us food, feed, fiber, and fuel. We heavily rely on agricultural production but at the same time, we need to reduce the footprint of agriculture production: less input of chemicals like herbicides, fertilizer, and other limited resources. Agricultural robots and other new technologies offer promising directions to address key management challenges in agricultural fields. To achieve this, autonomous field robots need the ability to perceive and model their environment, to predict possible future developments, and to make appropriate decisions in complex and changing situations. This talk will showcase recent developments towards robot-driven sustainable crop production. I will illustrate how tasks can be automatized using UAVs and UGVs as well as new ways this technology can offer.

Cyrill Stachniss is a full professor at the University of Bonn and heads the Photogrammetry and Robotics Lab. He is additionally a Visiting Professor in Engineering at the University of Oxford. Before his appointment in Bonn, he was with the University of Freiburg and the Swiss Federal Institute of Technology. Since 2010 a Microsoft Research Faculty Fellow and received the IEEE RAS Early Career Award in 2013. From 2015-2019, he was senior editor for the IEEE Robotics and Automation Letters. Together with his colleague Heiner Kuhlmann, he is a spokesperson of the DFG Cluster of Excellence "PhenoRob" at the University of Bonn. In his research, he focuses on probabilistic techniques for mobile robotics, perception, and navigation. The main application areas of his research are autonomous service robots, agricultural robotics, and self-driving cars. He has co-authored over 250 publications, has won several best paper awards, and has coordinated multiple large research projects on the national and European level.

# **ASSESSMENT OF KERNEL PRESENCE IN WINTER WHEAT EARS AT SPIKELET SCALE USING NEAR-INFRARED HYPERSPECTRAL IMAGING**

**DAMIEN VINCKE**<sup>1</sup>; PROF. DR. BENOÎT MERCATORIS<sup>2</sup>; DR. DAMIEN EYLENBOSCH<sup>1</sup>;  
DR. VINCENT BAETEN<sup>1</sup>; PHILIPPE VERMEULEN<sup>1</sup>

<sup>1</sup> Walloon Agricultural Research Centre (CRA-W);

<sup>2</sup> University of Liège, Gembloux Agro-Bio Tech

Obtaining varieties with higher yield is one of the aims of crop breeding. For winter wheat (*Triticum aestivum* L.), yield is the result of three components which are the number of ears per unit area, the number of kernels per ear and the kernel weight. The occurrence of stresses during crop growth can have a negative impact on the yield components. Assessing the physiology behind the number of kernels and the impact of stresses is a complex process requiring observations at specific critical periods of the crop cycle. The scope of this work is to propose a laboratory method based on Near Infrared Hyperspectral Imaging (NIR-HSI) to rapidly evaluate the presence or absence of kernels in winter wheat ears. This preliminary study has allowed evaluating the potential of the method using ears collected in a field trial in Belgium. The ears were scanned using a NIR-HSI camera in the spectral range 900-1700 nm. Hyperspectral images of the ears have been collected before and after manually removing the kernels from the ears. NIR-HSI combined with chemometric tools identified spectral bands potentially characteristic of the presence of kernels inside the ears. A chemometric model has been built using Partial Least Squares Discriminant Analysis (PLS-DA) to identify ears with and without kernels. The PLS-DA results obtained in this study allowed to track the presence or absence of kernels in winter wheat ears at spikelet scale. These results indicate that NIR-HSI might be a useful method to provide information assessing the status of kernels inside the ear which plays an important role in yield assessment.

# **LEAF SHAPE PHENOTYPING METHOD BY INSTANCE SEGMENTATION AND CURVE-BASED 3D RECONSTRUCTION**

**HIDEKAZU MURATA**

Kyushu Univeristy

The shape of plant leaves is believed to change to adapt to the surrounding environment. Therefore, measuring the shape of plant leaves can provide a better understanding of the effects of the external environment on plants. However, the thin shape of plant leaves makes it difficult to measure them in three dimensions, and quantification has conventionally been based on two-dimensional approximations. In this study, we developed a method to obtain morphological information of leaves in 3D space by acquiring leaf outlines from multi-view images of plants and reconstructing them in 3D based on the outlines. Masks and classes are created by instance segmentation of images, and 3D reconstruction is performed using the curve features of the contour image generated from the masks to obtain the 3D shape of the leaf. Deep learning can handle leaf contours because it is now possible to accurately obtain overlapping leaf contours from images. However, since the correspondence between leaves between images is not known by instance segmentation, the correspondence between leaves between images is also obtained by reprojecting the point cloud obtained by Structure from Motion (SfM), which is used to obtain camera parameters. The contour of the mask and the correspondence of the leaves can be used to correspond the fragments of the curve between the two images, allowing for stable reconstruction. In this study, it was shown that segmentation of images of actual plants taken with a camera and 3D reconstruction using curve features in simulated data is possible with high accuracy. We also examined the applicability of this method to leaves with holes, which we believe can be handled by this method, using simulation data.

## **RECENT DEVELOPMENT AND POTENTIAL USAGE OF MULTISPECTRAL LIDAR**

**DR. ANTOINE FOURNIER PHD**

ARVALIS-Institut du Végétal

Developed to provide ground truth reflectance for passive spectral imagery from automated high-throughput plant phenotyping field platform operated by ARVALIS, the multispectral LIDAR prototype is based on a supercontinuum source developed at Photonics-Bretagne and allow to scan successively seven waveband centred a 450, 530, 570, 680, 730, 750 and 850 nm of 15 nm width. It provide directional-directional reflectance values over a switchable footprint of 1 inch or 10 inches pointing on nadir sight. Actual work is focused on inverting and fusing information from passive and active sensors covering both visible and near-infrared range. PROSAIL model and its derivative are candidate for leaf chemical compound estimation and handling more finely with cover radiosity. Authors are open to collaborate on improving inversion and on listing operative needs from phenotyping platform to prioritize instrument evolution notably to other spectral ranges or scanning features.



# Informatics, Data & Analytics

## **KEYNOTE LECTURE 7**

### **DR. EMILIE MILLET**

Genetics and Breeding of fruit and vegetables, INRAE, Avignon, France

As a quantitative biologist with an initial training as an agronomist, Dr. Emilie Millet's activities focus on the integration of methods and data at different scales in order to identify the determinisms of quantitative plant responses to environmental conditions.

After a PhD thesis at LEPSE (MAGE 2012-2016) in the framework of the DROPS and Amaizing projects, she did a first post-doc at Wageningen University (WUR, the Netherlands) in the EPPN2020 project.

She joined the MAGE team again in July 2021 as a Post Agreenskills postdoc. In the EXPOSE project (EXploring PhenOtypic SpacE for Mining Genotypes and Alleles in Maize), she proposes to interface genetics and ecophysiology with ecology/evolution and data science. The objective is to define G×E interactions in terms of phenotypic space and to study their structure. The use of variables measured in platforms, which are predictive of the response of plants to the environment, will make it possible to identify combinations of traits and individuals adapted to specific conditions. She is currently researcher at the INRAE group Genetics and Breeding of fruit and vegetables (GAFL) in Avignon, France, working on tomato breeding. Her primary research interest focuses on integration of methods and data at different scales to identify the determinisms of plant responses to change in climate.

# **WEAKLY SUPERVISED SEMANTIC SEGMENTATION OF MULTI-SPECIES CANOPIES USING ACTIVE LEARNING AND FULLY CONVOLUTIONAL NEURAL NETWORKS**

**CHARLES RONGIONE**; PROF. DR. XAVIER DRAYE PHD<sup>1</sup>; PROF. GUILLAUME LOBET<sup>2</sup>;  
CELINE CHEVALIER; CHRISTOPHE DE VLEESCHOUWER

<sup>1</sup> Earth and Life Institute, Université Catholique de Louvain;

<sup>2</sup> Juelich Forschungszentrum

Monitoring ecophysiological characteristics of multispecies canopies is essential to understand how diverse plant species grow and interact together and develop agroecological practices. Image-based monitoring has become increasingly popular due to a high information:cost ratio. Semantic segmentation has shown great potential for canopy image analysis and has already been applied in a wide range of tasks, such as phenotyping, weeds and crops differentiation or leaves counting. However, it still suffers from a significant bottleneck: the acquisition of segmentation masks. Getting proper masks is a time-consuming task that often makes image segmentation unpractical. The majority of the work done to reduce the ineffectiveness of the annotation process has been done on monocrop images from homogenous fields. Despite being effective for such images, most proposed methods lose much of their performance when applied to heterogeneous canopies. Therefore, these methods are not transferable to multi-species cropping plots. In this work, we present an active learning method based on gradient boosted decision trees that allows getting high-quality binary plant/background segmentation masks in a few minutes by annotating only small regions of the images, even on very diverse mixed crops images. We then show that a fully convolutional neural network trained with masks generated by this method on monocrop images can generalize to mixed crop images and robustly classify each plant pixel according to their species. This approach drastically reduces the amount of human labor needed to semantically segment mixed crops images from hours to minutes and, therefore, greatly facilitates the study of eco-physiological characteristics of multispecies canopies.

# CHANNEL ATTENTION-GAN BASED SYNTHETIC WEED GENERATION FOR PRECISE WEED IDENTIFICATION

**TANG LI**<sup>1</sup>; PROF. YUYA FUKANO<sup>2</sup>; PROF. YOICHIRO KATO<sup>1</sup>; PROF. WEI GUO PHD<sup>1</sup>

<sup>1</sup> The University of Tokyo;

<sup>2</sup> Chiba Univeristy

Weeds are considered the main biological causative factor in declining crop yield. So far, large-scale herbicide dispersal remains the primary means of weed control. However, the heavy use of herbicides does not guarantee the effectiveness of weed control and leads to ecological diversity loss. Deep learning-based precise weeding is the best solution in the context of big data, which usually needs large datasets coping with real-world conditions. However, cultivating and labeling weeds manually is challenging, requiring specialized knowledge and skills in weed science and agronomy. To address such data problems, our research proposed a data farm that consists of 1) standardized weed data cultivation and collection scheme; 2) a generative model-based synthetic data generation pipeline. Our data farm collects data automatically, remotely, and efficiently, then generates more diverse synthetic data by learning the latent representations of real-world data. To be detailed, first, the weed cultivation and data collection scheme were designed by combining farmbot (Farmbot Inc, San Luis Obispo, USA), NVIDIA Jetson Xavier NX (NVIDIA Corporation, Santa Clara, USA), and multiple ELP 13mp cameras (Ailipu Technology Co., Ltd, Shenzhen, China). And a Google Cloud was used to achieve real-time synchronization of data. Our scheme can collect tens of thousands of weed data within a month. Second, the synthetic data generation pipeline contains data cleansing and a GAN model driven by a channel attention mechanism. A metric called Frechet Inception Distance (FID) score was used to assess the quality of the synthetic data (a lower FID score indicates higher fidelity). Our proposed method scored 25.67, better than current state-of-the-art networks SNGAN (35.78) and WACGAN-GP (51.01). Besides, in a class discriminability test, our method achieved an average identification accuracy of 91.02%, better than SNGAN (90.94%) and WACGAN-GP (83.92%).

# USING DEEP LEARNING (DL) TO IMPROVE SEGMENTATION FROM RGB AND HYPERSPECTRAL IMAGING DATA

**JASON WALSH**<sup>1</sup>; PATRICK LANGAN<sup>1</sup>; JOEY HENCHY<sup>1</sup>; EMILIE JACOB<sup>2</sup>;  
GAËLLE MONGELARD<sup>2</sup>; STÉPHANIE GUÉNIN<sup>2</sup>; HERVÉ DEMAILLY<sup>2</sup>; DR. LAURENT GUTIERREZ<sup>2</sup>;  
DR. SONIA NEGRÃO PHD<sup>1</sup>; DR. ELENI MANGINA<sup>1</sup>

<sup>1</sup> University College Dublin;

<sup>2</sup> Université de Picardie Jules Verne (Amiens, France)

To study how plants respond to their environment researchers use imaging phenotyping technologies. The use of image-based phenotyping has enabled researchers to analyse plants and produce data at a large scale. However, this large influx of data has created a 'big data' problem to emerge causing researchers to search for new innovative ways to tackle the challenges of processing and analysing their data in a reasonable timeframe. To address such issues, deep learning and data science techniques are being used to perform a comprehensive analysis. Here we use a Plant Screen™ compact system to image a series of barley plants using two different imaging sensors. This compact system contains an RGB top and side view camera and a hyperspectral visible near infrared (VNIR) camera that captures a spectral cube of spatial resolution 500 x 500 pixels and a spectral resolution of 480 pixels which covers a wavelength range from 380 – 900nm on the electromagnetic spectrum. To streamline the processing and analysis of RGB and hyperspectral imaging, we are building a pipeline using a lightweight implementation of the U-Net architecture to improve the accuracy of semantic segmentation based on the raw images captured via the compact system. Several models were designed and developed, each of which was tailored to either the type of imaging sensor being used or the angle for which the images been provided were taken (e.g., top-down, side-view). Results showed that each model regardless of sensor or perspective produced an accuracy greater than 90% and could accurately segment cereal crops regardless of their size, shape or colour. These results demonstrate the feasibility of using DL models to semantically segment cereal crops imaged using either RGB or hyperspectral imaging sensors.



# Poster presentations



# Climate Change & Photosynthesis

# **THE EFFECT OF DROUGHT AND HEAT STRESS COMBINATION ON THE PHYSIOLOGICAL TRAITS AND YIELD RESILIENCE OF SPRING WHEAT GENOTYPES**

**DR. LAMIS ABDELHAKIM PHD; PROF. CARL-OTTO OTTOSEN PHD**

Aarhus University

Warmer and drier weather patterns as a consequence of climate change are negatively affecting the productivity of global cereal crops such as wheat. Thus, it is highly in demand to select robust crops to maintain food supply. Various responses among wheat genotypes are dependent not only on their susceptibility to the stress but also on their biomass and duration of grain-filling. This study aimed to investigate the yield resilience in spring wheat genotypes with different yield potential under the combination of heat and drought stress at ambient CO<sub>2</sub> level. In addition, understand the underlying mechanisms of morpho-physiological and biochemical responses of the different genotypes. Our hypothesis that the resilient genotypes with better photosynthetic activity and water consumption will show less yield reduction compared to the susceptible ones. To assess the performance during anthesis and yield of the genotypes under abiotic stress, gas exchange, water use efficiency, chlorophyll fluorescence and targeted metabolomic profiling are applied. The different mechanisms among the selected genotypes under the combined drought and heat to reduce yield losses are presented. These findings will provide a better understanding of the physiological responses for selecting climate-resilient wheat genotypes. In addition, highlight the necessity of investigating the effect of combined stress types in revealing the genotype potential to cope with stress.

## **INTEGRATIVE “OMICS” TO STUDY THE ROLE OF TERPENOIDS IN DROUGHT STRESS TOLERANCE OF NORWAY SPRUCE**

**MUHAMMAD AHMAD MSC.**<sup>1</sup>; CLARA PRIEMER<sup>2</sup>; DR. DEBOJYOTI CHAKRABORTY<sup>3</sup>;  
DR. MARCELA VAN LOO<sup>1</sup>; DR. CARLOS TRUJILLO-MOYA<sup>1</sup>

<sup>1</sup> Unit of Provenance Research and Breeding, Department of Forest Growth, Silviculture and Genetics, Federal Research Centre for Forests, Natural Hazards and Landscape (BFW);

<sup>2</sup> Unit of Molecular Systems Biology, Department of Functional and Evolutionary Ecology, University of Vienna;

<sup>3</sup> Unit of Forest Growth, Department of Forest Growth, Silviculture and Genetics, Federal Research Centre for Forests, Natural Hazards and Landscape (BFW)

Norway spruce (*Picea abies* (L. Karst.)) is one of the ecologically and economically most important forest tree species in Europe. Based on predicted climate change and the high sensitivity of Norway spruce to drought, its distribution is expected to decrease drastically in most European regions. However, it is unclear which populations may be more tolerant to the upcoming drought. Furthermore, there is a distinct lack of knowledge on the metabolic, molecular and genetic basis of drought tolerance in this tree species, especially on the role of terpenoids, which have hardly been studied. In this project, we aim to investigate the adaptive potential and underlying mechanisms of drought tolerance in Norway spruce populations throughout the species' range. For this purpose, seedlings of Norway spruce from 80 different populations (seed sources) covering the entire climatic range will be subjected to drought stress in a highly controlled environment (climatic chamber) and phenotyped using a joint automated high-throughput phenotyping platform at PHENOPlant (Vienna BioCenter, Austria). We will first integrate 1) high-throughput phenotyping data obtained from RGB, chlorophyll fluorescence and hyperspectral imaging; 2) secondary metabolites (terpenoids, phenolics, and phytohormones) and 3) mRNA-seq profiles. Next, genomic data of populations exhibiting extreme phenotypes will be explored by XP-GWAS, and finally, our integrative approach will allow us to identify adaptive populations in nature to examine our results and define the underlying basis of drought tolerance.

# **PLANT PHYSIOLOGICAL RESPONSES TO DIFFERENT INTENSITIES OF COMBINED DROUGHT AND HEAT STRESS IN DIVERSE WHEAT CULTIVARS**

**NAGASHREE AKULA NAGESHBABU<sup>1</sup>**; DR. EVA ROSENQVIST PHD<sup>2</sup>;  
PROF. CARL-OTTO OTTOSEN PHD<sup>1</sup>

<sup>1</sup> Aarhus University;

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The anomalies of climate change increase the frequency of hot and dry spells. These repeated episodes of high temperatures and drought has a broad impact on wheat production. The climate models also predict the intertwining of high temperatures with drought stress during the critical crop developmental stages, hence, staking global food security. Considering the future climate scenarios, it is important to shift the focus from individual abiotic stress to combined abiotic stress (drought and heat stress) to understand the intricate tolerance mechanisms. Hence, the main aim of my study is to capture differential plant responses to the different severities of combined stress at stem elongation stage in old and modern wheat cultivars. For this study, six wheat cultivars including old and modern cultivars are subjected to different severities of combined temperature and drought stress at stem elongation stage. The plants were exposed to three severities of combined temperature (25/18, 31/24 and 37/30 °C) and drought (40% pot holding capacity) stress. Physiological parameters like CO<sub>2</sub> assimilation rate (A), stomatal conductance (gs), maximum quantum efficiency of photosystem II (FV/FM) and chlorophyll content were recorded on fifth day of combined stress. All physiological parameters (A, gs, FV/FM) showed reduction with increasing severity of combined stress, except for chlorophyll content among the cultivars. The percent reduction of A and gs at different intensities of stress was higher in old cultivars compared to modern ones. However, the FV/FM was similar in all the cultivars at all stress intensities. Thus, overall results portray that at stem elongation stage, modern wheat cultivars show tolerance to combined stress compared to old cultivars.

## **DELVING INTO THE GENETIC ARCHITECTURE OF EARLY VIGOUR IN AUSTRALIAN BARLEY**

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Australian rainfall is highly variable year-to-year with phenomena such as El Niño, La Niña, and the Indian Ocean Dipole predominately driving variability. Despite this, long-term trends of drier conditions during April-October, especially across south-western and south-eastern Australia are evident. This increasing frequency of drought events challenges Australian crop production. To tackle this obstacle and better adapt crops to the changing Australian environment, we need to better understand the genetic eco-system modulating drought adaptation. Yet, drought adaptation is complex and made up of a network of traits and trait-interactions. Early seedling vigour, defined as the rapid development of leaf area, is one such trait and has the potential to improve water-use efficiency and enable drought avoidance. Vigorous early growth in climates which experience late drought reduces evaporative water loss by shading the soil surface from direct solar radiation, allowing more water to be available for crop transpiration. However, if early growth is excessive in a terminal drought, insufficient water may be available to optimise grain-filling. Early vigour also provides other agronomic benefits, such as improved phosphorus and nitrogen uptake as well as enhanced weed competitiveness. This study explored variation for early vigour in barley (*Hordeum vulgare*) and determined its physiological and genetic components. Normalised difference vegetation index (NDVI) was used as an objective, quantitative measure for early vigour across six field trials conducted over two years. Variation for NDVI was explored in a panel of 334 barley individuals, comprised of an Australian relevant doubled haploid population and a subset of a nested association mapping population, and genotyped with 14,984 polymorphic DArT-seq<sup>TM</sup> markers. Single-marker and haplotype-based genome-wide association analyses were performed, and significant QTLs were identified on chromosomes 2H, 3H and 5.

# **THE IMPORTANCE OF PLANT ANATOMY IN HIGH-THROUGHPUT PHENOTYPING STUDIES TO UNRAVEL CROP MORPHO-PHYSIOLOGICAL ACCLIMATION UNDER CHANGING VPDs AND WATERING LEVELS**

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Plant plasticity in adaptation relies on the development of structural and functional traits which affect plant hydraulic and photosynthesis and is deeply influenced by environmental factors and cultivation management. Since today about 50% of the global yield loss are due to climate changes, understanding plants' ability to respond to fluctuations in environmental conditions is critical to tackle climate change and unlock the agricultural potential of crops. Considering that air (increasing VPD) and soil drought are among the principal environmental stressors, the aim of this high-throughput phenotyping experiment was to study how stomatal regulation and water use affect carbon gain and biomass allocation in lettuces acclimated to two different VPDs (low and high) in combination with two watering regimes (well-watered and low-watered) and then subjected to sudden changes in the VPD. To fulfil this purpose plants were characterized using an automated high-throughput phenotyping station at the IPK in Gatersleben by means of an infrared, RGB and fluorescence cameras. At the end of the experimental trials, leaf samples were collected and chemically fixed to analyze the leaf morpho-anatomical development in terms of mesophyll organization, stomata and vein densities and dimensions. The main purpose of the study was to detect the early stress signals in lettuces and to highlight the different degrees of plants' eco-physiological acclimation to the change in VPD, as influenced by anatomical traits. Results demonstrate that well-watered plants under low VPD developed a morpho-anatomical structure which more efficiently drove the acclimation of these plants to sudden changes in environmental conditions. Therefore, to unravel the mechanisms for crop acclimation and forecast possible physiological behavior after climate change-driven sudden environmental fluctuations it is pivotal to complement high-throughput phenotyping with the analysis of anatomical traits.

## EXOGENOUS ORNITHINE APPLICATION IMPROVES WATER STRESS RESILIENCE OF ABA-DEFICIENT BARLEY MUTANT

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The use of amino acids as small molecule-based biostimulants is considered an excellent strategy to enhance plant performance under stress conditions such as drought. Among amino acids, ornithine (Orn) plays a central role in connecting C and N metabolism and is a direct precursor of stress-related metabolites such as polyamines and proline (Pro). However, there is limited information about the effect of the exogenous Orn application regulating water stress tolerance. In this work, the exogenous Orn application (1 mM) was evaluated in two different barley lines [tolerant; *Hordeum vulgare* L. cv. Steptoe (WT) and sensitive ABA-Deficient mutant (AZ34)] under two water regimes [well-watered (WW) or water restriction (WR) until the substrate achieved 40%], combining plant phenotyping (performed in Olophen platform), metabolomics, and gene expression. The Orn application enhanced the growth of AZ34 mutant to the WT levels under both tested growth conditions. The WR significantly increased the foliar temperature of WT compared to AZ34 due to the lower stomatic closure in the mutant. At the beginning of this phase, the WR reduced the photosynthesis rate ( $< V_{cmax}$ ). However, the Orn treatment minimized this damage. The metabolic profile showed increased Orn endogenous levels in both lines treated with Orn under WW conditions but not under WR, pointing to rapid consumption of Orn to synthesize other compounds as polyamines. Contrarily, the treated plants significantly accumulated spermidine and proline (Pro) under WR. Regarding the gene expression, HvP5CS2 [a gene involved in the proline (Pro) biosynthesis] was upregulated in both lines under WR and by the Orn application. HvOAT gene was only upregulated in AZ34 under WR. This result pointed to glutamic acid as the main precursor for Pro synthesis in WT but not in the AZ34 lines. Additionally, the  $\gamma$ -aminobutyric acid (GABA) was significantly accumulated in water-stressed AZ34 lines treated with Orn.

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## **ROOT PHENOTYPING: THE USE OF MINIRHIZOTRON IN FIELD PLATFORMS**

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ARVALIS-Institut du Végétal

In the current context of adaptation to climate changes, while taking into consideration multi-plant, agro-ecological approaches, observations of the aerial part are no longer sufficient and a better understanding of the root function of the cultivated plant in interaction with its environment is needed.

In order to characterise root system under field conditions, ARVALIS invested in the use of minirhizotrons. The measurement consists of positioning transparent tubes in the soil at a 45° angle and introducing a rotative scanner that acquires images at different depths. A set of processing algorithms allows first the detection of the percentage of root impacts per cm of soil depth by a segmentation based on deep learning and secondly to extract physical measure like root length density (mm o roots per cm<sup>2</sup> of soil) and the average root diameter per unit of soil and per depth.

From a methodological point of view, the experiments, carried out in 2020 and 2021 on various locations, enabled us to streamline data processing, standardise graphic outputs, compare the « impact » approach with the « tracing » approach, to measure the impact of hydric stress on root development and to evaluate the genetic variability of a small panel of wheat.

Initially, this tool allows us to evaluate the resilience of varieties to constraining conditions (deficiencies, excesses) and will soon be deployed to evaluate the impact of biointrans or biostimulants on root development, to evaluate innovative cropping systems, etc.

# **SENSOR-BASED ANALYSIS OF GRAPEVINE VITALITY AND GRAPE QUALITY AFTER TREATMENT WITH SOIL ORGANIC AMENDMENTS**

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A wide range of phenotypic traits can be used to assess grapevine vitality of which leaf chlorophyll content is among the most important. It is known to be negatively affected by stress and can thus be used as an indicator for plant vigor. Several sensors are currently available to determine chlorophyll content of which the Dualex sensor provides high correlation to ground truth data. Other important aspects are grape quality and resilience towards Botrytis bunch rot – one of the most important fungal diseases in cool climate viticulture. Since no resistance loci to Botrytis have been described, resilience to the pathogen is based solely on physical barriers. Novel sensor techniques such as 3D scanners can be used to assess these traits objectively and non-invasively.

In this study, the effects of different organic soil amendments on the above-described traits should be investigated. In the context of climate change mitigation, vineyard subsoils could perform as long-term carbon storages, since they are tilled only once before planting and are then left at rest for several decades, which might prevent a rapid turnover of organic matter. In order to test this hypothesis, two organic amendments (greenwaste compost and biochar compost substrate) were incorporated in 30-60 cm depth in a vineyard before planting with the fungus-resistant grapevine cultivar 'Calardis Musqué'.

The impact of this deep incorporation on grapevine vitality and grape quality was investigated using several sensor-based approaches. The Dualex sensor revealed significant differences in chlorophyll content between plants grown in control rows (no amendment) and the two amendments with the latter having higher chlorophyll contents. However, chlorophyll did not vary significantly between plants grown on the two amendments. Preliminary results further indicate variations in berry physical traits and thus in Botrytis resilience depending on the respective organic amendment.

# HIGH THROUGHPUT HYPERSPECTRAL PHENOTYPING OF PHOTOSYNTHESIS RELATED PARAMETERS FROM LEAF TO CANOPY SCALE

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Increasing crop yield is critical to meet growing agricultural demands. One opportunity is to increase the efficiency of photosynthesis at scales from the leaf to the ecosystem, however this necessitates high-throughput phenotyping techniques to explore both natural and genetic variation among crop species and cultivars. The non-invasive nature of hyperspectral imaging is increasingly used to phenotype photosynthesis and photosynthetic physiology and its use has shifted research efforts into efficiently mining spectral information for high-throughput phenotyping. Here, we describe the development of data analytic pipelines that leverage proximal sensing platforms and data sources including both reflectance spectra and solar-induced fluorescence (SIF) for quantifying photosynthetic performance at leaf and canopy levels for multiple crop genotypes. Specific phenotypes included photosynthetic physiology parameters that are represented by the maximum carboxylation rate ( $V_{cmax}$ ) and the maximum electron transport rate ( $J_{max}$ ). Time-synchronized hyperspectral point-based measurements and canopy-based images from 400 to 1000 nm and irradiance measurements of sunlight under clear-sky conditions were collected for capturing reflectance spectra and solar-induced fluorescence (SIF) and SIF-related parameters. Within 30 minutes of spectral measurements, ground-truth  $V_{cmax}$  and  $J_{max}$  were obtained from portable leaf gas exchange system. Our results suggested both reflectance spectra and SIF can provide accurate estimations of  $V_{cmax}$  and  $J_{max}$ . The developed platforms and techniques offer a high-throughput screening tool for crop trials aimed at identifying increased photosynthetic capacity. The results from a range of wild-type cultivars and from genetically modified germplasm demonstrate the relative benefits in using proximal sensing at multiple scales to accelerate crop breeding processes based on traditional or gene editing techniques to enhance photosynthetic efficiency.

## **A VISUAL AND UAV BASED COMBINED PHENOTYPING METHODOLOGY REVEALS MULTIPLE AND DIVERSE RESPONSES TO DROUGHT IN A EUROPEAN SOYBEAN COLLECTION**

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ILVO

Current soybean production in Europe is insufficient to cover the increasing demand for proteins. Breeding cultivars that are adapted to current and future European climate conditions is required to support the expansion of cultivation area. Increased frequency and severity of drought spells implies that newly bred soybean cultivars should be drought-resistant. However, breeding for drought resistance is not simple as drought scenarios are diverse, plant responses are complex and the impact on yield and quality varies with developmental stage and drought spell duration. Therefore, we need field phenotyping methodologies to screen drought resistance traits under variable conditions and drought scenarios relevant for Western Europe.

In the EUCLEG EU project at ILVO, the performance of a large collection of soybean accessions was evaluated combining traditional phenotyping methods (e.g. canopy wilting or developmental stage scores) with imagery captured with a UAV. Two trials (control = well-watered conditions; drought = rain-out shelters) were sown with 359 soybean accessions from diverse origins in an augmented row-column design for two growing seasons determining two different drought conditions (3-4 weeks and 6-7 weeks)[1]. Multi-temporal UAV imagery was used to screen our collection for growth in terms of canopy coverage and height. Thereto, biologically interpretable growth and development parameters were inferred from fitting growth curves[2]. Then, we identified the plant traits that could be used to select for drought resistance. To quantify the accessions' responses, a drought response index was calculated for every trait. As expected, varied responses were observed across conditions. As an example, the reduction in seed number per plant was moderate (17%) to severe (38%) for short and long drought duration. Here we present the results of both years and a screening and data processing pipeline to phenotype soybean for growth and drought resistance parameters.

## **DIGITAL TWIN OF A PLANT FACTORY/RESEARCH FACILITY : SIMULATION OF RGB-D DATA WITH THE CPLANTBOX FSPM TO SOLVE OCCLUSION ISSUES IN NADIR VIEW THROUGH DATA ASSIMILATION**

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The Digital Twin (DT) concept in plant science can be considered at the border between phenotyping and modeling. While a DT is characterized by several characteristics and can be declined in any or all of the six components detailed by Verdouw et al. (2021), the common denominator is the twinning process at the core of the interaction between the physical and virtual entity of the DT. In the context of research on photosynthesis response to variable frequency LED lighting in controlled environment horticulture, we present advances in the twinning process between individual *Lactuca sativa* L. plants monitored through RGB-D cameras and their virtual counterparts modeled in the CPlantBox functional-structural plant model (FSPM). In the growth system, the phenotyping RGB-D cameras are fixed with a nadir point of view over the growing plants. While making observations very stable, this angle tends to generate occlusions of lower leaves by newly generated leaves. To address occlusion issues, we hypothesize that the CPlantBox FSPM parametrization can be automated through data assimilation. To do so, we simulate RGB-D data through in silico experiments to reproduce, plant geometry and occlusions in similar growth conditions. The RGB-D data can then be matched with the output of the in silico experiments to compare features extractible through image analysis (e.g. projected area, optical flow) and 3D point cloud analysis (e.g. convex hull volume, leaf orientation).

## HIGH-THROUGHPUT PHENOTYPING OF MAIZE GROWTH DYNAMICS UNDER NITROGEN AND WATER STRESS

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Limited water availability and nitrogen deficiency are the most restricting factors for maize growth. Although maize susceptibility to drought is the highest at the reproductive stage, water shortage during vegetative development can significantly reduce grain yield. The aim of this study was to assess the genetic variation of growth dynamics in 20 maize inbred lines (ILs) through automated phenotyping based on visible light (RGB) imaging in a greenhouse under optimal (C), nitrogen (N), water (W), and combined nitrogen and water stress (NW) conditions. Thirteen biomass-related and morpho-physiological image-derived traits acquired at 33 time points were selected, covering developmental stages from five fully developed leaves to 10-13 fully developed leaves, depending on treatment. Estimated biomass volume (EBv), a proxy for biomass in our study, showed to be the most sensitive image-derived morphological trait to water and combined water and nitrogen stress with the maximum reduction of 53 and 54% in W and NW compared to C at the time of maximum water stress. Genotypic variation of EBv within each treatment was consistently high and over 80%, while the analysis across treatments showed that as stress got higher the genetic variation got lower (32-38%) and the interaction term became more prominent. Phenotypic correlations between EBv and other image-derived traits at early stages showed that under no stress or mild stress conditions, morphological traits were more appropriate than color-related traits for the prediction of biomass accumulation, while under more severe stress conditions, color-related traits and chlorophyll fluorescence are more useful to differentiate genotypes for high biomass. To investigate whether ILs can express EBv in similar patterns, temporal profiles were clustered by using the fuzzy c-means clustering algorithm, and two temporal dynamics of EBv patterns among the studied ILs in each treatment were identified.

# **HIGH-THROUGHPUT PHENOTYPING REVEALS DIFFERENTIAL TRANSPIRATION BEHAVIOUR WITHIN THE BANANA WILD RELATIVES HIGHLIGHTING DIVERSITY IN DROUGHT TOLERANCE**

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Crop wild relatives, the closely related species of crops, may harbour potentially important sources of new allelic diversity for (a)biotic tolerance or resistance. However, to date, wild diversity is only poorly characterized and evaluated. Banana has a large wild diversity but only a narrow proportion is currently used in breeding programmes. The main objective of this study was to evaluate genotype-dependent transpiration responses in relation to the environment. By applying continuous high-throughput phenotyping, we were able to construct genotype-specific transpiration response models in relation to light, VPD and soil water potential. We characterized and evaluated six (sub)species and discerned four phenotypic clusters. Significant differences were observed in leaf area, cumulative transpiration and transpiration efficiency. We confirmed a general stomatal-driven 'isohydric' drought avoidance behaviour, but discovered genotypic differences in the onset and intensity of stomatal closure. We pinpointed crucial genotype-specific soil water potentials when drought avoidance mechanisms were initiated and when stress kicked in. Differences between (sub)species were dependent on environmental conditions, illustrating the need for high-throughput dynamic phenotyping, modelling and validation. We conclude that the banana wild relatives contain useful drought tolerance traits, emphasising the importance of their conservation and potential for use in breeding programmes.

## **A PHENOTYPER'S VISION FOR IMPROVING PHOTOSYNTHESIS AND YIELD OF C3 CROPS UNDER RISING CO<sub>2</sub> LEVELS**

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Atmospheric [CO<sub>2</sub>] will soon have doubled compared to pre-industrial levels while temperatures rise. C<sub>3</sub> crops do not fully use the potential benefits of elevated [CO<sub>2</sub>] (e-CO<sub>2</sub>) for greater C assimilation. A main bottleneck is sink limitation reducing photosynthesis (acclimation). More vigorous and plastic sinks throughout the life cycle (branching, organ number and potential size) can increase biomass and yield. Recent evidence also suggests that adaptive plasticity of specific leaf area can improve utilization of e-CO<sub>2</sub> and high radiation levels. Phenotyping and genetic analysis of such traits will open opportunities for grain, biomass, and also root production contributing to soil C sequestration ("4p1000") and drought avoidance. Natural variation and synthetic traits (e.g., via T6P-based sink modification) should be systematically explored.

As CO<sub>2</sub> becomes more abundant, we also propose a renewed focus on light interception and quantum efficiency (QE). As QE is greater at sub-saturation, enhanced photosynthetic contribution of lower leaf strata would be efficient, namely in high-LAI crops. Such traits already contributed to the Green Revolution but may be revisited with respect to pigment (antenna) distribution in the canopy and electron transport efficiency. Lower canopy strata also benefit heat avoidance via transpiration cooling and partial shading. Crop microclimate thus deserves more attention in tackling global warming.

This vision of desirable adaptations calls for improved functional-structural models that may guide new phenotyping strategies, genetic research, and ultimately quantitative concepts for ideotypes addressing future crop environments.

## **EARLY SELECTION OF WHEAT GENOTYPES USING ROOT AND SHOOT TRAITS AT SEEDLING STAGE**

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Screening genotypes at early growing stage is considered important to breeders and researchers in crop improvement. A set of 101 wheat genotypes were grown in optimum environment at the onset of two-leaf stage, 10 days after germination, in a hydroponic phenotyping system. This study aimed to characterize phenotypic variability in wheat root and shoot morphological traits at early vegetative stage and to determine the relationship among shoot and root traits. Considerable phenotypic variation existed for seminal roots length, root angle (measured between the first pair of seminal roots) and branching depth of primary root. Strong positive correlations were identified for some key root traits (i.e., root length, root dry mass, and root specific weight) and shoot traits (i.e., shoot dry mass and shoot specific weight). The cluster analysis, based on observed traits, showed the homogeneity of genotypes originating from the region of the South-East Europe. A subset of 18 genotypes with different/exceptional root and shoot characteristic have been chosen as parents for 16 targeted crosses to produce novel germplasm in wheat for improving early vigour and tolerance to drought. The average hybrid vigour across all combinations and traits under osmotic stress was -2.3% and 8.5% in non-stress and osmotic stress conditions, respectively. No one crossing combination showed positive hybrid vigour for all traits.

## **NATURAL GENETIC DIVERSITY IN PHOTOSYNTHESIS AND GROWTH OF ARABIDOPSIS THALIANA UNDER DIFFERENT NIGHTTIME TEMPERATURE**

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Plants coordinate the balance between carbon assimilation and utilization (e.g. growth). Understanding the mechanisms that regulate carbon acquisition, storage, mobilization and usage in changing environments is critical to enhance crop yield by manipulating these processes. We investigated natural genetic diversity in photosynthesis and growth of *Arabidopsis thaliana* at two different nighttime temperatures (20°C or 15°C) while keeping the same daytime temperature (26°C). Phenotypic traits related to photosynthesis and rosette growth were collected for GWAS to identify candidate genes that influence the observed natural variations. Photosynthetic traits were monitored in 3-week-old plants using the light-induced fluorescence transient (LIFT) method under the growth conditions in a climate chamber at predawn (in a dark-adapted state at the nighttime temperature of 20°C or 15°C) and around midday (under 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity and at 26°C). In parallel, relative growth rate was determined by 2-D image analysis of projected leaf area. Rosette growth differed between the two nighttime temperatures, with plants grown in 26°C/15°C (day/night) having higher or lower relative growth rate than those grown in 26°C/20°C. The chlorophyll fluorescence parameters also showed variations in photosystem II (PSII) quantum yield and reduction/re-oxidation kinetics of the primary quinone acceptor (QA) between the two temperature regimes. Plants in 26°C/15°C had on average slightly higher maximum quantum yield but lower effective quantum yield of PSII than plants in 26°C/20°C. As expected, QA reduction and re-oxidation were slower in dark-adapted plants at 15°C than 20°C. The results of GWAS related to the natural variations of photosynthetic and growth traits in the two nighttime temperature conditions will be discussed.

# **TOWARDS PHENOTYPING OF SOURCE-SINK RATIO AS A PROXY FOR PHOTOSYNTHESIS AND YIELD RESPONSE TO ELEVATED ATMOSPHERIC CO<sub>2</sub> IN C<sub>3</sub> CROPS**

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The increase in atmospheric CO<sub>2</sub> concentration (e-CO<sub>2</sub>) associated with climate change will significantly impact agricultural crop productivity. Varieties of C<sub>3</sub>-type cereals vary greatly in their photosynthetic response to e-CO<sub>2</sub>, in large part because of a variable capacity to adjust sink capacity to an increased C source. Optimizing C source-sink relationships might provide gains in photosynthesis response to e-CO<sub>2</sub> and thereby increase biomass and yield.

An experiment was carried out on IR64 rice to study the diurnal dynamic of photosynthetic parameters at mid grain-filling stage under modified of C source-sink balance. Control plants were compared to panicle-pruned plants (sink limitation) at two CO<sub>2</sub> levels: ambient (400ppm) and e-CO<sub>2</sub> (800ppm for 14d from heading) (source boosting). Flag leaf and internode NSC (starch, sucrose and hexose concentrations) were measured, as well as photosynthetic parameters on the flag leaf of the main stem.

TPU (triose phosphate utilization) was identified as the main biochemical driver of photosynthesis down-regulation by sink limitation, occurring predominantly in the afternoon. A negative correlation was found between TPU and markers of sink limitations: leaf [sucrose] and the local C source-sink ratio (LSSR), computed as the ratio between flag leaf area and grain number of the adjacent panicle on the main stem.

A second experiment was carried out in order to confirm these results among 5 indica genotypes having constitutive variation of LSSR: Plants were compared under two continuous CO<sub>2</sub> treatments, 400 and 800 ppm. A negative relationship between genotypic LSSR and photosynthetic capacity under e-CO<sub>2</sub> was confirmed. Plant biomass and grain yield response to e-CO<sub>2</sub> were negatively correlated with LSSR as well, suggesting a key role of C sink capacity in enhancing C<sub>3</sub> plant productivity under e-CO<sub>2</sub>.

Further studies are under way to confirm LSSR and similar proxy traits as phenotyping and selection tools for improved e-CO<sub>2</sub> response.

## EXPLORING PHOTOSYNTHESIS RATES ACROSS THE BRASSICACEAE FAMILY

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After demonstrating that Brassicaceae species *Hirschfeldia incana* can achieve very high photosynthesis rates at high irradiances ( $>1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to many other species with C3 or even C4 photosynthesis, we proceeded to further explore photosynthetic efficiency in the Brassicaceae family by means of high-throughput phenotyping. The hypothesis underlying this experiment was that light-use efficiency differences could be related to the phylogenetic architecture of the Brassicaceae family. Furthermore, we hypothesized that the well-documented divergence between the Rapa/Oleracea and Nigra clades of tribe Brassiceae may have given rise to higher photosynthetic capacity species in the Nigra clade, to which *Hirschfeldia incana* belongs to. We thus grew a panel of 11 Brassicaceae species and one C4 reference from family Amaranthaceae in a climate chamber equipped with the PSI PlantScreen™ Robotic XYZ System. We measured photosynthetic performance twice a day over 28 and 21 days of growth for slower- and faster-growing species, respectively. We complemented this dataset with a series of measurements conducted after destructive sampling of all species, aiming to further explore specific aspects of the photosynthetic process. In detail, we extracted leaf pigments to estimate photosystems' antenna sizes, we studied the distribution and dimensions of stomata to determine each species' capacity for gas exchange, and we investigated the correlation between a number of leaf anatomical parameters and photosynthetic performance. We hereby provide the first summary of the extensive datasets we generated, explore correlations between the phylogenetic architecture of the Brassicaceae family and photosynthesis efficiency, as well as the other parameters we studied, and reflect on the limitations of using a system developed for phenotyping smaller plant species to study larger, more complex species.

# **SPECTRAL ESTIMATION OF FIELD CROPS DROUGHT RELATED TRAITS FROM SPACE AND AIR**

**DR. ITTAI HERRMANN**

The Hebrew University of Jerusalem

Drought is a limiting factor that can reduce grain yield in rainfed crops. In order to isolate drought effect on the plants there is a need to minimize the effect of all the other parameters. In Israel wheat is rainfed grown while chickpea is rainfed in the beginning of the season and then, in most cases, irrigated. To meet this challenge of controlled water stress, a rainout shelter facility was built to automatically cover part of the field only while it rains. During the 2022 winter, about 1800 wheat plots (more than 200 cultivars) were grown in one field under two irrigation regimes: well-watered (rainfed) and water-limited (rainout shelter), with three replicates. The plants were just harvested, while throughout the season imaged by hyperspectral UAV-borne line scanner camera and measured for leaf area index (LAI) on ground. During the spring of 2019 and 2020, commercial chickpea fields, one per year, were irrigated in five levels each, to strive meeting the drought effect isolation. VENUS (French-Israeli satellite, 11 spectral bands) imagery was acquired throughout these two seasons, in parallel to ground LAI and water leaf potential ground (LWP) data collection. The spectral data was analyzed by calibrating and independently validating partial least squares regression (PLS-R) models for LAI and LWP resulting in validation R<sup>2</sup> values of 0.62 and 0.68 and root mean square error (RMSE) values of 0.74 m<sup>2</sup> m<sup>-2</sup> and 0.18 MPa, respectively. The wheat data is yet to be fully analyzed but with preliminary promising results. It was concluded that spectral data from space and air is a valid phenotyping tool to assess drought effect on field-grown crops.

## PHENOTYPING FOR HEAT TOLERANCE IN CHICKPEA USING TOLERANCE INDICES AND STABILITY PARAMETERS

**PHD AVINALAPPA HOTTI<sup>1</sup>; DR. RAGHUNATH SADHUKHAN SADHUKHAN<sup>2</sup>**

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High temperature is an important factor affecting chickpea growth, development and grain yield. As there is huge gap between the potential and actual yield, understanding plant response to high temperature is a key strategy in breeding chickpea for heat tolerance. Population or lines developed by prebreeding program contains diverse sources of useful traits, e.g. heat tolerance, and such population were used in the current investigation. These populations are among 2200 introgression lines, which were recently developed by complex three way crosses involving two wild Cicer species at ICRISAT, Hyderabad. A large set of chickpea accession comprises 100 prebreeding population including four standard checks (Annigeri-1, BG 256, DCP 92-3 and Anuradha), were phenotyped for specific traits (days to flowering, pods per plant, harvest index and CTD) under different planting conditions (heat stressed and non-stressed) to identify heat tolerance over two years, 2017-2019. Identified twenty diverse breeding lines were further subjected to stability analysis to achieve yield stability in such planting conditions. Days to flowering found negative association with maximum temperature, indicates lines were exposed to temperatures up to 37 °C. Pods per plant and harvest index were highly related to improve seed yield under stressed conditions ( $R^2$  0.50). Two way ANOVA revealed significant interaction of genotype and environment for these traits, designates lines responded differently to the different planting conditions over the years. RSG 963, Vijay, ICCV 10, Annigeri 1, JG 14, RSG 888, Pusa 372, RSG 974, JAKI 9218 and RSG 945 were found adaptable, stable, adaptable and stable for studied traits and highly promising due to their presence of G x E interaction. Also, produced greater CTD values (>1) further highlighting their resistance to heats tress. Average CTD of ICCV 10, JAKI 9218, when regressed against grain yield under heat stress showed a positive relationship.

# **NOVEL GERMLASM SOURCE FOR SUSTAINABLE INTENSIFICATION OF CHICKPEA CULTIVATION IN INDIA**

**PHD AVINALAPPA HOTTI**

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

As the identification of phenological traits associated to yield improvement under conservation agriculture (CA) can throw some light on adaptation pattern to changing climatic condition. In reference to it, germplasm carrying genetic desired traits is only possible strategy to sustain intensification of chickpea cultivation towards increasing demand for production in the context of growing population. As a result, an investigation was conducted to identify promising lines, valuable germplasm for CA, and effective molecular tools to support knowledge-based breeding new valuable varieties in collaboration with ICRISAT, Hyderabad. A total set of 600 accessions comprising reference collection, including varieties, elite lines and landraces were evaluated for their yield performance (biomass as main trait for yield maximisation in CA) over two years under CA across India, using augmented design. Selected set of accessions (300) were phenotyped for studied traits under the same conditions. Site regression analysis, generated phenotypic data related to respective environments for studied traits were identified elite lines to be used in pre-breeding purposes to CA. Simultaneously, further set was phenotyped for designated pests and disease (wilt and pod borer) reaction in hot spot field areas in controlled conditions. Then, in-depth statistics for analysis of multi-environment phenotypic variation was identified the best genotypes, with best for target traits. Based on grain yield performance of each accessions, variance components due to genotypes ( $g^2$ ) and error ( $e^2$ ), G x E interaction ( $ge^2$ ) were estimated and significance determined, thereby adaptability and yield stability were assessed. Correspondingly, meta-analysis was performed to investigate grain yield differences of different lines grown under different environments of CA. Phenotypic performance of target germplasm panels would reveal CA-based agronomy for adaptation to specific cropping system.

## **MICROCLIMATE-EFFECTS OR GENETIC DIFFERENCES? WHAT IS DRIVING THE CORRELATION BETWEEN CANOPY TEMPERATURE AND HEIGHT OF WHEAT?**

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DR. HELGE AASEN

<sup>1</sup> ETH Zurich

Thermography is a potentially powerful tool to assess canopy temperature (CT) and identify beneficial plant traits related to stomatal conductance. In field phenotyping experiments, taller varieties generally tend to have cooler canopies. It is yet unclear, to what degree this effect is driven by a microclimate effect or genetic factors. Microclimate effects relate to the fact that smaller varieties are more frequently surrounded by taller canopies while tall varieties frequently stand out. Due to the resulting confounding effect of neighbouring plant heights on temperature, integrating height as a covariate is challenging. To disentangle the neighbouring effects from genetic effects we selected 40 tall and 40 small varieties from the GABI wheat panel. The varieties were grown in the field in a split-plot design in which the arrangement (intermixed or separated according to height) was the whole-plot factor and variety was the split-plot factor. Thus, within a replication, the tall and small varieties were either mixed in a chessboard-like arrangement or grouped in two large blocks. CT measurements taken by a drone indicate that there is a dual effect of height on CT. In the mixed arrangement, the small varieties were on average 0.66 C° warmer, while in the uniform arrangement, they were only 0.38 C° warmer compared to their tall counterparts. We conclude that both microclimate effects and genetic effects drive the relationship between height and CT. We will discuss the efficiency of using the height differences as a covariate for correcting for microclimate effects.

## UNCOVERING THE POLYAMINE METABOLISM ROLE IN ARABIDOPSIS MUTANT RESILIENCE TO WATER STRESS

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JONATHAN CÁRDENAS-GALLEGOS<sup>1</sup>; PROF. DR. MARGARITA RODRÍGUEZ-KESSLER PHD<sup>2</sup>;  
PROF. DR. LUKÁŠ SPÍCHAL PHD<sup>1</sup>; DR. NURIA DE DIEGO PHD<sup>1</sup>

<sup>1</sup> Palacky University;

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Plants are sessile organisms exposed to several environmental stimuli; biotic and abiotic stresses. To deal with stress plants have developed different responses; molecular, metabolic, and physiological. Drought is one of the most studied abiotic stresses because it compromises plant growth, seed germination, development, and production. In the last years, interest in polyamines (PAs) has come back due to their potency as very effective stress alleviators. PAs are low molecular weight aliphatic amines; the diamine putrescine (Put), the triamine spermidine (Spd), and the tetraamine spermine (Spm) are the most studied ones. However, little is known about how polyamine metabolism can help to avoid stress. In *Arabidopsis thaliana*, two genes encoding arginine decarboxylase (ADC) partially regulate PA synthesis, whereas five genes of polyamine oxidase (PAO) are involved in PA back-conversion and terminal catabolism. We phenotyped seven *Arabidopsis* knockout lines (Atadc1-1, Atadc2-1, and Atpao-1 to Atpao5-1) under different water regimes [(well-watered (WW) or drought (D) conditions] using our Olophen platform equipped with RGB, chlorophyll fluorescence, and IR sensors. The complex phenotyping showed that mainly Atadc2-1 and Atpao5-1 lines reduced the rosette growth under WW and D, whereas Atadc1-1 was only affected by the water restriction. These three lines also reduced the photosynthesis efficiency and increased leaf temperature under D. This finding suggests that both ADC and PAO5 genes are involved in plant stress response, and hence, *Arabidopsis* resilience under D. Besides, our results showed that ADC2 must also be regulating plant growth under WW conditions. Altogether, we demonstrate that PAs' synthesis and catabolism are essential for *Arabidopsis* resilience to water stress.

## **GRASS LEAF ADAPTATIONS DUE TO THE LOW TEMPERATURE**

**KANYANAT KASETSUNTORN**; PROF. COLIN OSBORNE; DR. PASCAL-ANTOINE CHRISTIN

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C4 grasses originated from the tropical and subtropical regions which have a high average temperature all year. However, there is some evidence showing that some C4 grasses can survive and grow in areas that experience low temperatures, and that some species have adapted to tolerate chilling and freezing events. Although alternations in leaf anatomy in relation to cold tolerance have been found among some C4 grasses, previous studies have been limited to particular species and we lack general understanding. In this study, we study the alterations in leaf anatomy that may be involved in freezing tolerance among C4 grass lineages, testing whether they share the same changes or not. The results show that Constitutive freezing resistance was associated in leaves with a small metaxylem vessel diameter, but thick minor veins, which reduce the risk of freeze-thaw embolisms disrupting hydraulic supply. Cool climate species using C4 photosynthesis have also evolved larger bundle sheath cells than their tropical counterparts to compensate for the thermal limitation of enzyme activities. Finally, small mesophyll, epidermis and bulliform cell sizes increase relative cell wall thickness, reducing the risk of cells collapsing under freezing. Structural modifications of leaves have therefore evolved consistently and correlated with cold tolerance across multiple temperate or alpine grass lineages, providing evidence that they are adaptative at low temperatures.

## **EVALUATING WATERLOGGING STRESS RESPONSE IN BARLEY (HORDEUM VULGARE L.): AN IMAGE-BASED PHENOTYPING APPROACH**

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Waterlogging is expected to become a more prominent yield restricting stress for barley production as rainfall frequency increases in many regions with climate change. The duration of waterlogging events in the field is highly variable throughout production, this variation is also observed in experimental waterlogging studies. To screen for waterlogging tolerant varieties an optimal duration and setup of simulated waterlogging stress in controlled conditions is presented using image-based phenotyping. Six protocol durations, 5, 10, 14 days of stress with and without 7 days of recovery were tested. Two genotypes of spring barley grown in glasshouse conditions were subjected to each of the six protocols. Stress was applied at the three leaf stage. Waterlogging conditions were applied by maintaining the water level at 1cm above soil level. Top down and side view RGB images were taken daily over the course of the different protocols using the PSI PlantScreen™ imaging platform. Shoot biomass and root imaging data was also analysed to determine the optimal stress protocol and determine the physiological responses of barley in response to waterlogging. Time-series comparison of stress response allows determination of the ideal period of stress and recovery for comparing waterlogging tolerance. The most recent results will be presented to propose an optimised protocol, aiming to improve comparison among waterlogging studies.

# **PRECISION PHENOTYPING ACROSS LIFE CYCLES TO DECIPHER DROUGHT-ADAPTIVE QTLs OF WILD EMMER WHEAT (TRITICUM TURGIDUM SSP. DICOCOIDES) INTRODUCED INTO ELITE WHEAT VARIETIES**

**MADITA LAUTERBERG**<sup>1</sup>; PROF. DR. YEHOSHUA SARANGA; MATHIEU DEBLIECK;  
TAMAR KRUGMAN; DRAGAN PEROVIC; FRANK ORDON; ANDREAS GRANER;  
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Drought events or the combination of drought and heat conditions are expected to become more frequent due to global warming, and wheat yields may fall below their long-term average. One way to increase climate-resilience of modern high-yielding varieties is by their genetic improvement with beneficial alleles from crop wild relatives.

In the present study, the effect of two beneficial QTLs introgressed from wild emmer wheat and incorporated in three wheat varieties was studied under well-watered conditions and under drought stress using non-destructive High-throughput Phenotyping (HTP) throughout the life cycle.

The yield-enhancing effect of the introduced QTLs under drought conditions that were previously demonstrated under field/screenhouse conditions in Israel, could be confirmed in a greenhouse experiment using HTP. Daily phenotyping enabled to decipher the mode of action of the QTLs in the different genetic backgrounds, and yielded information about which image-derived traits are informative at which developmental stage of the entire life cycle.

The estimated biovolume and plant height were used to allow a precise comparison of the severity of the effect and the timing of the effect on the tomb stress between the recurrent parent and the corresponding Near Isogenic Line (NIL) and also serve to better explain interrelationships between manually measured parameters.

Furthermore, the color parameters in this study allow meaningful conclusions to be drawn about the effect of drought stress on the ripening time. The color parameter hue showed that the higher thousand grain weight (TGW) was due to the stay-green effect, i.e. slower senescence associated with slower chlorophyll degradation in the final ripening stage of NIL under drought stress.

## **GROWING PLANTS IN A DIURNAL CONSTANT LIGHT ALTERS DIURNAL NPQ CAPACITY AND NPQ KINETICS**

**MARTINA LAZZARIN**; DR. STEVEN DRIEVER; MAARTEN WASSENAAR;  
PROF. DR. LEO MARCELIS; PROF. DR. WIM VAN IEPEREN

Wageningen University

Photoprotection by non-photochemical quenching (NPQ) prevents damage to the photosynthetic systems when absorbed sunlight is in excess. Many processes in plants follow diurnal patterns, either externally induced or regulated by endogenous clocks. NPQ is often investigated on plants grown in climate chambers under a diurnal constant white light intensity. This diurnal constant artificial light regime usually facilitates plant growth well, but strongly deviates from a natural parabolic-shape sunlight's intensity profile over a day. If and how this artificial growth light regime influences NPQ during the day is not known. Here, we grew plants in diurnal parabolic and diurnal constant intensity. We then assessed diurnal changes in steady-state NPQ capacity and the NPQ decay rate towards steady state after the initial rise in response to a switch from low to high-light. We measured the violaxanthin de-epoxidation state (DES) and leaf gas-exchange prior and after a 30 min high light period at different time points in the day. Plants grown in diurnal constant light regime showed less diurnal variation in DES, stomatal conductance and NPQ capacity, and reached NPQ steady state earlier. We conclude that using a diurnal constant intensity during growth in climate chambers limits progress in understanding the physiological bases of NPQ flexibility.

# **THE ESSENCE OF NON-PHOTOCHEMICAL QUENCHING IN HIGHER PLANT PHOTOSYNTHESIS**

**LOUISE LOGIE**

Wageningen University & Research

Plants constantly adapt to changing environments. A plant that is able to adapt to these changes more rapidly than its neighbor, is a plant that will perform better than its neighbors. Robustness of a crop is going to be increasingly important when facing climate changes in agricultural settings. One of the mechanisms to which this is pertinent is photosynthesis. The photosynthetic apparatus is constantly subjected to light fluctuations in the field. In the light reaction of photosynthesis, adaptability is regulated in the chloroplast through a mechanism called non-photochemical quenching (NPQ). NPQ protects a plant against photodamage caused by changes in light intensity by decoupling the light harvesting centers from the photosynthetic systems.

The sensitivity of the system for when to start NPQ and how strong the NPQ response is, varies from plant to plant. Genetic variation for NPQ is of great value to improve our understanding of NPQ and other adaptability traits, and also to increase crop productivity. In order to gain genetic insights into NPQ, high-throughput phenotyping is necessary. The NPEC facilities at Wageningen University & Research provide this opportunity. Pilot experiments in other phenotyping facilities from Photon System Instruments have already yielded interesting insights in response to light fluctuations. Using *Arabidopsis thaliana* as a crop model we have identified different parts of the photosystems, which seem to be subject to feedback loops that influence the presence and strength of NPQ.

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## **FIELD PHENOTYPING THE FUTURE CROPS: NON-DESTRUCTIVE HIGH THROUGHPUT PHENOTYPING ABOVE AND BELOW GROUND (FABPS) COMBINING CLOSE REMOTE SENSING AND ELECTRICAL RESISTIVITY TOMOGRAPHY**

**DR. PETER LOOTENS**; DR. IRENE BORRA-SERRANO; DR. TOM DE SWAEF;  
DR. GUILLAUME BLANCHY; DR. TOMMY D'HOSE; BART VLEMINCKX;  
PROF. DR. SARAH GARRÉ; PROF. DR. ISABEL ROLDÁN-RUIZ

ILVO

Continued improvements in crop performance are needed to cope with climate change and to keep pace with population growth. To tackle these global challenges, novel approaches are required that help to investigate the genetics of agriculturally important crop traits and to accelerate the development of superior plant varieties. Plant phenotyping covers the description and characterization of complex plant traits using non-destructive tools in the context of plant research and crop breeding. As part of the Emphasis-Belgium project (funded by FWO) and the FutureAdapt project (funded by Government of Flanders), FABPS, a state-of-the-art high throughput field phenotyping installation is being built. This infrastructure will enable investigating the response of crops to drought and salinity stress using mobile rain-out-shelters (6 x 300 m<sup>2</sup>). Aboveground phenotyping will be based on the use of a UAV equipped with multiple sensors (e.g. De Swaef et al. 2021 (forage grasses)) and belowground phenotyping on the use of electrical resistivity tomography (e.g. Blanchy et al. 2020) at high spatial resolution (0.2-4 cm and 10 cm, respectively). Regular crop monitoring throughout the entire growing season will allow describing the plant responses to the stress applied but also investigating the recovery in a realistic crop production environment. A control field with the same trial serves as reference. Growth curves per plant/genotype/plot (e.g. Borra-Serrano et al. 2020 (soybean)) are fitted to decipher plant responses and to extract the trait of interest in a high-throughput manner for large sets of genotypes (up to 800 mini-plots can be evaluated simultaneously, e.g. Saleem et al. 2022 (soybean), Muylle et al. in prep. (red clover)). Access modalities are currently being defined, but it is our intention to open this facility to universities, research institutes and industry. The installation is being set up and will be tested in 2022-2023, being operational from 2023 onward.

## PHENOTYPING ION-INDEPENDENT SALT STRESS IN GRAPEVINE

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Salt stress is increasingly threatening crop productivity also because of the use of salt irrigation water imposed by water scarcity. Initial plant response to soil salt stress depends not on the accumulation of ions in the plant tissues but on the osmotic effects of the increased salt concentration in the rhizosphere. Early identification of salt stress on plant might support adaptation strategy to counteract salinity issues in agriculture.

Image-based phenotyping (LemnaTec 3D Scanalyzer phenotyping platform, LemnaTec GmbH, Aachen, Germany), was used to identify RGB traits (Dark Green, Green, Yellow and Brown colour) related to physiological traits changed by early salt stress. At the ALSIA-Agrobios glasshouse, potted vines (*Vitis vinifera*) were irrigated for 10 days with salt water (NaCl 100 mM). Concurrent physiological traits measured included stomatal conductance (gs), photosynthesis (A), photosystem II efficiency (Fv/Fm), stem water potential (SWP). Chlorophyll concentration was also determined.

Salt treatment did not increase leaf Na<sup>+</sup> but it did lower gs, A, and SWP (less negative) Fv/Fm. Analysis of images of the lower (older) leaves of salt-treated vines reveals the Dark Green colour correlates well with soil electrical conductivity ( $R^2 = 0.73$ ) vines. Using a dose-response approach, this paper concludes that a Dark Green threshold (about 1% deviation from that of unstressed vines) corresponds well with the impaired physiological traits and, hence, it might be a promising proxy for early detection of salt stress in grapevines within a digital agriculture domain.

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# **DIURNAL STOMATAL APERTURES AND DENSITY RATIOS AFFECT WHOLE-CANOPY STOMATAL CONDUCTANCE, DROUGHT RESPONSE, WATER-USE EFFICIENCY AND YIELD**

**PROF. DR. MENACHEM MOSHELION<sup>1</sup>; DR. SANBON GOSA<sup>2</sup>**

<sup>1</sup> Hebrew University of Jerusalem;

<sup>2</sup> The Hebrew University of Jerusalem

Key physiological traits of plants, such as transpiration and stomatal conductance, are usually studied under steady-state conditions or modeled using only a few measured data points. Those measurements do not reflect the dynamic behavior of the plant in response to field conditions. To overcome this bottleneck, we used a gravimetric functional-phenotyping platform and a reverse-phenotyping method to examine the dynamic whole-plant water-regulation responses of tomato introgression lines and compared those responses with several years of yield performance in commercial fields. Ideotype lines had highly plastic stomatal conductance and high abaxial–adaxial stomatal density ratios and the size of their stomatal apertures peaked early in the day under water-deficit conditions. These traits resulted in dynamic daily water-use efficiency, which allowed for the rapid recovery of transpiration when irrigation was resumed after a period of imposed drought. We found that stomatal density, the abaxial–adaxial stomatal density ratio and the time of maximum stomatal apertures are crucial for plant adaptation and productivity under drought-stress conditions. Abaxial stomatal density was also found to be strongly correlated with the expression of the stomatal-development genes SPCH and ZEP. This study demonstrates how a reverse functional phenotyping approach based on field yield data, continuous and simultaneous whole-plant water-balance measurements and anatomical examination of individual leaves can help us to understand and identify dynamic and complex yield-related physiological traits.

## **A DIFFERENT BEERSPECTIVE: IMAGING BARLEY UNDER WATERLOGGING STRESS**

**SÓNIA NEGRÃO**

University College Dublin

In Ireland, spring barley is the most cultivated cereal, and production must meet an increased demand of a flourishing malting industry. Climate change is causing extreme weather events such as excessive rainfalls. Barley is the most susceptible cereal to waterlogging with yield losses of approximately 20-25% and improving waterlogging tolerance has been flagged as a major goal for future breeding programs.

Modern barley cultivars have been bred under optimal conditions, leading to a reduced stress tolerance. In contrast, robust stress tolerance can be found in heritage germplasm. Here we explore the core European HerItage Barley collectiOn (ExHIBiT), a collection comprising 230 two-row spring-barley accessions originating from across Europe, including landraces, formerly bred cultivars and modern cultivars, which has been genotyped with the 50K SNP array. To investigate waterlogging tolerance, the ExHIBiT collection is being phenotyped in controlled conditions using RGB, chlorophyll fluorescence and hyperspectral sensors as well as in field conditions at the UCD Lyons Research Farm using Unmanned Aerial Vehicles (UAV) coupled with RGB and multispectral sensors. Phenotyping of the core- ExHIBiT population include imaging data, but also agronomic and quality traits. Waterlogging has a significant impact on several traits ranging from flowering time to grain protein content. The most recent data will be presented.

## **PHENOTROLLEY: AFFORDABLE PHENOTYPING SYSTEM FOR CONTROLLED GROWTH CONDITIONS**

**DR. AJIT NEHE PHD<sup>1</sup>**; VISHNUKIRAN THURAGA<sup>1</sup>; LINDA ÖHLUND<sup>2</sup>;  
DR. AAKASH CHAWADE PHD<sup>1</sup>

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In plant science research, a controlled growth environment is used for inducing abiotic and biotic stresses to facilitate germplasm evaluation and comparison. Challenge, however, is that under controlled growth facilities where space is limiting, there is a need to screen maximum numbers of genotypes in a short time, repetitively and non-destructively. Moreover, high-throughput phenotyping is challenging mainly because of the need for suitable equipment, infrastructure and software skills. Although commercial phenotyping platforms are being developed, there is a need for simple to use, custom-made, affordable phenotyping systems designed for the local growth infrastructure. To address this, a simple, manually controlled, affordable system called "Phenotrolley" is proposed. Phenotrolley consists of a manually pushed aluminum gantry trolley mounted with up to two DSLR cameras at a zenithal angle. Phenotrolley is moved over the potted plants placed on a bench while the cameras can be triggered using a simple mobile-controlled app. A detailed blueprint of the phenotyping system and image analysis pipeline will be presented using two case studies in two different plant species - barley (*Hordeum vulgare*) and forage grasses. The digital plant biomass (DM) and conventional biomass (CM) in two barley genotypes and growth analysis under drought stress conditions in 87 forage breeding populations per species were studied. Forage species of tall fescue (*Festuca arundinacea*) and white clover (*Trifolium repens*) were subjected to one or two drought situations over time. Different growth parameters like early vigor, the percent decrease in DM, percent recovery after re-irrigation, and area under the growth progression curve were estimated for all genotypes. There was a significant association between DM and CM for barley ( $R^2=0.74$ ), white clover ( $R^2=0.42$ ) and tall fescue ( $R^2=0.87$ ).

## **PLANT GENE BANK: FROM PHENOTYPING OF SINGLE CROPS TO AGRO-ECOLOGIES**

PRINCIPAL RESEARCHER **KIEN NGUYEN VAN MSC**

Plant Resources Centre (PRC)

Vietnam National Plant Genebank is conserving 45,000 accessions of 450 crop species and CWRs in Vietnam. With more than 1,000,000 records of description and evaluation forms in various crops in line with over 100,000 records of collection forms in whole Vietnam agro-ecological regions during the over 40 years ago, this has been providing and depicting a concise and clear overview of agro-biological diversity situation, agro-ecological transformation, agro-production system transition as well as changes of food system, consumption behaviors and impacts of climate change. At general level, we could see change, evolution of agro-biological diversity, agro-ecologies and food systems, production behaviors under natural, socio-economic, and cultural and environmental drivers in various ecological regions of Vietnam. At specified level, we could see change and evolution of single crop or multi-crops in a specified agro-ecological region in the context of various changing drivers. In fact, Vietnam National plant genebank is keeping invaluable genetic materials and non-materials that could strengthen adaptation to future challenges in the agriculture and rural sector of Vietnam. Under phenotypic science, if we have holistic, multi-disciplinary and interdisciplinary and logistic approaches from plant genebank, we could face and overcome challenges and reach sustainable development goals with lowest cost. In this presentation, we please touch into potential opportunities of plant phenotyping under plant genebank's lens to against future pressures such as food, diet, health, resources, nature, environment, education, employment and livelihood.

## **NATURAL GENETIC VARIABILITY IN MULTISTEP PHOSPHORELAY AS A TOOL FOR ELUCIDATING DROUGHT ADAPTATION IN ARABIDOPSIS THALIANA**

**KATRINA LESLIE NICOLAS MSC.**<sup>1</sup>; DR. JAN SKALÁK PHD<sup>1</sup>;  
DR. IOANNIS SPYROGLOU PHD<sup>1</sup>; DR. JAN ZOUHAR PHD<sup>2</sup>; DUŠAN TUREK<sup>2</sup>; STIJN DHONDT<sup>3</sup>;  
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Using publicly available resource 1001 Genomes, we explored the natural variation in three CK-responsive histidine kinases, AHK2, AHK3, and AHK4/CRE1. Accessions with SNPs located near a sequence previously shown to have an impact on protein function were chosen for further investigation. Using root-elongation assay and targeted transcriptomic analysis of CK signaling reporter genes, we demonstrated that identified SNPs affect plant responsiveness to CK. Further analysis of the root morphology upon exogenous CK treatment showed a stronger reduction of root apical meristem (RAM) size in the AHK variants revealing higher CK sensitivity compared to WT. The size of the RAM, previously shown to be CK-controlled, correlated well with the reduced root length of particular accessions, supporting the observed altered CK sensitivity. AHKs activity assay revealed higher responsiveness, while no significant changes in the CK affinity were observed in the more sensitive AHKs variants using ligand binding assay. This suggests that the discovered SNPs may affect AHKs' ability to transduce the signal downstream of the pathway rather than the ability of the sensor to bind CK. To study the role of altered CK sensitivity in the drought stress responses, we used the automated plant phenotyping platform WIWAMxy. The data analysis using a machine learning-based algorithm revealed a correlation between the decreased or enhanced CK responsiveness and drought stress responses. Accessions with high CK sensitivity grew bigger than Col-0 under well-watered conditions but showed a higher reduction of growth under drought. Furthermore, accessions with high CK sensitivity perceived drought much earlier than Col-0 in contrast to accessions with low CK sensitivity which becomes insensitive to drought when exogenous CK is applied before the onset of drought. The information generated can be used for targeted improvement of important Brassicaceae species for higher yield and abiotic stress tolerance.

## **HIGH-THROUGHPUT PHENOTYPING MULTIPLATFORM FOR WHEAT BREEDING AND PHYSIOLOGICAL PRE-BREEDING IN CIMMYT**

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International Maize and Wheat Improvement Center (CIMMYT)

High-throughput phenotyping (HTP) has shown great potential to accelerate genetic gain in wheat breeding programs. Through the efficient screening of large populations, HTP approaches not only can assist in the selection of elite germplasm based on highly heritable agronomic traits, but also enables the exploitation of more complex integrative traits for complementary strategies such as physiological pre-breeding, calibration of genomic selection models, screening of genetic resources and gene discovery.

The Global Wheat Program at CIMMYT is adopting different complementary HTP platforms that enable the assessment of several traits while keeping a balance between resolution, cost, and throughput. These breeder-friendly platforms range from simple handheld low-cost devices up to aerial imagery. Ground-based high-resolution imagery is used for object detection and counting, as well as for characterization of canopy structure and biomass accumulation, whereas aerial HTP allows fast screening for indices related to changes in pigment composition, radiation use efficiency and canopy temperature.

We report our latest advances in using HTP for key complex physiological traits and how we use them for the evaluation and selection of germplasm under field conditions, assisting in the design of strategic crosses targeting different environments. For instance, we are developing an index—using thermography and spectroscopy—that captures genotypic differences in root capacity. Similarly, spectroscopy has been used for indirect estimations of photosynthetic traits and radiation use efficiency across the growing cycle. Other studies using HTP data include finding genotypic differences for the dynamics of photoprotection-related pigments as an adaptation to drought and heat stress, the parameterization of crop models, the improvement of genomic selection models and the implementation of deep learning models for accurate predictions of key agronomic and physiological traits.

## **ADVANCES IN PHENOTYPING TECHNIQUES AND CONTROLLED ENVIRONMENTS TO OVERCOME THE CHALLENGES CROP PLANTS ARE FACING IN AUSTRALIA**

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DR. GONZALO ESTAVILLO<sup>3</sup>; PROF. JOHN EVANS<sup>1</sup>; DR. ANTONY CONDON<sup>3</sup>;  
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The lack of rapid in-field measurement of traits contributing to yield is a significant roadblock to the inclusion of those traits in crop breeding programs. An example is the measurement of photosynthetic traits that are notoriously complex and slow to measure in the field using gas exchange or fluorescence techniques. To address this problem, we have developed a high-throughput model to predict photosynthetic parameters from hyperspectral reflectance spectra in wheat leaves and wheat ears. As part of this work, the Australian Plant Phenomics Facility (APPF) has developed a web portal where scientists and breeders can upload their wheat reflectance spectra gathered from a range of instruments and obtain accurate predictions for a range of photosynthetic parameters.

Further, we are combining measurements of reflectance spectra with Machine Learning and Deep Learning techniques to identify plant growth patterns, morphological parameters, photosynthetic parameters, and disease susceptibility before visible lesions can be measured.

To explore plant responses to climate regimes – including extreme stress events - the APPF is using an open-source framework in its controlled environments to simulate the climate based on single or multi-year historical data or synthetic data. We use dynamic environmental control and multispectral LED fixtures to better mimic natural sunlight in controlled environments for various seasons and geographical locations. The APPF hosts a range of international collaborations that leverage its unique mix of controlled environments, phenotyping tools and data analytics capabilities to tackle grand challenges.

## **EXPLORING NATURAL VARIATION OF PHOTOSYNTHESIS TO IMPROVE CROP YIELD IN TOMATO**

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Photosynthesis is an essential component of plant productivity. Photosynthesis is physiologically complex and natural genetic variation for photosynthesis in many crop species is yet unexplored. Environmental fluctuations often enhance the effects of genetic variation on the photosynthetic performance of plants, improving the ability to identify the genetic causality of variations in photosynthetic phenotypes in their environment. These variations can be explored to improve photosynthesis and provide insights into the genetic regulation of this complex trait leading to a potential for crop improvement. The use of high throughput phenotyping and genomics to identify Quantitative Trait Loci for photosynthesis phenotypes can be used in marker-assisted breeding to improve crop photosynthesis and yield.

This approach was tested using backcross inbred lines (BILs) and wild accessions of tomato, the second-largest horticultural crop grown in temperate and tropical regions. Light intensity affects plant growth and other physiological responses in plants. High light intensities can be a major constraint on photosynthesis by the accumulation of reactive oxygen species (ROS), which damage photosystems I and II (PSI/PSII) termed photoinhibition. Plants respond to high light stress by converting the excess light energy into heat through non-photochemical quenching (NPQ). We found that the wild species performed better than the cultivated species under high light conditions with a high quantum yield of PSII electron transport ( $\Phi_{PSII}$ ) and low NPQ values. We are currently exploring the genetic nature of this difference in segregating populations.

## **PHYSIOLOGICAL MECHANISMS UNDERLYING WATER STRESS RESPONSE IN A DIVERSE GRASS PEA (*LATHYRUS SATIVUS*) GERMPLASM COLLECTION**

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Plants have evolved diverse strategies to avoid and/or cope with drought and flooding events and the various stresses associated with them. In the current climate change scenario, a deeper understanding of the underlying mechanisms of water stress tolerance in crops and their incorporation into breeding programs is a priority.

The grain legume *Lathyrus sativus* L. (grass pea) has high economic importance for food and feed in Asian and African developing countries. Interest has been raising also in the Mediterranean region, where this crop is part of cultural heritage of more marginal areas, due to its outstanding robustness under adverse environmental conditions.

The aim of this work was to screen the natural diversity within a grass pea worldwide germplasm collection concerning their physiological responses to drought and flood, as a starting point to the clarification of the associated genetic control mechanisms and to establish once and for all this crop potential as a model for multiple abiotic stress resistance studies.

Following a split-plot design, the physiological responses of 193 grass pea accessions were evaluated under three water treatments (well-watered, waterlogging and water deficit), using several parameters including net CO<sub>2</sub> assimilation rate, stomatal conductance, water use efficiency, maximum quantum yield of photosystem II, leaf relative water content, total dry biomass, and root to shoot ratio.

Linear mixed models were applied for univariate statistical analysis of the phenotypic data, with significant variation detected among grass pea accessions for all the traits and water treatments. Using best linear unbiased estimators (BLUEs), the performed multivariate analysis provided important insights on the diversity of water stress-coping strategies in grass pea, and allowed the identification of a few multiple contrasting (susceptible vs. tolerant to both drought and flood) accessions among the collection that can be exploited in future breeding.

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# **HIGH THROUGHPUT SCREENING PLATFORMS FOR A DROUGHT TOLERANCE TRAIT: THE LIMITED TRANSPIRATION RATE (TRLIM) IN MID-SOUTH SOYBEAN GENOTYPES**

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Identification and integration of genetic traits enabling increased water conservation in plants could provide increased water availability and plant performance during drought. This research was initiated to provide a high-throughput protocol for phenotyping 122 recombinant inbred soybean lines (RILs) of crossing ('Jackson' × 'KS4895'), using aquaporin inhibitor and thermal imaging. The genetic material was tested under high vapor pressure deficit (VPD) leading to identify drought tolerant soybeans in the mid-south of the U.S. The transpiration rate (TR) responses of soybean shoots before and after application of AgNO<sub>3</sub> were measured under 37°C and >3.0 kPa VPD. The decrease in transpiration rate (DTR) for each genotype was determined. Based on DTR rate, a diverse group (slow, moderate, and high wilting) of 26 RILs were selected and tested for the whole plant TRs under varying levels of VPD (0.0–4.0 kPa) at 32 and 37°C. The phenotyping results showed that 88% of slow, 50% of moderate, and 11% of high wilting genotypes expressed the TRlim trait at 32°C and 43, 10, and 0% at 37°C, respectively. Genetic mapping with the phenotypic data we collected revealed three QTL across two chromosomes, two associated with TRlim traits and one associated with leaf temperature. Ten soybean lines that showed limited water loss during whole plant transpiration test in controlled environments were studied under field conditions in 2020-2021. A correlation was found between water use efficiency intrinsic (WUE<sub>k</sub>) and lower stomatal conductance. Genotypes that showed higher WUE<sub>k</sub> also had lower stomatal conductance. In average genotypes with expressing the TRlim (RILs #91, #22, #151, and #93) had 20-22% higher yield than other soybean genotypes. Superior lines tolerated not only dry conditions but also produced higher yields under prolonged periods of dry weather.

## **PHENOTYPING INNOVATIONS TO HARNESS GENETIC DIVERSITY FOR CLIMATE RESILIENCE**

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The University of Queensland

Climate change is threatening crop production globally and is particularly impacting smallholder farmers in the Asia Pacific region. The inclusion of genetic diversity into crop improvement programs is key to ensuring crops are adapted to current, and future, environments. However, traditional phenotyping approaches are a bottleneck that limit the evaluation of genebank accessions at the scale required for plant breeding without impacting the rate of genetic gain. Phenotyping strategies can be adapted to suit the needs of crop improvement programs and will vary depending on the goal, trait of interest, project budget and access to germplasm and facilities. Case studies for staple crop taro (*Colocasia esculenta*) and mungbean (*Vigna radiata*) will be shown to demonstrate how high-throughput (UAV-based) and low-cost (RasPi) based platforms can be developed and applied for key traits associated with adaptation to abiotic stress environments (salinity, drought). In mungbean, UAV fitted with multi-spectral cameras were flown over diverse nested association mapping populations grown in the field in Queensland, Australia in 2021 and 2022. A high degree of genetic variation was observed for UAV traits including canopy temperature and NDVI. We anticipate that these results and this platform will be translated with partners through the International Mungbean Improvement Network for use in Australia and Asia. In taro, low-cost, high-throughput protocols were developed to screen diverse germplasm for adaptation to salinity tolerance. This is currently being optimised for adoption with partners in the Pacific to screen genebank accessions. With appropriate capacity building in-country adoption with partners in the Asia Pacific will take place to ensure genetic gain for staple food crops currently impacted by climate change.

## **IPUPIL: IOT-BASED PLATFORM OF UNMANNED PHENOTYPING WITH IMITATED LAND CONDITION**

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Drought stress negatively affect crop yields. To develop drought-resilient crops, we need to elucidate the relationship between plant phenotypes and gene regulatory network mediating drought adaptation. However, it is difficult to accurately quantify plant response to the stress, for the following reasons: 1) Inadequate control of the environmental conditions such as soil moisture. 2) Incapacitation in acquiring the environmental data for individual plant. 3) Inadequate acquisition of the phenotype data for individual plant. To solve this problem, we have developed a high-precision plant phenotyping platform, IoT-based Platform of Unmanned Phenotyping with Imitated Land condition (iPUPIL), comprising of a growth chamber with controlled environmental conditions. The iPUPIL has the following features: 1) It has an equipped irrigation system which controls the soil moisture level. Drought stress can be adequately mitigated using this system. 2) It can also monitor the environmental parameters of each pot, such as soil moisture, air temperature, humidity, and light intensity. 3) It has high-resolution RGB and far infrared cameras to evaluate the plant growth and physiological responses.

Using the iPUPIL, we evaluated the drought stress responses of cultivated rice species (*Oryza sativa*) and its wild relatives which are grown under various environmental conditions, such as the dry climate region. We will conduct transcriptome analyses on these rice to reveal the relationship between gene expression and plant phenotype under the drought stress conditions. From the resulted multi-omics data, we will consequently identify the regulatory genes; mediating drought stress tolerance in the wild rice species.

This work was supported by Cabinet Office, Government of Japan, Moonshot R&D Program for Agriculture, Forestry and Fisheries (funding agency: Bio-oriented Technology Research Advancement Institution) Grant Number JPJ009237.

## **HEAVY DUTY LEGUMES: GETTING TO THE ROOT OF WATER STRESS TOLERANCE IN COWPEA**

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Boyce Thompson Institute

Cowpea (*Vigna unguiculata*) is an important crop in dry-land agriculture and a source of protein for many subsistence farmers. Cowpea exhibits high environmental resilience to many abiotic stresses, such as drought, and significant natural variation was previously observed for agronomic traits. However, the genetic constituents of cowpea's drought stress resilience are yet to be identified. Our work focuses on cowpea responses to drought during the early vegetative growth phase, and aims to identify genetic components of drought stress resilience through forward genetics. We developed a non-destructive phenotyping protocol to monitor cowpea's growth rate, evapotranspiration and photosynthetic efficacy. The protocol utilizes low-cost computers and microcontrollers, such as Raspberry Pi and Arduino, and provides insight into multiple processes occurring during drought stress exposure. The preliminary results indicate a number of genetic loci associated with drought tolerance. In the future, we aim to verify the identified loci using reverse genetics, as well as increase the genomic prediction of growth maintenance by combining the current protocol with a metabolome analysis. The obtained results will inform improvement of high-value legumes.

# **IMAGE FACILITATED ASSESSMENT OF INTRA-SPIKE VARIATION IN GRAIN SIZE IN WHEAT GENOTYPES EXPOSED TO TERMINAL HIGH-TEMPERATURE STRESS AND TERMINAL DROUGHT STRESS**

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<sup>1</sup> ICAR-NIASM

Exposure to drought and high-temperature stress during grain development in wheat (*Triticum aestivum* L.) reduces grain size, which varies across the length of the spike. Several factors might be contributing to this variation. We hypothesized that genetic variability for intra-spike variation in grain size (ISVGS) could be one of the opportunities to improve the grain weight per spike and improve wheat productivity. However, this task is tedious as the size of each grain has to be measured in a large set of germplasm or breeding lines. Hence we explored the phenomics approach involving image-based tools to assess the ISVGS. Sixteen wheat genotypes were subjected to well-watered, water-stressed, and high-temperature conditions during grain development in 2018-2019 and 2019-2020. At physiological maturity, spikes were manually threshed. The grains were arranged corresponding to the spikelet position on the rachis for each spike, and the Samsung Galaxy J2 Cell Phone was used to acquire 288 images. Open-source software ImageJ was used to analyze and interpret the features of grains in spike and their relevance to ISVGS. The image-derived parameters were used to compute ISVGS, and two significantly distinct genotypes were selected for transcriptomics based on this analysis. The results prove that it is possible to quantify ISVGS by employing an image-based phenomics approach. We demonstrated an economic plant phenomics approach for ISVGS by using images acquired by mobile phone and open source software for image analysis. The study could differentiate sensitive and tolerant genotypes with respect to their ISVGS under high temperature and insufficient soil moisture conditions relative to plants grown under optimum temperature and sufficient soil moisture. The phenomics approach developed may be useful for developing stress-tolerant wheat varieties for agro-ecologies featured by the terminal drought and high temperature.



## **MATCHING WHEAT GENOTYPES AND FUTURE CLIMATE SCENARIOS: MULTIVARIATE MODELLING OF HIGH THROUGHPUT FIELD PHENOTYPING DATA IS KEY**

**FLAVIAN TSCHURR; DR. LUKAS ROTH; PROF. DR. ACHIM WALTER**

ETH Zuerich

Ongoing climate change leads to severe challenges for agriculture. Adapted crop varieties for future climates are needed. Breeding towards adapted varieties requires an ideotype - an idealised stature and developmental program of the crop - that is defined by a certain number of distinct parameters. Defining such parameters according to environmental conditions needs vast amounts of data, enabling to build genotype specific growth models. High Throughput Field Phenotyping (HTFP) shows major potential for this purpose as a non-destructive way to gather high-resolution RGB images over the whole growing period. The FIP, a cable suspended Field Phenotyping Platform of ETH Zurich, equipped with a 21 MP full frame DSLR camera (EOS 5D Mark II, 35 mm lens) has acquired time series of images of various winter wheat genotypes for 5 years in intervals of a few days to weeks. Using semantic segmentation with deep learning, canopy cover is extracted as a trait from these high-resolution images. The high precision allows an alignment over time of a few mm and hence the observation of single sowing rows. With such high precision, establishing a growth model of even the very early growing phase can be achieved, contrary to other phenotyping approaches with coarse resolution. These advantages can be shown in a novel genotype x environment model approach describing the early development of winter wheat. This approach allows extraction of the parameters of a 'dose-response-function' that describes genotype-specific response to multiple environmental covariates. After this extraction, a selection process considering the importance and weight of each covariate response is applied. This multivariate dose response model allows dynamic modelling according to different environmental conditions in a genotype specific manner by using high temporally and spatially resolved HTFP data. This, in turn, is a prerequisite to establish future ideotypes optimally suited for certain climate scenarios.

# **GENOME WIDE ASSOCIATION STUDY UNCOVERS THE QTLOME FOR OSMOTIC ADJUSTMENT AND RELATED DROUGHT ADAPTIVE TRAITS IN DURUM WHEAT**

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Osmotic adjustment (OA) is a major component of drought resistance in crops. The genetic basis of OA in wheat and other crops remains largely unknown. In this study (Condorelli et al. 2022), a panel of 248 field-grown durum wheat elite accessions grown under well-watered conditions until flowering, underwent a progressively severe drought treatment started at heading. Leaf samples were collected at heading and 17 days later. The following traits were considered: flowering time (FT), leaf relative water content (RWC), osmotic potential ( $\psi_s$ ), osmotic adjustment (OA), chlorophyll content (SPAD) and leaf rolling (LR). The high variability (3.89-fold) in OA among drought-stressed accessions resulted in high heritability (72.3%) of the trait. Notably, a high positive correlation ( $r = 0.78$ ) between OA and RWC was found under severe drought conditions. GWA analysis revealed 15 significant QTLs for OA, as well as 8 major QTL hotspots where a higher OA capacity was positively associated with RWC and/or SPAD, and negatively with LR, indicating a beneficial effect of OA on the water status of the plant. The comparative analysis with the results of 15 previous field trials conducted under varying water regimes showed concurrent effects of five OA QTL cluster hotspots on normalized difference vegetation index (NDVI), thousand-kernel weight (TKW) and/or grain yield (GY). Gene content analysis of the cluster regions revealed the presence of several candidate genes, including bidirectional sugar transporter SWEET, rhomboid-like protein and S-adenosyl-L-methionine-dependent methyltransferases family protein and DREB1. Our results support OA as a valuable proxy for marker-assisted selection (MAS) aimed at enhancing drought resistance in wheat.

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# **DEVELOPING PHENOTYPING METHODS TO UNRAVEL THE GENETIC ARCHITECTURE OF HEAT STRESS TOLERANCE IN SOYBEANS**

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Iowa State University

Climate change is predicted to bring about rising global temperatures, including in the temperate regions where soybeans are heavily cultivated. In addition to rises in the average temperature, current climate predictions for the end of the century also indicate an increased frequency and duration of extreme weather events such as heat waves and droughts. The combination of increased average temperature and frequency of heat waves means that in the coming decades soybeans are increasingly likely to encounter higher ambient temperatures resulting in higher incidences of heat stress. Present research in soybean heat tolerance is primarily limited to quantifying yield losses, and not to in-season measurements. A panel of 450 diverse soybean genotypes was grown in two different greenhouse conditions: optimal and supra-optimal temperatures. Four weeks after planting, seedlings were phenotyped for chlorophyll content, canopy temperature, and biomass to determine if there was a genotypic response of these traits under abiotic stress. Additionally, a genome wide association study was conducted for each of these traits. Here we report on which of these traits appear useful for studying soybeans under heat stress, as well as the underlying genetics of this tolerance. A repeat of this study is scheduled for the summer of 2022, with an addition of new physiological and photosynthetic traits planned. In addition to the greenhouse study, we are also working towards developing a mobile unit that can be deployed in the field so that soybeans may be tested for heat stress tolerance in as close to field conditions as possible. Prototypes have been constructed and will be tested for use in the upcoming summer of 2022.

## **A PHENOTYPING PLATFORM TO EVALUATE ROOT PLASTIC RESPONSES TO SOIL WATER HETEROGENEITY**

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Under changing environment, being able to select plants that with good agricultural performance under conditions of heterogeneous soil water availability is becoming increasingly relevant. A general agreement is that the structural and functional plasticity of root systems should contribute to crop performance in heterogeneous environments, although the mechanisms of this contribution remain unclear. Our objective is to investigate experimentally how plants adjust their hydraulic properties and system architecture when facing soil water heterogeneity. We developed a novel rhizotron platform, wherein constant spatial patterns can be imposed while monitoring water consumption and its spatial distribution. The platform comprises a set of 15 independent rhizotrons, equipped with control units. Each rhizotron is split into 3 × 3 cells that are isolated hydraulically while the roots are able to grow freely. The control units allow to impose constant but different water potentials in each of the 9 cells. The volumetric water consumption is also recorded in each cell with a minute time resolution. A scanner will be added to monitor root growth and local soil moisture by light transmission. Maize plants (cv. B104) have been grown in these rhizotrons during four weeks at constant and homogeneous water potential, followed by a fifth week during which three water potentials were imposed. Morphological and hydraulic plant responses to these different levels of water availability has been observed using manual root annotation, continuous leaf psychrometer measurements, and transpiration. These results allowed to compute root system growth and real-time changes of whole root system conductance. The root hydraulic conductance was also measured directly at the end of the experiment with the hydraulic conductance flow meter to see local hydraulic adaptation. The results will allow us to better understand the complex response of maize plants in heterogeneous and changing environments.

## **STOMATAL RESPONSES TO A FLUCTUATING ENVIRONMENT DEPEND ON GUARD CELL SIZE AND PHOTOSYNTHETIC PATHWAY**

**DR. YANMIN ZHOU PHD**; PROF. COLIN OSBORNE

University of Sheffield

Stomatal behaviour controls the balance between carbon fixation and water loss in plants. Faster stomata responses to a fluctuating environment can improve intrinsic water use efficiency and reduce the risk of transient water deficits. The speed of stomatal responses depends on guard cell size, and recent work has highlighted that C4 plants have smaller and faster stomata than C3 species. In this study, we used *Alloteropsis semialata* as a system to investigate how natural variation in guard cell size and photosynthetic pathway impact stomatal responses to a dynamic environment. We measured the variation of guard cell size and responses to light fluctuations in C3 and C3-C4 grasses in diploid populations, and C4 grasses in diploid, hexaploid, and dodecaploid populations. The results show that C4 grasses have smaller guard cells than C3 and C3-C4 grasses in diploid populations. C4 grasses in hexaploid populations have a similar guard cell length to C3 and C3-C4 grasses. By testing the relationships between guard cell length and the parameters of stomatal speed in opening and closing, we have found smaller guard cell length is associated with faster stomatal response to increasing light in C4 grasses. However, for a given guard cell length, C4 grasses have a slower speed of stomatal opening than both C3 and C3-C4 grasses. Conversely, when exposed to decreasing light, stomata in C4 grasses close as fast as C3 and C3-C4 grasses. Overall, stomata take a shorter time for closure than opening. The findings indicate that the speed of stomatal opening changes in C4 plants independently of guard cell size.

## **APPLICATION OF CHLOROPHYLL FLUORESCENCE IMAGING IN PHENOTYPING OF DROUGHT STRESS TOLERANCE IN LETTUCE BREEDING LINES**

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MÁRIA BARBORIČOVÁ; DOMINIKA MLYNARIKOVA VYSOKA; MAREK KOVÁR

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Chlorophyll fluorescence imaging (CFI) represents a promising tool for screening the vitality and the photosynthetic functions of plants exposed to environmental constraints. It enables recognizing the spatial heterogeneity and the severity of the stress. Our study tested the opportunity to apply CFI to recognize drought sensitivity in lettuce genotypes. Eight genetically distinct parental lines of cultivated lettuce (*Lactuca sativa* L.) and wild lettuce (*Lactuca serriola* L.) were grown in a growth chamber under limited/non-limited water supply. At the end of the experiment, plants were exposed to severe drought stress by withholding irrigation for three more days. CFI was recorded regularly in light-exposed plants at the actinic light intensity set on the ambient level. Depending on genotypes, total dry mass in drought-stressed plants decreased by 20-50% compared to control; the relative plant dry mass decrease was used to measure the drought sensitivity of genotypes. CFI analyses have shown a significant decline in the apparent electron transport rate, ETR, in all genotypes. However, contrary to expectations, the moderate drought stress led to a negligible decrease or even a slight increase in ETR, which did not correspond to the observed reduction in photosynthetic performance. It indicates that the electron transport was efficiently re-directed to alternative energy-consuming pathways. On the other hand, we observed a significant decrease in steady-state fluorescence intensity ( $F_s$ ), both in moderate and severe drought. The steady-state fluorescence signal ( $F_s$ ) reflected well the effects of water deficit on the photosynthetic apparatus better than parameter ETR, which is often referred to as the most useful parameter for assessing the photosynthetic functions. That is important information emphasizing the need to test the techniques in individual crops and different stress scenarios. (Supported by VEGA 1-0683-20, VEGA 1-0664-22, APVV-18-0465).

# Plant Development and Allocation

## **MODIFICATION OF METABOLITE PROFILE BY ASCORBATE AND HYDROGEN PEROXIDE IN WHEAT**

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The effect of ascorbate (ASA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (5 and 20 mM for 3 and 7 d) treatments on metabolome was compared in wheat seedlings. Surprisingly, ASA treatment resulted in a more oxidising redox environment, while addition of H<sub>2</sub>O<sub>2</sub> in a more reduced one as indicated by the ratios of the oxidised and reduced forms of the two compounds. While ASA inhibited, H<sub>2</sub>O<sub>2</sub> activated the oxidative pentose phosphate based on the changes in glucose-6-phosphate content. This difference can be also responsible for the decreased synthesis of several amino acids from the compounds of glycolysis after ASA supplementation and their increased levels after H<sub>2</sub>O<sub>2</sub> treatment. Interestingly, ASA induced a 5-fold greater increase in Pro accumulation compared to H<sub>2</sub>O<sub>2</sub>. Differences in Pro metabolism may influence the NADPH formation/use during this process and consequently the redox environment and redox-dependent biochemical processes in the leaf tissues. Indeed, ASA treatment modified the expression of several genes related to the ascorbate-glutathione cycle and the pentose phosphate pathway. The present results demonstrate the special effect of ASA and H<sub>2</sub>O<sub>2</sub> on the tissue redox state and adjustment of metabolism and gene expression in wheat.

## **BIOSTIMULANTS & BARLEY; A GREENHOUSE, GROWTH RATE AND GRAIN QUALITY STUDY USING REDUCED FERTILISER**

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Global crop production relies upon exogenous applications of synthetic nitrogen to maintain yield. The production and use of fertilisers cause the long-lived greenhouse gasses carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) to accumulate in the atmosphere, destroying stratospheric ozone and aggravating climate change. Human-induced N<sub>2</sub>O emissions derived from croplands have increased 30% over the past four decades, underscoring the need to reduce our dependency on fertilisers. The new European fertilising products regulation recognises plant biostimulants as an agricultural input that can potentially mitigate fertiliser use. These products are incorporated into the soil or sprayed on plants to enhance nutrient use efficiency, abiotic stress tolerance or crop quality traits.

Spring barley is Ireland's most widely sown tillage crop accounting for more than 150,000 ha, providing feedstock for animals and malt for brewers and distillers. Here, we test three plant biostimulants for their effect on spring barley growth and development. Biostimulant treatments compared to control plants increased growth and net assimilation rates at booting, and two of the three products prolonged the growing period of barley. However, biostimulant treatments did not increase yield or improve grain quality compared to controls. We will next analyse to which extent biostimulants can improve abiotic stress resistance and photosynthetic gas exchange. The latest results from our trials will be presented.

## **PHENOTYPING OF MAIZE YIELD TRAITS VIA PROXIMAL SENSING TECHNIQUES**

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Phenotyping, via proximal and remote sensing techniques, has been increasingly used to provide information about plant properties in a large range of crops. A large number of spectral reflectance indices (SRI) computed from proximal sensing data has become useful tool for agricultural monitoring, assessing within-field crop variability which can support the right decisions in field management. The objectives of this study were to evaluate the potential of using an active, portable, multispectral proximal canopy sensor, namely Plant-O-Meter, to detect the influence of different nitrogen (N) supply on maize yield traits. The Plant-O-Meter device possess the multispectral source, which integrates four light sources in one optical module and based on four band combinations (465, 535, 630 and 850 nm) allows calculation more than 30 different SRI. In order to estimate the influence of N applications on variability of grain yield traits of maize, four maize hybrids (*Zea mays* L.) of different maturity were selected (P9074, P97557, P9889 and P0725). The study included five different N treatments (0, 70, 140 and 210 kg N ha<sup>-1</sup>), as pre-plant and top-dress applied. The sensor measurements were performed at throughout the season, from V4 (4 fully developed leaves) until R2 (blister) corn growth stages. The relationships between yield traits and SRI were determined using Pearson correlation coefficient. The results revealed that maize genotypes responded differently to different N conditions and significant correlations were found between certain SRI and yield traits. However, since that significance of examined SRI highly differed depending on growth stage of maize, the results of this study highlight the need for careful selection of suitable indices and optimal timing of the measurements to increase the accuracy of in-season spectral phenotyping for grain yield and yield traits, respectively.

## **QTL ANALYSIS OF ROOT TRAITS IN SEEDLINGS OF A BARLEY RIL POPULATION**

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Root systems have not been thoroughly explored in breeding programs. Plant breeding will benefit from the study of root diversity. Studying root system architecture (RSA) will lead to a better understanding of the patterns of root development in relation with soil exploration for the acquisition of water and nutrients. The aim of this study is to evaluate root system diversity in a population of barley (*Hordeum vulgare* L.) recombinant inbred lines (RILs), and to identify quantitative trait loci (QTLs) for root traits potentially useful in breeding programs. A population of 114 RILs from the Orria x Plaisant cross, an elite Spanish breeding population, was tested for RSA traits under controlled and repeatable conditions. The RILs were genotyped with barley OPA1. The lines were evaluated at seedling stage, using a rhizoslide system, which is low-cost, medium-throughput method, amenable to breeding operations. A sandwich composed of a PVC plate, black cardboard sheet, filter paper, and a plastic sheet, A-4 size, with the lower end submerged in a container with distilled water, was used to grow the seeds. The population was previously characterized for germination speed, to plan sowings. Six pre-germinated seedlings for each RIL were grown, one per sandwich, in a growth chamber for 7 days at 22/18°C and 12/12 h photoperiod. After that, roots were scanned using a flatbed scanner, at 330 ppi, and analyzed using the software RootNav. The set of morphological and quantitative traits were then subjected to QTL analysis. QTL for total root length, root number, root angle and other traits were found and will be reported. A field trial with the 10% families showing extreme values for root angle, to validate the results in the field, is ongoing. Comparisons of RSA in seedlings and adult plants will be reported.

## **NITROGEN CONTENT ESTIMATION USING MULTISPECTRAL IMAGES AND CNN**

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Recent advances in deep learning and more specifically in convolutional neural networks (CNN) bring new avenues for plant phenotyping. Most of the applications use RGB images but few studied CNN with multispectral imagery. Yet, plant spectral information is known to bring information on plant health and development. This research aimed to investigate the use of CNN for nitrogen content estimation, using close-range multispectral images in wheat field trials.

Two wheat field trials were studied for two years. Nitrogen content (N kg.ha<sup>-1</sup>) of the leaves, the flag leaves, the tillers and the ears was manually determined for different growth stages. Nadir images were acquired by a six bands multispectral camera array at a height of 1.6 meter above the canopy. A registration algorithm was applied to align the frames pixel to pixel. Then, data from an incident light spectrometer were used to compute the bi-directional reflectance factor. The size of the database is the main key in deep learning, that is why several training scenarios were tested for plant nitrogen estimation. Ground truth data interpolation, pseudo labelling, and data augmentation methods were tested on an adapted VGG16 and a proposed CNN architecture. Preliminary results showed potential with a RMSE of 28.72 and a R<sup>2</sup> of 0.81 for plant nitrogen content estimation. Transfer learning was finally applied to model the different organ nitrogen levels. It provided the dynamics of wheat nitrogen distribution from tillering to maturity.

# **FRUITPHENONET: AUTOMATED FRUIT DETECTION AND TRACKING USING TIME-SERIES VISIBLE LIGHT AND HYPERSPECTRAL IMAGERY FOR TEMPORAL FRUIT PHENOTYPING USING A DEEP NEURAL NETWORK**

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A phenotype is the composite of an observable expression of a genome for traits in a given environment. Phenotypes represented as a function of time computed by analyzing time-series imagery are called temporal phenotypes that provide important information on plant vigor. The research introduces a novel method called FruitPhenoNet, which performs automated fruit detection using deep learning for temporal fruit phenotyping analysis using time series visible light and hyperspectral imagery. The method uses a multimodal image analysis approach to address the challenge of detecting fruits that assume the same color as that of the leaves (e.g., pepper) to compute temporal phenotypes. We first demonstrate that the reflectance spectra of fruits show a distinct pattern in comparison to other parts of the plants. Then, FruitPhenoNet, an artificial neural network, is trained to learn the characteristics of fruits from their reflectance spectra derived from hyperspectral imagery. FruitPhenoNet detects the fruits as soon as they become visible and hence, accurately performs the fruit emergence timing detection followed by fruit growth tracking. The resolution of the visible light camera is significantly higher than that of the hyperspectral camera. The fruit regions from the hyperspectral images are then aligned with the visible imagery to extract the color features that may codify color transition event in pepper plants. FruitPhenoNet generates a status report consisting of a set of temporal phenotypes, including the day of emergence of the first fruit, the total number of fruits present in the plant at a given time, the total number of fruits produced by the plant, and the timing of fruit color transitions. FruitPhenoNet is trained and evaluated using a benchmark dataset called FruitPheno which contains multimodal image sequences of the full life cycle of pepper plants captured in a high throughput plant phenotyping platform from multiple views to demonstrate its efficacy.

## **DEVELOPMENTAL PHENOTYPING OF ARABIDOPSIS THALIANA UNDER CONTROLLED ENVIRONMENTAL CONDITIONS**

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Plant growth and development are largely influenced by temperature. Morphological adaptations in response to elevated ambient temperature are termed thermomorphogenesis. In *Arabidopsis thaliana*, measurable thermomorphogenic adaptations occur already in the early seedling stage. These include elongation of hypocotyls and petioles, as well as hyponastic leaf growth, resulting in an open rosette arrangement, which is thought to promote leaf cooling. These morphological processes are well-described in early development of *Arabidopsis* seedling growth. To be able to generate quantifiable morphological and phenotypic data of further developing plants, we installed a phenotyping unit, including a CropScore top-view RGB camera system in two environmentally-controlled phytochambers. Additionally, a LemnAIexplorer Pro (LemnaTec) imaging box equipped with top- and side-view RGB cameras plus a chlorophyll fluorescence camera, was installed to monitor selected plants. The phenotyping unit is complemented with a mobile thermal imaging module. The whole setup allows us to monitor and quantify the impact of different temperature regimes on phenotypic traits of hundreds of plants grown simultaneously in the two phytochambers. First results obtained with a set of different *Arabidopsis* genotypes (mutant lines and ecotypes) are presented and discussed.

## **SEED YIELD STABILITY ASSESSMENT OF CHICKPEA GENOTYPES THROUGH AMMI AND BI PLOT ANALYSIS**

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In order to assess seed yield stability of twelve chickpea genotypes, an elite crosses and popular varieties were evaluated under four designated locations (Kalaburagi, Bidar, Raichur, and Sirguppa) during 2019-20. All the experiments were conducted in randomised complete block design with three replications. The interaction analysis (AMMI ANOVA) indicates large variations for seed yield due to highly significant (more than 0.01) environment and genotype, thus the genotypes were diverse with large sum of squares for their seed yield and environment. The differential response of chickpea genotypes for seed yield across the environments was observed. IPCA1 and IPCA2 together captured 4.27 percent of the interaction mean squares. JG11× WR315 (F7)-57, and ICCV 191106 showed higher average yields and adaptable to favourable environments. Based on AMMI bi-plot analysis, genotypes (JG11×WR315 (F7)-57, JG11) and (JAKI 9218, RGV 203) were stable and unstable respectively. Similarly, best performer genotypes and three mega environments were noticed through the analysis. Environments (Kalaburagi, Sirguppa) were found with most selective for seed yield estimation. In terms of seed yield stability, the genotypes (JG11×WR315 (F7)-57, JG11 and JG11×WR315 (F7)-49) were the best among all.

## **PHENOTYPIC AND GENETIC DIVERSITY OF ROOT TRAITS IN MAGIC WHEAT POPULATION FOR SELECTION IN BREEDING**

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Root system architecture (RSA) is a target for plant breeding because of the interest to develop crops with roots that use nutrients and water more effectively. Breeding for root traits requires phenotypic diversity in populations amenable to quantitative trait loci (QTL) identification to provide markers for large breeding programs. Our research aims to identify the genetic variation for root traits across a Multi-parent Advanced Generation Inter-Cross (MAGIC) wheat population by implementing the use of a state-of-the-art phenotyping platform, which is an automated non-invasive high throughput phenotyping system, GrowScreen-Rhizo III (based on Nagel et al., 2012). With the help of the platform, we will be able to quantify root traits of plants grown in rhizobox filled with a substrate providing mechanical impedance to root growth and record the dynamics of both root and shoot development during an early plant developmental stage. The NIAB eight-parent winter wheat MAGIC population (Mackay et al., 2014) will provide high-density and high-resolution genotypic information, and reliable pedigree map to help with the association study. The phenotypic data will subsequently be correlated to genotypic data and QTLs linked to the traits of shoot and root are identified by Genome-Wide Association Study (GWAS). Newly detected QTLs and candidate genes will require further validation. Our findings will not only increase the knowledge about root traits and their roles at early stage of crop establishment, a critical stage for crop productivity, but also provide novel genetic material for selection in breeding. The root phenotyping of the young wheat plant in the controlled environment will also provide valuable reference data for future studies in the field for exploring root development and its correlation to the stress/disease tolerance. We suggest that genes and QTLs revealed in our study could be a target in the wheat breeding practice when introduced in breeding programs.

# **INTEGRATED PHENOTYPING OF PEARL MILLET FOR DROUGHT RESISTANCE**

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University of Nottingham

Pearl millet is a staple crop in the Sahel region of Western Africa with 90% of it grown by smallholder farmers. Drought represents a major constraint to rain-fed agriculture and food security in the Sahel as a whole. Selecting new varieties of pearl millet with increased drought tolerance and yield stability is urgently required given altered weather patterns and increased temperature due to climate change in the next decades. One approach is to develop crops with less costly root tissues better able to explore deeper soil profiles for water to deliver greater yield stability under drought conditions. To do this, a panel of pearl millet lines have been grown under drought stressed and well watered conditions over three years of field trials in Senegal. These lines are phenotyped for yield, physiological, root architectural, root and leaf anatomical traits, and leaf and seed ionome. This will assess variation between lines, and the effect of drought stress on these traits within lines. We utilise and have developed a set of integrated phenotyping platforms for these traits, giving an extremely thorough overview of the effect of drought stress on our panel of pearl millet. Root and leaf anatomy will be analysed using the novel method of laser ablation tomography scanning, a high throughput method of sectioning and imaging. To analyse the images a neural net is being trained to be able to segment and annotate the root images to fully automate the root image analysis process. We will investigate the relationships between these traits, and how they may be used to identify drought tolerant lines, and the traits and potentially candidate genes responsible for drought tolerance traits. Through this we aim to accelerate the selection of new pearl millet varieties with improved drought tolerance and yield stability for use in Senegal.

# **NATURE AND NURTURE: GENOTYPE-DEPENDENT DIFFERENTIAL RESPONSES OF ROOT ARCHITECTURE TO AGAR AND SOIL ENVIRONMENTS**

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Root development is crucial for plant growth and therefore a key factor in plant performance and food production. *Arabidopsis thaliana* is one of the most commonly used systems to study root system architecture (RSA). Growing plants on agar-based media has always been routine practice, but this approach poorly reflects the natural situation, which has led to a dramatic shift toward studying RSA in soil. We directly compared RSA responses to agar-based medium (plates) and potting soil (rhizotrons) for a set of redundant loss-of-function plethora (plt) CRISPR mutants with variable degrees of secondary root defects. Using both trait-based and shape-based quantification approaches, we demonstrate that plt3plt7 and plt3plt5plt7 plants, which produce only a handful of emerged secondary roots, can be distinguished from other plt mutants on plates and in rhizotrons. However, the secondary root density and the total contribution of the side root system to the RSA is increased in these two mutants in rhizotrons, effectively rendering their phenotypes less distinct compared to WT. On the other hand, plt3, plt3plt5, and plt5plt7 mutants showed an opposite effect by having reduced secondary root density in rhizotrons. This leads us to believe that plate versus rhizotron responses are genotype-dependent. Our study demonstrates that the type of growth system affects the RSA differently across genotypes. Importantly, this finding emphasises the need for phenotyping methods in different growth conditions to understand how plant development is regulated.

## **EVALUATION OF DROUGHT TOLERANCE IN PLANT GENETIC RESOURCES OF CHICKPEA (*CICER ARIETINUM* L.) BY NON-INVASING PHENOTYPING**

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Chickpea is one of the most widely grown legumes in the world and is experiencing increasing demand. In the context of climate change and nutritional trends, chickpea is becoming more important as it is high in protein and adapted to low-input conditions. However, the breeding use of germplasm from different genebanks is limited, mainly due to the lack of available characterization data. Although chickpea is adapted to terminal drought, not much is known about tolerance at pre-anthesis stage as it may occur when grown in Central European conditions.

High-quality phenotypic data from High-throughput Phenotyping (HTP) experiments, such as estimated biovolume, plant height and several color parameters are suitable to detect the genetic architecture of growth and drought tolerance in GWAS studies. The genetic factors of biomass accumulation can be precisely dissected in a spatiotemporal manner and can be complemented with metabolomic and chlorophyll fluorescence data.

200 chickpea accessions are studied on a HTP system under contrasting water regimes to exploit genetic resources and precisely determine growth under optimal and spring drought conditions under European conditions. The accessions originate from IPK and USDA genebank and were single-seed descended and genotyped by whole genome shotgun sequencing (WGS). The collection is balanced for the two chickpea types, desi and kabuli.

The first results of the HTP experiments show a high data quality, and a large phenotypic diversity for growth in the investigated chickpea genetic resources. The drought impacted biomass formation and affected photosynthetic capacity. Color-related traits showed stress progression.

The data are suitable for GWAS that will now be performed to unravel in detail the underlying genetic architecture of growth and drought tolerance in chickpea.

## **QUANTIFICATION OF PHENOTYPIC RESPONSES TO ROOT-ROOT INTERACTIONS AMONG COMMON BEANS IN A SPECIALIZED MESOCOSM**

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Root-root interactions alter the architectural profiles of individual root systems, therefore affect nutrient foraging (O'Brien et al., 2005). Past reports have shown detrimental and beneficial effects to the yielding abilities of crops as they avoid or prefer belowground competition (Li et al., 2006; O'Brien et al., 2005). With little research done into this nuanced system there is still much to discover about the intricacies of root-root interactions and their impact on root development. Quantifying architectural traits of root system interactions would provide invaluable insight to root researchers worldwide. We have begun to develop a mesocosm system to perform a series of preliminary studies that utilize 3D imaging to quantify metrics of root-root interaction using common beans (*Phaseolus vulgaris*). Common beans have a relatively fast growing adventitious and basal root system, making them a suitable organism for this study. Our first mesocosms were fruitful but were not a suitable environment for quality imaging. To fine tune our mesocosm we are focused on improving the mesh system to provide better root support and utilizing light-weight, low-visibility materials to allow our imaging systems better access to the roots. Traits that we aim to extract include root growth angle, rooting depth, and root volume relative to neighbors, because these spatial qualities determine the soil areas that the root system will be foraging in. Our data will allow for the quantification and association of root plasticity in the presence of belowground competition.

## **SPECTRAL PROXIMAL SENSING IN PHENOTYPING YIELD TRAITS OF WHEAT GROWN IN DIFFERENT NITROGEN FERTILIZER CONDITION**

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Wheat (*Triticum aestivum* L.) is a staple crop worldwide and yield improvement was mostly attributed to nitrogen fertilizer application. Since that precision nutrient management requires accurate assessment of crop nutrient status, spectral proximal sensing could presents the promising tool for non-destructive methods to estimate plant status and yield traits. In order to estimate the influence of nitrogen applications on variability of grain yield and yield related traits, four winter wheat genotypes (Pobeda, Futura, NS40S and Ingenio) were selected. The wheat genotypes were grown in field trials of control and three nitrogen levels in amounts of 60, 120 and 180 kg of N ha<sup>-1</sup>. In this research, an active portable multispectral optical device, named Plant-O-Meter, for spectral measurements was used throughout the vegetation season until full maturity of wheat. The device possess an integrated multispectral source of light in four most indicative wavelengths (465, 535, 630 and 850 nm) and allows simultaneously illumination of plant. Large number vegetation indices (VI) based on four spectral bands combinations were calculated to estimate the influence of N supply on yield and yield traits. Across vegetation seasons, phenotypic variability and genotype by environment interaction (GEI) for yield traits of wheat across different N treatment were studied. The additive main effects and multiplicative interaction (AMMI) models were used to study GEI. AMMI analyses revealed significant genotype and environmental effects, as well as GEI effect, while positive correlations between certain VI obtained by the sensor encourage the potential for rapid detection crop nutrient status and its influence on yield using proximal sensing. The results of this study could provide guidelines for rational use of fertilizers, minimize possible harmful effect on environment, as well as in identification of genotypes with stable reaction adapted across environments for the grain yield traits.

## **USING HIGH-THROUGHPUT IMAGING AND ANATOMICAL ANALYSIS TO SCREEN BRACHYPODIUM DISTACHYON ROOT SYSTEM IN RESPONSE TO DIFFERENT NITROGEN SOURCES AND CONCENTRATIONS**

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Nitrogen uptake indicators are a possible framework for evaluating the variation of N utilization efficiency. Crop remote and proximal sensing are widely using the correlation between leaf protein N, chlorophyll content, and leaf reflectance to predict N uptake by the plant, whereas N uptake is a dominant feature of the root system and its ability to soil exploring is the major determinant of N uptake efficiency. In this context, we supposed specific physiological and anatomical attributes associated with root morphology as the potential predictors for N acquisition by crops. This study aimed to explore and Identify root phenotypes function as well as develop specific molecular markers as the potential predictors for N acquisition. In order to quantify root growth and monitor root phenotype, the experimental setup established in the GrowScreen-Agar system that is providing an automated and non-destructive quantification of root and shoot growth and root architecture by using high-throughput imaging. To establish a dose-response curve and anatomical analysis, *Brachypodium distachyon* seedlings were grown for up to 16 days in a medium containing 0 mM, 0.18 mM, 0.37 mM, 0.75 mM, 1.5 mM, 3 mM, 6 mM of ammonium and nitrate as a sole N source. Interestingly, plants reduced their total root length by increasing the N concentrations in both mediums. However, plants grown on a medium containing ammonium appeared to produce a more branched root system. Growing plants on a nitrate medium reduced root length while maintaining root mass by increasing N concentration, resulting in lower specific root length and showing about 6 times more N uptake per mm of their root length. On the other hand, plant roots grown in ammonium were observed to perform aerenchyma and cortical senescence. Quantitative measures of RSA showed that *Brachypodium* seedlings were highly responsive to N concentrations and sources, and drastically reduced most RSA parameters by increasing N concentration.

## DETECTING EARLY DROUGHT STRESS AT DIFFERENT GROWTH STAGES USING SIF IN RAPESEED

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Drought decreases crop yield worldwide, and with ongoing climatic changes the negative impact of drought on yield is likely to increase in future. Brassica is one of the most economically important crop families grown globally, producing not only rapeseed oil, but also many leaf and root vegetables. Therefore, breeding for drought tolerance in Brassica is essential to secure yield. In order to effectively breed for improved drought tolerance, accurate phenotyping for drought response is necessary. However, measuring drought response in early plant development stages is challenging, particularly using non-invasive techniques.

Solar induced fluorescence (SIF) may offer a promising future technique for assessing drought response. SIF serves as a proxy for photosynthetic activity, which is highly correlated to drought response. In contrast to previously used reflectance and fluorescence methods, SIF could be used as a stress indicator measuring from leaf level up to satellite images from unmanned aerial vehicles, aircraft and satellites), providing additional information to captured reflectance.

Here, we aimed to assess the correlation of SIF with other drought response measurements under controlled water stress conditions in the glasshouse. Ten contrasting Brassica genotypes, including *B. rapa*, *B. juncea* and *B. carinata*, as well as two novel allohexaploid hybrids and *Sinapis alba*, were subjected to drought conditions at the four-leaf stage (BBCH 14), rosette stage (BBCH 30) and at flowering (BBCH 50) and kept under drought conditions until the wilting point. The genotypes were then watered again until harvest. Soil moisture and temperature as well as radiation was monitored continuously. Plants were taken outside to measure solar-induced fluorescence using point measurements through a fluorescence box (FLOX) and image-based measurements through HyScreen. Additional measurements with light induced fluorescence (LIFT, MiniPam) measuring active light induced fluorescence, and PolyPen to capture the reflectance, were done. Differences in drought response between the genotypes as well as between time points of drought induction were observed. The SIF signal, measured on leaf level, was shown to be elevated under drought conditions compared to the control group, also before visible drought symptoms could be recorded. Yield and seed quality trait measurements will be analysed at the end of the vegetation period.

SIF shows promise as an early detection method for drought response in Brassica. This experiment is therefore a step towards high-throughput phenotyping for drought in the future. However, further experiments to validate these results at the field level and with different drought events still need to be done.

# UTILIZING UAV BASED HIGH THROUGHPUT PHENOTYPING, CONTINUOUS IN-FIELD CEPTOMETRY AND CROP GROWTH MODELS TO ESTIMATE RADIATION USE EFFICIENCY IN WHEAT

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Radiation use efficiency (RUE) describes the ability of a crop to convert photosynthetically active radiation into biomass. Biomass acts as an important source of resources that can be translocated into harvestable yield. High throughput phenotyping techniques have been demonstrated to strongly correlate with biomass across plant development in several species. These techniques utilise vegetation indices and the 3-dimensional properties of crop canopies and can be applied at higher temporal frequency than destructive approaches. RUE requires a measure of the fraction of intercepted photosynthetically active radiation (FiPAR) in the crop canopy over time. Modelling techniques can be applied to estimate FiPAR, however, these approaches may not adequately capture the variability of light dynamics in row crops, especially when canopy closure occurs. In this study we explore the use of UAV based high-throughput phenotyping approaches in combination with continuous ceptomety measurements to non-destructively estimate RUE in wheat. Trials were grown in three distinct Australian environments and a novel experimental approach to modelling biomass was applied in these experiments. A 'biomass calibration' trial, containing genotypes of varying maturities and densities, was grown to produce a wide range of biomass values. Destructive harvests were taken at five growth stages, and biomass was modelled as a function of UAV derived vegetation indices, crop 3d properties, and additional parametrizations using the APSIM crop growth model. At the Gatton site, a network of continuously logging ceptometers was also deployed to directly measure FiPAR and make a comparison against traditional modelling approaches for this trait. These models were then applied to a larger adjacent trial containing late-stage breeding lines and commercial wheat varieties to predict RUE and its heritability.

# HIGH-THROUGHPUT VISUALIZATION AND QUANTIFICATION OF RICE ROOT SYSTEM ARCHITECTURE USING X-RAY COMPUTED TOMOGRAPHY

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Root system architecture (RSA) is one of the key traits determining plant growth. Plants modify their RSA in response to environmental stresses to avoid or minimize damage. This response is called RSA plasticity, and it is one of the key targets of breeding aimed at the development of crops that can adapt to major environmental stresses. To evaluate RSA plasticity, root growth in the soil must be visualized and quantified in a nondestructive manner. Recently, nondestructive observation of RSA in the soil was achieved using X-ray computed tomography (CT). CT generates massive 3-D image data; however, high-throughput processing of these images is challenging, because rapid scanning of large pots degrades image quality, rendering RSA extraction and quantification difficult. Therefore, in the present study, we developed two software to analyze RSA plasticity. RSAvis3D is an RSA visualization software for monocots. Focusing on thick roots, such as the radicle and crown roots, which constitute the RSA skeleton, RSAvis3D can be used to visualize RSA in large pots (20 cm in diameter). In addition, noise reduction using 3-D median filtering and simple RSA extraction using edge detection enable rapid CT scanning within just 33 s to 10 min to visualize RSA. RSAtrace3D is an RSA vectorization (connecting adjacent root voxels to create a path representing the RSA shape) software. Vectorization allows effortless quantification of RSA traits. RSA can be vectorized in 10 min because this software is GUI-based and, therefore, easy to operate. We demonstrated that RSAvis3D visualized RSA growth in rice (*Oryza sativa*) over 3 weeks and that RSAtrace3D quantified RSA traits, such as root length and rooting angle, of three rice cultivars. Overall, RSAvis3D and RSAtrace3D can be integrated to evaluate RSA traits in a nondestructive manner, enabling high-throughput quantification of RSA plasticity. This work was supported by JST CREST Grant Number JPMJCR1701, Japan.



# Microbiome, Ecology & Biotic Interactions

# **AUTOMATED QUANTITATIVE MEASUREMENT OF SYMPTOM CHARACTERISTICS IN THE ZYMOSEPTORIA TRITICI/WHEAT PATHOSYSTEM TO CHARACTERIZE HOST-PATHOGEN INTERACTION**

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The availability of accurate measures of crop disease intensity is a prerequisite for efficient selection of genotypes with improved quantitative resistance. Besides differences in disease intensity among genotypes, variability in disease symptom appearance is frequently observed, particularly where crops are exposed to genetically diverse pathogen populations, as occurs in the *Zymoseptoria tritici*/wheat pathosystem. The detailed characterization of such variability has led to a much-improved understanding of host-pathogen interactions in several pathosystems. We hypothesized that frequently observed chlorotic yellow halos surrounding necrotic lesions caused by *Z. tritici* may be attributable to the action of diffusible host selective toxins in susceptible cultivars. We developed an image-based phenotyping method to quantitatively measure the occurrence of chlorotic yellow halos surrounding lesions caused by *Z. tritici* on detached wheat leaves. We applied the developed method to more than 10,000 images of leaves infected with a diverse natural population of *Z. tritici* isolates to investigate the genetic basis of yellow halo occurrence in wheat. The overall tendency of wheat genotypes to form chlorotic halos was found to be a quantitative but highly heritable trait. Genome wide association mapping identified six chromosome segments associated with the phenotype, with most segments containing gene families known to be involved in plant-pathogen interactions in other pathosystems. Our results suggest that interactions between pathogen toxins and host sensitivity genes may contribute significantly to the observed variation in quantitative disease resistance under field conditions. Identification and elimination of the responsible sensitivity genes using the occurrence of chlorotic halos as a readily accessible phenotypic marker may therefore significantly improve quantitative disease resistance.

# **CHARACTERIZATION OF FUNGI ASSOCIATED WITH DETERIORATION OF PITAHAYA CROP IN THE AMAZON REGION, ECUADOR**

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In Ecuador, Pitahaya (dragon fruit) is an exotic and economically significant fruit. This crop is a new alternative for agricultural production with a significant economic potential for producers. The yellow variety is the most popular globally, and its demand has increased in the European and United States. However, the fruit is prone to many diseases incited by fungi, bacteria, viruses, and nematodes leading to heavy losses. Among these, losses due to diseases play a significant role in fruit-yield reduction and profitability. Globally, there are 17 genera and 25 species of plant pathogens currently infecting dragon fruits. Mainly fungal pathogens attack various plant parts from cladodes, flowers, and fruits. It is known that 21 fungal species cause various dragon fruit diseases. Most of these pathogens have been identified macroscopically. Nevertheless, in Ecuador many other species have not been identified by applying molecular techniques. The aim is to determine the main strains of fungal species in pitahaya growing ranches in the Ecuadorian Amazonia and use molecular and morphometric traits to identify the pathogenic and endophytic strains. The study was located on farms in the Ecuadorian Amazon, specifically in the provinces of Morona Santiago, Pastaza, and Napo. The isolated fungi were found associated with fruit and cladodes of pitahaya and differentiated in cultural and morphological characteristics. The fungi were identified on the basis of classical microbiological methods based on cultural and morphological characters. Moreover, DNA from pathogen fungi was extracted using the E.Z.N.A. Fungal DNA Kit (Promega). Afterward, the samples were amplified by PCR methods using primers for ITS region and TEF1 $\alpha$ , and PCR product were purified using the Wizard Genomic DNA purification Kit (Promega). The clean PCR products were sequenced using the Sanger method. The sequences were analyzed using the Ugene and MEGA software and were compared with databases using the NCBI BLAST (Basic Local Alignment Search Tool). Our results have provided not only the pathogenic fungal information, but also endophytic fungal identification that will contribute to address sustainable and practical management strategies.

Keywords: Dragon fruit, diseases, fungal pathogens, endophytic fungal, Ecuadorian amazon

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# INVESTIGATION OF THE EFFECT OF PLANT BIOSTIMULANTS ON NITROGEN USE EFFICIENCY USING A TRANSCRIPTOMIC AND PHENOMIC APPROACH

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Approaches to sustainable agriculture are increasingly required and aim to consider an eventual reduction of external input, particularly N mineral fertilizers. Consequently, the research goal to improve Nitrogen Use Efficiency (N-UE), especially in key industrial crops such as corn, has become a priority. Use of plant biostimulants (PBS) are among the most valuable tools to ensure sustainable production with economic benefits for farmers, considering the challenges of future agriculture.

This work proposes a "multi-omics" approach to rapidly and efficiently screen plant or microorganism-based PBS for N-UE enhancement. This approach involves investigating N-UE through gene expression and multi-spectral image analysis by adopting distinct transcriptomic and phenomic platforms.

We monitor differential marker gene expression comparing PBS-treated vs. untreated model plants. Simultaneously, image acquisitions using the HTS system (LemnaTec) on the model plants are performed. Based on the first screening results, the best performing PBS are tested in a second step, using the Scanalyzer 3D (LemnaTec) and PlantEye (Phenospex) phenomics platforms on target crops. The output is the selection of the best prototype for further field test validation on the crops of interest. Our approach, which analyzes gene expression changes and phenotyping, either on model plants or target crops, makes it possible to fast screen PBS candidates while characterizing their "Mode of Action". In turn, new formulations can be leveraged to preserve, stabilize, and increase crop yield.

## **AUTOMATED PHENOTYPING PLATFORMS ARE AN INDISPENSABLE TOOL TO EVALUATE THE EFFECTS OF BIOSTIMULANTS AND BIOCONTROL PRODUCTS**

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The ecological awareness on the excessive agricultural use of chemical fertilizers and pesticides increases, and restrictions on the use of these agrochemicals are being imposed in Europe. Therefore, the real-time monitoring of crop growth and health status in order to respond appropriately to possible (a)biotic stresses and to avoid the overuse of agrochemicals are the main incentives towards precision agriculture. In the past, plant phenotyping was primarily done by scoring traits by the naked eye. As this approach is time consuming, has a low resolution and is prone to human bias, plant phenotyping has evolved towards a new scientific discipline: phenomics, which refers to the characterization of plant phenotypes through the acquisition and analysis of high-dimensional phenotypic data. Phenomics is especially interesting when investigating the effect of potential biostimulant and/or biocontrol products (BBP), as their impact is often small and/or transient.

Within our research group, research and industry work tightly together towards a more sustainable future by screening potential BBP in close association with the host plant. By incorporating a high-resolution multispectral imaging technique, the effect of novel BBP can be monitored on seeds, seedlings and plants in their natural environment, or under extreme conditions. Due to the highly automated sensor-to-plant principle, the spread of a disease or the effect of an agrochemical or stressor can be traced throughout the plant in time. Using a combination of RGB, chlorophyll fluorescence, anthocyanin, NIR and GFP/RFP imaging, the impact of these BBP can be visualized in multiple ways. Recently, our research group expanded its equipment with a hyperspectral camera designed for trait discovery. Moreover, researchers with both biological and image processing expertise can be consulted to compute scientifically relevant measures from these images, thus reaching beyond the mere visualization of phenomena.

# **APPLICATION OF DIGITAL PHENOTYPING METHODS TO QUANTIFY DISEASE RESISTANCE**

**LAURA GROENENBERG**

Wageningen University & Research

The project "Phenotyping 4 profit" aims to determine which sensors can be exploited to qualify and quantify plant-pathogen interactions using the state-of-the-art high-throughput digital phenotyping NPEC facility. From different types of interactions we will test the ability of several sensors to detect (early) symptoms caused by pathogens. Furthermore, we aim to find a correlation between sensor data output, visual resistance scoring and molecular methods such as RT-PCR. Finally, we aim to combine this data for marker-assisted breeding and correlate the phenotypic data to the genotypic data, to assist in disease-resistance breeding.

# **CHARACTERIZING THE PLANT GROWTH-PROMOTING ACTIVITY OF A NEW RHIZOBACTERIUM STRAIN EAOP14 TO SUPPORT SUSTAINABLE CROP PRODUCTION**

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In the last decade, EU regulations (EU Directive 2009/128/EC) have oriented the region towards more sustainable crop production, limiting the use of chemical crop improvement products. Moreover, an increase in volatile weather patterns linked to climate change impacts production systems and highlights the need for more resilient cropping systems if we are to achieve the European Farm to Fork goals for 2030. In light of this, the biostimulants field has received considerable attention. Microbial inoculants (i.e. plant growth-promoting bacteria) have proven to improve plant growth and yield via entering the plant nutrient cycle and actively enriching nutrient availability, and activating defence mechanisms.

This project focuses on characterising the biostimulant activity of a new rhizobacterium strain EaOp14, previously isolated from the *Brassica napus* rhizosphere. Using methods that analyse root and shoot growth, initial phenotypical results indicate that this bacterium has the potential to improve seedling vigour by up to 40% in oilseed rape and up to 50% in wheat under in-vitro conditions. Under glasshouse conditions, strain Op14 enhanced plant development in oilseed rape by 7%, 6%, and 14% based on the assessment of height at the nine-leaf stage, stem diameter and the number of inflorescences at the flowering stage, respectively. Trying to understand the impact of EaOp14, we built rhizoboxes to observe root development in real time and noticed that EaOp14 promotes root establishment for both crop species, oilseed rape and wheat.

Further investigations are underway to test the growth-promoting effect on more crop species (i.e. faba beans, lupins) and to evaluate the promotional effect of the bacterium in field trials of spring oilseed rape. Future work includes root phenotyping using rhizoboxes and RhizoVision Explorer software.

## **METABOLICOFFEE – HOW PRIMARY METABOLISM MODULATES COFFEA SPP. - HEMILEIA VASTATRIX INTERACTIONS?**

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Coffee leaf rust (*Hemileia vastatrix*) is the most important disease of *Coffea arabica*. This project aims to identify coffee’s primary metabolic components essential for growth and development that are simultaneously involved in plant defense responses. We studied the interaction of two contrasting coffee genotypes [*C. arabica* S4 Agaro and Kawisari hybrid (*C. arabica* × *C. liberica*)] inoculated with different *H. vastatrix* races to establish compatible (susceptible - S) and incompatible (resistant - R) interactions. Mock-inoculated leaves were used as control (C). Leaves were collected during the infection process, and the fungal colonization was cytologically evaluated. Inoculated and mock-inoculated coffee leaves were prepared and, metabolites, proteins, and starch were extracted from a single sample using a fractionated extraction method. At IPK, metabolite analysis by GC-TOF was undertaken while using the Golm Metabolome Database (GMD). The microscopic evaluation of *H. vastatrix* pre-penetration stages revealed a high rate of urediniospore germination and appressoria differentiation over the stomata which ensured the success of the infection. For both genotypes, starch content increased along the infection process. A preliminary Principal Component Analysis (PCA) was performed on GC-TOF data showing a clear separation of the two coffee genotypes. In the Kawisari genotype, the PCA analysis of sugar-related features revealed an evident split between samples C, R and S, at each time point. The role of these metabolic features in the coffee defense responses will be discussed.

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# **DISSECTING THE ROLE OF THE TOMATO ROOT ARCHITECTURE IN THE RESISTANCE TO RALSTONIA SOLANACEARUM**

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The root system architecture (RSA) provides critical functions to plants. Understanding the regulation of RSA will enhance our ability to develop crops with higher agronomic performance. Our knowledge of RSA in the soil is limited due to the challenges of imaging and describing the complex root network in an opaque medium. Recent developments in non-destructive root phenotyping enable significant breakthroughs in our understanding of RSA regulation and response to stress. *Ralstonia solanacearum* (Rs) is a devastating soilborne pathogen of major crops such as tomatoes. RSA is known to provide partial resistance to tomato wilt disease caused by Rs, but its mechanisms are not well understood.

We used X-ray Computed Tomography (XCT) and a mini-rhizotron (MR) system to characterize the impact of Rs on tomato root growth. We developed two image analysis pipelines to extract 3D root features from the XCT root models and quantify root growth from the 2D MR images. Our experimental design quantified the roots at different soil depths. Images close to the soil surface quantified large lateral roots, while other images quantified thin roots more profoundly in the pot. The complementary information extracted from these two methods provided a detailed characterization of root system architecture.

We showed different impacts of Rs on root growth and topology of susceptible and resistant genotypes. The disease induced a homogeneous inhibition of the root growth on susceptible genotypes while it caused only a reduction of the root growth for the deepest and thinnest parts of the root system of the resistant genotypes. Our results suggest the role of lateral root emergence in tomato resistance to Rs. The inoculation by a mutant of Rs showed the role of Type 3 effectors secretion on root growth inhibition. Our study represents a baseline for future characterization of the role of Type 3 Effectors during wilt disease and tomato root-driven resistance to other vascular pathogens.

# **PHENOTYPING AND IDENTIFYING VIRAL INFECTION USING A HIGH-THROUGHPUT PHENOTYPING PLATFORM**

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Plant viruses represent a major threat to current agriculture, thus new effective ways are required to monitor and identify them. Novel image-based technologies that allow non-destructive analysis of sample material are emerging from multiple fronts, bringing fast development of lean and digital technologies in plant disease identification. Previously by taking advantage of the University of Helsinki National Plant Phenotyping Infrastructure (NaPPI) we established morphological and physiological differences between plants single and co-infected by Sweet potato feathery mottle virus (SPFMV) and Sweetpotato chlorotic stunt virus (SPCSV). Identified parameters were later successfully used to assess co-infection severity to confirm Sweet Potato Virus Disease (SPVD) reversion after chemical treatment. Recently, the increase of computer power and artificial intelligence makes it clear that machine learning will be part of the next revolution of pathogen identification. In that sense we are currently building a comprehensive image dataset of Potato virus Y (PVY) viral symptom in potato for developing image classification model(s) by machine learning approaches. This dataset encompassing visible RGB images from different sources includes highly-standardized images generated by NaPPI, semi-standardized images taken with a camera and non-standardized images manually taken with a smartphone. By combining images from different sources, we aim to develop image classification model(s) allowing broader classification of PVY-infected potato that could be directly used by farmers in their field.

## **HIGH THROUGHPUT PHENOTYPING FOR EARLY DETECTION OF BIOTIC STRESS USING ARABIDOPSIS THALIANA × PSEUDOMONAS SYRINGAE PATHOSYSTEM**

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*Arabidopsis thaliana* - *Pseudomonas syringae* pv. *Tomato* (Pst) interaction constitutes an efficient model for studying the host-microbe interaction associated with the pathogenicity mechanisms and plant signaling defense. Recently, digital sensors coupled to plant phenotyping platforms have been promissory to determine early damages caused by pathogen infection. Our study aimed to develop a new non-invasive high throughput screening (HTS) for studying biotic stress through a simple readout based on red-green-blue (RGB) and chlorophyll fluorescence (Ch/FI) images. Three *Arabidopsis thaliana* ecotypes (Columbia, Landsberg erecta, and Wassilewskija) grown in vitro into multi-well plates and infected with Pst were evaluated. True leaves from 14-days-old seedlings were inoculated with a Pst suspension at three concentrations. From RGB images obtained at 24, 48, and 72 hours after inoculation was possible to record the lesion progress caused by Pst infection in the three concentrations. All infected plants significantly reduced the growth compared to non-inoculated plants. On the other hand, fluorescence imaging showed alterations in the photochemical and non-photochemical energy dissipation parameters suggesting reductions in the photosynthetic capacity of the plants due to the Pst infection. Our results demonstrate that a simple RGB-based HTS is suitable for early detection of plant infection and an up-and-coming method to study host-pathogen interaction, including testing chemical libraries for plant resistance improvement.

## ULTRAVIOLET IMAGE-BASED PHENOTYPING FOR LETTUCE DOWNY MILDEW RESISTANCE

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Lettuce downy mildew (*Bremia lactucae*) is an oomycete pathogen responsible for large losses in lettuce production. We observed that illumination of infected lettuce leaves with ultraviolet light (365 nm) induces blue-green fluorescence at areas of pathogen infection, likely from secondary metabolites, which provide a quantifiable image-based biomarker. Using a custom UV imaging system, young lettuce plants across six different genotypes with varying levels of *Bremia* resistance were imaged from 5 to 9 days after inoculation. After plant segmentation, six metrics including the mean, median, mode, variance, skewness, and kurtosis from individual histograms of the RGB, HSV, LAB, and other channels were calculated, resulting in 66 features. For multiple features, statistically significant changes over time were found in the susceptible or partially susceptible genotypes that were not observed in the resistant genotype. Principal component analysis also showed a clear separation between *Bremia* and mock treated plants, indicating that there are strong signals from biotic stress that can be visually captured by these UV image-based features using unsupervised methods. Using a supervised method, random forest, a classification accuracy of 96.4% was achieved (from a 70:30 training/test data split) between *Bremia* versus mock treated plants at 9 dpi with susceptible and partially-susceptible genotypes. Additionally, the percentage of infected leaf area based on blue-green fluorescence also generally correlates to sporangia levels and qPCR assays. Finally, comparison between UV imaging and chlorophyll fluorescence imaging of infected lettuce also shows a correspondence between the two methods, providing an additional form of validation. Taken together, this demonstrates that UV imaging can function as a fast and inexpensive method for screening and genetic mapping of quantitative resistance against *Bremia* and potentially other stress-associated traits in lettuce.

## THE USE OF PHENOTYPING PLATFORM IN INVESTIGATION OF TRICHODERMA-TOMATO INTERACTION IN DROUGHT CONDITIONS

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The use of plant phenotyping platforms in investigation of interaction between plants and symbiotic microorganisms is a new approach. Trichoderma spp. are cosmopolitan soil and rhizosphere inhabiting fungi providing many advantages in agriculture. In this experiment, the adaptive responses of tomato plants (cv. Gružanski zlatni) to progressive drought were studied. In the pot experiment realized at environmentally controlled conditions of PlantScreen phenotyping platform at SUA in Nitra, tomato plants grown in substrate without and with addition of *T. harzianum* and *T. brevicompactum*. Structural (leaf area and shape of plants) and functional (chlorophyll a fluorescence and hyperspectral vegetation indices) responses were simultaneously complemented with measuring of other biochemical and leaf water status parameters. Morphometric RGB-based imaging analysis revealed that water shortage resulted into the reduction of plant growth measured as a planar plant area without significant differences between effects of Trichoderma species. On the other hand, application of Trichoderma caused longer maintenance of photochemical efficiency during water stress conditions than control plants. This phenomenon was confirmed by higher concentration of chlorophylls in Trichoderma treated plants. Abrupt changes of flavonol index, observed 24h after the Trichoderma application, indicate the participation of flavonols in signalization. The recovery of flavonol content from 48h to 96h after the application was the slowest in plants treated with *T. brevicompactum*. Photosynthetic parameters indicate that the effects of drought were more significant than the Trichoderma treatment. *T. harzianum* treatment had a positive effect on the rate of fruit formation, both in optimal water supply and in drought.



# Modelling of Physiological & Ecological Processes

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# **HIGH RESOLUTION DATA, NATIONAL RESEARCH INFRASTRUCTURE AND ECOSYSTEM MODELLING TO ENABLE A CARBON NEUTRAL DIGITAL AUSTRALIA**

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The explosion of capabilities created by the Internet was only possible because the core design intent for the Internet was to optimise information movement to improve research outcomes. This approach was open by design and didn't seek to monetize this framework. Rapidly transitioning to a carbon negative, sustainable economy requires creating similarly open architectures for digitising the world that support a rapid iteration model for research and solutions development within a shared, interoperable, auditable data framework. Thus it is essential that our tools for monitoring, mapping and modelling agricultural and natural systems be developed in a manner that prioritises common standards and FAIR principles.

Due to the perceived economic value of digital agriculture (DA), many commercial players are seeking to monetize full-stack solutions rather than supporting collaborative development of open data architectures similar to what enabled the success of the Internet. Solutions not built on open architectures lead to walled gardens and vendor lock-in which actively stifles competition and inhibits knowledge discovery. Despite efforts in academia to create FAIR data frameworks, there is no internationally adopted equivalent of Internet architecture for DA.

National research infrastructure (NRI) can play an essential role in countering this trend by creating open source full-stack solutions for common technical & data challenges. These solutions can be made widely available at lower cost and further improve interoperability by supporting multiple research domains under a common framework.

NRI can also provide shared platforms on which nextgen modelling and MLAI can be developed and rolled out at national scale. We will review ongoing work at the ANU node of the Aus. Plant Phenomics Facility to support phenomics and ecosystem monitoring in Australia. Projects include open source tools for controlled environments, field data and a national platform for drone research.

## IN PURSUIT OF BETTER WORLD: CROP GROWTH MODELS-GUIDED PHENOMICS FOR AGRI-SYSTEM IMPROVEMENT

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The climatic changes are projected to threaten food security especially in the most fragile agri-systems such as Semi-Arid Tropics (SAT). The stabilization/improvement of such complex agri-systems requires a quantitative understanding of the system dynamics at the level of the soil-plant-atmosphere continuum as well as the system components interplay. This can be efficiently done in-silico using the crop growth models (CGMs) which can integrate knowledge of the soil-plant-atmosphere continuum and enable analysis of the agri-systems dynamics across the relevant spatio-temporal scales. We will demonstrate several cases of cereals and legume cropping systems where CGMs (i.e. APSIM, SSM) guided the effective design of the environment-specific cultivars (along with the crop management practices suiting the particular stakeholder's needs; i.e. GxExMxS optimization). Consequently, such crops can be rapidly developed in-vivo using range of phenomics tools and sensors (e.g. Lidar, RGB, multi-spec, hyperspec sensors carried by UAVs or gantry). Nevertheless, despite of the rapid boom in sensor-based phenotyping, the efficient, standardized evaluation of the target traits from the TBytes of sensor-generated data has become a significant bottleneck. Hence, we will also showcase our current research to overcome the gaps in big data analytics with the focus on analysis of crop features in 3D data. We will highlight several cases where high-throughput phenomics already enabled rapid development of climate-smart crops and our ongoing efforts to effectively integrate the relevant technology into the ICRISAT (International Crops Research Institute for SAT) breeding programs to practically support improvement of dry-land cereals and legumes-based cropping systems. We will take an opportunity to discuss the integration of CGM with the phenomics tools and data to develop the climate-smart crop products for the target stakeholders in the future.

## **DEVELOPING A ROOT INDEX FOR INDIRECT PHENOTYPING OF ROOT CAPACITY UNDER FIELD CONDITIONS**

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In order to maximize crop productivity, investment in roots must be optimized to permit evapotranspiration (ET) rates that are non-limiting to photosynthesis. However, breeding pipelines usually neglect root traits in their selection process, driven mainly by the challenges associated with phenotyping below-ground structures. Despite the latest advances, root phenotyping under field conditions is still limited and relies on direct observations of the root system, restricting their application at breeding scale.

To estimate root function, we propose the use of a root index derived from remote sensing of canopy temperature (CT) and the water-related spectral index (WI) to screen for genetic variation in root capacity in stressed and unstressed wheat plants. This development builds on our previous findings, where CT is tightly related to root biomass and depth under drought and overall root capacity under heat conditions. Complementary measurements of WI provide a proxy for canopy biomass and water status, both important when evaluating the capacity of a given root mass.

We set a bread wheat panel consisting of 13 genotypes that were grown under yield potential, under an increasing water stress imposed after maximum root development, and under a high VPD. These experimental conditions were strategically imposed to maximize the association of CT and WI with ET, allowing the discrimination of different root capacities. The yield potential and the water stress environments showed significant correlations for root biomass, indicating that maximum root development was achieved. The gradual water stress increased the ability of CT for to predict root biomass, indicating that this environment can be used for predicting root capacity under yield potential. However, the high VPD environment showed the best relationship between CT and root biomass. The Root Index may eventually give a true estimate of functional root capacity since it is integrative of the whole root system.

## REDUCING THE PHENOTYPING BOTTLENECK OF SOURCE TRAITS IN WHEAT

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To boost crop yields it is necessary to improve source traits such as photosynthesis, biomass accumulation and light conversion efficiency. These are key physiological traits that link plant primary metabolism with yield, but their study is characterized by low throughput methodologies limiting the measurement of such traits at breeding scale. High-throughput phenotyping (HTP) was used in spring wheat genotypes with contrasting RUE and photosynthesis rates in three field seasons. We built predictive models of RUE, biomass and photosynthetic traits using hyperspectral data collected at leaf and canopy level during the vegetative and grain filling growth phases. RUE was predicted using linear regression with spectral indices with 70% accuracy and it was found that spectral indices related to chlorophyll and water content were the main predictors of RUE, biomass and intercepted PAR during the vegetative stage while gas exchange and senescence related spectral indices were the best predictors during grain filling. For photosynthetic traits partial least squares regression was used to build models for the top, middle and bottom layers of the canopy and a combined layer model approach was also applied. Our predictions for photosynthesis ( $R^2 = 0.48$ , RMSE =  $5.24 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $R^2 = 0.36$ , RMSE =  $0.14 \text{ mol m}^{-2} \text{s}^{-1}$ ) were more robust when the three layers were combined compared to using individual layers. Using HTP methods allowed us to increase the phenotyping capacity by 40 times compared to conventional growth analysis and 30 times compared to conventional gas exchange measurements. The approach presented in this study can be used to screen elite breeding progeny and exotic genetic resources for source traits and help improve our understanding of wheat physiology by adding several canopy layers to physiological models.

# **A SPATIAL APPROACH OF WHEAT GRAIN YIELD VARIATION UNDER CLIMATE CHANGE SCENARIOS, ECOLOGICAL AND PHYSIOLOGICAL IMPLICATIONS**

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Climate and global change place crops sustainability at risk. This is especially relevant in the Mediterranean basin where increasingly irregular rainfall and extreme temperature are expected. This study aims to find ecological, physiological and land-use aspects that may help to contribute finding wheat ideotypes in the Iberian Peninsula for the next decades. On R, a Random Forest algorithm was used with current climatic data (rainfall and temperature) to define, at district level (~600 units), marginal low-yielding areas (less than 2000 kg/ha), middle-low yielding areas (between 2001-3000 kg/ha), middle-high yielding areas (3001-4000 kg/ha) and high-yielding areas (over 4001 kg/ha). Subsequently, the model was applied for the projected 2041-2060 scenario for two IPCC shared socioeconomic pathways (SSP), SSP2-4.5 (middle of the road, 2.7C increase of temperature) and SSP5-8.5 (fossil-fueled development, 4.4C increase of temperature). Irrigation and ecological intactness maps were matched to districts yields to see their influence in each scenario. The results suggest that in the SSP5-8.5 scenario there might be an increase of marginal producing districts. While in the SSP2-4.5 there might be a reduction of top producing districts. Regarding districts features changes, several relevant wheat physiological traits are spatially described. We observed that, currently, most of marginal districts for wheat production in the Iberian Peninsula have less than 10% access to irrigation. The districts with middle-high yields have the highest access to irrigation. A similar trend is observed for the two IPCC scenarios studied. Regarding ecological impacts of agriculture, the most intensive (middle to high-yielding producing sites) have higher ecological intactness loss. In conclusion, the Iberian Peninsula could experience a generalized reduction in wheat yield and an increase of marginal low-yielding districts, which urges to spatially define relevant physiological traits.

## **DROUGHT RESEARCH IN THE DRY AREAS OF AFRICA: THE CASE OF PHENOMA THE HIGH THROUGHPUT PHENOTYPING PLATFORM IN MOROCCO**

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Climate change and increasing population poses serious threats to food and nutrition security in Africa. Accelerated selection of germplasm that is resistant to major abiotic stresses (drought, high temperatures, mineral deficiencies) is needed. Advances in the large-scale automated characterization of plants, also known as high throughput phenotyping is accelerating the progress of genetic improvement of crops. The African continent has been lacking high throughput phenotyping platforms. Here we present a unique close-to-field high-throughput plant phenotyping platform "PhenoMA" built in Benguerir Morocco by the Mohammed VI Polytechnic University (UM6P). PhenoMA is located in an arid region of Morocco and consists of 1440 fully automated lysimetric mini-plots system which allows to follow the dynamics of water use and simulate any drought scenario. PhenoMA is equipped with a fully autonomous and versatile phenotyping robot (PhenoMobile V2) allowing to make advanced plant measurements of the canopy level. The PhenoMobile V2 is equipped with many sensors: RGB, multispectral, IR, and LiDAR cameras allowing a real-time characterization of the canopy development. The combination of lysimetric data (Transpiration) and canopy development analyses allows the assessment of a realistic water budget of crops under drought and temperature stress.

# **A NEW FARMING PROGRESS CLASSIFICATION METHOD IN RICE–WHEAT ROTATION FIELDS BASED ON UAV RGB IMAGES AND THE REGIONAL MEAN MODEL**

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Extraction of farming progress information in rice–wheat rotation regions is an important topic in smart field research. In this study, a new method for the classification of farming progress types using unmanned aerial vehicle (UAV) RGB images and the proposed regional mean (RM) model is presented. First, RGB information was extracted from the images to create and select the optimal color indices. After index classification, we compared the brightness reflection of the corresponding grayscale map, the classification interval, and the standard deviation of each farming progress type. These comparisons showed that the optimal classification color indices were the normalized red–blue difference index (NRBDI), the normalized green–blue difference index (NGBDI), and the modified red–blue difference index (MRBDI). Second, the RM model was built according to the wholefield farming progress classification requirements to achieve the final classification. We verified the model accuracy. The proposed method was then applied to predict UAV RGB images of unharvested wheat, harvested wheat, and tilled and irrigated fields. The results were compared with those obtained with traditional machine learning methods, that is, the support vector machine, maximum likelihood classification, and random forest methods. The results indicate a significantly higher accuracy of the proposed method than those of the traditional machine learning classification methods for the identification of farming progress type, with the Kappa coefficient of up to 0.9179, while traditional machine learning methods all produced a Kappa coefficient less than 0.7. The proposed work provides an important reference for the application of UAV to the field classification of progress types.

## **TOMATO CROP DIGITAL TWIN: DESIGN AND DEVELOPMENT OF A TOMATO PLANT SIMULATION MODEL**

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Tomato breeders and growers need efficient strategies to increase the sustainability of tomato production, because of the new restrictions on CO<sub>2</sub> emissions and the need to reduce energy consumption. To optimize tomato agronomic management, production and breeding strategies, a digital twin for tomato crops was designed, in the project "Virtual Tomato Crops" (VTC). Here, we present the design of a tomato plant simulation model, which is at the core of this digital twin. The plant model is based on functional-structural plant (FSP) modelling concepts, and it simulates 3D architectural development and functioning of individual tomato plants in response to climatic conditions. The key climatic factors are temperature, CO<sub>2</sub>, and light. Indoor climate is modelled using outdoor climate data and greenhouse properties. An essential feature of the model is the feedback between light absorption by leaves and assimilate production. To maximize the precision of the calculation of light absorption, the model includes leaf optical properties, and a detailed shape and orientation of leaves and leaflets. For a realistic simulation of light distribution, the greenhouse construction and planting pattern of the simulated crop were incorporated. The FSP model was calibrated for four tomato cultivars based on two phenotyping experiments, conducted in the Netherlands Plant Eco-phenotyping Center (NPEC) facilities. Simulations were performed to test model performance for a selection of cultivar dependent traits. The tomato digital twin will provide users with a decision-support tool for determining the optimal cultivation strategy, selecting appropriate cultivation measures, and for supporting genotype selection and design.

# **VARIATIONS BETWEEN TEMPERATURE AND RELATIVE HUMIDITY INFLUENCE TOMATO GROWTH INDICES DURING GREENHOUSE CULTIVATION**

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Tomato growth is mainly defined by genetic factors and influenced by environmental factors, such as temperature and relative humidity (RH). Global warming is recently gaining significance as a threat to the natural ecosystem, and there is a strong demand to increase productivity. Therefore, deep learning, a non-destructive method was used to detect and predict tomato (*Solanum lycopersicum* L. cv. Definis) growth indices influenced by environmental factors in two experimental sites during the 2020/2021 cropping season, and vegetative and reproductive development were investigated. The significant correlation between tomato growth indices and environmental conditions revealed that temperature had a strong effect on plant height and fruit yield whereas relative humidity affected plant height and the number of flowers. The higher variation between temperature and RH in site 1 affected pollen formation and their translation to fruit set as compared to site 2 with lower variations. Phenotypic markers are used to steer plant growth balance during cultivation. In this study, the diameter of the stem (SD), and the distance between the flowering truss and the head (FH) was used to predict the vegetative and generative growth of tomato. The relationship between SD and FH correlated well with leaf width and leaf length in both experimental sites. Leaf width and length are known to be the main determinant of plant photosynthesis, hence, the increase in flower truss and fruit sets. This study shows that optimum variations in environmental factors can be used to predict plant growth and productivity.

# Food & Bio-economy

## **NORDPLANT - A CLIMATE AND PLANT PHENOMICS UNIVERSITY HUB FOR SUSTAINABLE AGRICULTURE AND FOREST PRODUCTION IN FUTURE NORDIC CLIMATES**

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Climate change is a global challenge, and new plant breeding and protection efforts are urgently needed. Advanced and precise methods for rapid screening of plant material is central to identify suitable crops for current conditions and future climate scenarios. Therefore, five Nordic universities with versatile and complementing research and infrastructures established NordPlant in 2018 to promote education, research mobility and technological development to meet challenges in Nordic agriculture and forestry. Core activities include the application and development of novel methods for plant phenotyping and climate facilities.

NordPlant stimulates knowledge-exchange by seminars, site-demos and education. To better build on each other's strengths and knowledge, it supports joint research projects between the founding universities by reduced user-fees and mobility grants. NordPlant has five focus groups: Phenotyping methods in field, greenhouse, and cell physiology; Data handling and integration related to phenotyping and modelling; Emerging and increasing plant pathogens and pests in the Nordic countries; Abiotic stress relevant for future climate change in the Nordic countries; and Demands of breeders and precision farmers. It especially focuses on plants important for the Nordic countries both economically and culturally.

NordPlant will run until the end of 2023 and we currently have a strong focus on joint data standards for plant phenotyping. Previously, we have promoted forum discussions for plant facility managers in the Nordic countries, which identified challenges and possibilities in running these facilities.

NordPlant is funded by NordForsk, which supports research as part the Nordic Council of Ministers. The founding universities are University of Copenhagen, University of Helsinki, Lund University, Swedish University of Agricultural Sciences and UiT The Arctic University of Norway. More information is available at [www.nordplant.org](http://www.nordplant.org)

## RGB-DERIVED VEGETATION INDICES TO ESTIMATE SOIL COVERAGE ABILITY IN DURUM WHEAT

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Among cereals, durum wheat has the least competitive ability against weeds due to its weak early vigor that limits soil coverage during early-growth stage. We report here the application of a series of RGB-derived vegetation indices (VIs) from Unmanned Aerial Vehicle (UAV) to evaluate the soil coverage ability in a large collection of durum wheats, and its validation under different agronomic management. To achieve this goal, two field experiments were carried out, collecting digital RGB images from seedling emergence to late grain filling. RGB-based Green Area (GA), Greener Area (GGA), Normalized Green Red Difference Index (NGRDI), Triangular Greenness Index (TGI) and crop senescence index (CSI) were calculated during four consecutive growing seasons. In the first experiment a collection of 450 durum wheats was evaluated during 2015-16, with a standard agronomic management; in the second experiment two contrasting genotypes for soil coverage ability, were managed by two sowing rates (200 and 400 seeds m<sup>-2</sup>) and six Nitrogen (N) treatments (ranging from 0 to 240 kg N-1) over three growing-seasons (2017-19). Statistically significant differences between genotypes were observed for all VIs for durum wheat collection. GA, GGA, NGRDI and TGI explained more variability than CSI, when recorded at the end of tillering stage. In the second experiment, the multivariate analysis of variance (MANCOVA) confirmed a good discriminating capacity of VIs for genotypes and for the two sowing densities, starting from the tillering phase ( $\eta^2=0.50$ ), whereas for the N levels the differences were significant only starting from heading date, i.e. only after the absorption and utilization of N by the crop. Genetic correlations of RGB-derived indices vs. grain yield and other traits (plant height, biomass) were also significant. These results highlight the potential use of RGB-derived VIs for phenomic selection of soil coverage ability in durum wheat under different agronomic conditions.

# **TOWARDS INCREASED GENETIC GAIN: UTILIZING SPECTRAL DATA IN A LARGE SCALE WHEAT BREEDING PROGRAM UNDER A DROUGHT YEAR**

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Multispectral imaging with unmanned aircraft systems (UAS) is a promising high-throughput phenotyping technology that has been shown to help understand the causal mechanisms associated with crop productivity. This imaging technology can accurately predict complex agronomic traits like grain yield within a given generation, creating the potential to fast-track selections in plant breeding and increase genetic gains. The objective of this study was to determine the effectiveness and efficiency of prediction on grain yield in an abnormal drought year across locations within a breeding program. Eleven spectral reflectance indices (SRI) including NDRE, NWI, NDVI, and percent canopy cover were used to evaluate Washington State University winter wheat breeding lines between 2018 and 2021. Data was collected using a DJI Inspire 2 drone, equipped with a Sentera Quad Multispectral Sensor, and collected at the heading date. Lines were observed from single location, single replication preliminary yield trials to multi-location, replicated advanced yield trials. Lines advanced in the breeding program were evaluated across 13 different location-year trials. The calculated SRIs and canopy cover were used individually and in combination with genomic data to model prediction for grain yield under drought conditions. Models were independently validated with 2021 data. Across locations, SRIs are shown to improve the prediction performance for grain yield under abnormal drought conditions by as much as 40% in the case of NDRE. This research is vital for plant breeders to understand the utility of UAS imaging in variety improvement when dealing with abnormal growing seasons.

## **CARNOSIC ACID CONTENT ESTIMATION BY HYPERSPECTRAL REFLECTANCE OF FIELD GROWN ROSEMARY PLANTS**

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Rosemary (*Salvia rosmarinus* (L.)) extracts are widely used as natural preservatives due to the antimicrobial and antioxidant properties attributed to the phenolic diterpenoid carnosic acid (CA). Growers are rewarded based on CA content in their rosemary leaf harvested. Conventional methods for estimating leaf CA content are destructive and often time-consuming. Identifying cultivars with high CA content as well as quick and non-destructive CA content estimation in rosemary field are needed. This study presents a spectral non-destructive approach for in vivo estimation of CA content in different rosemary cultivars, based on the reflectance spectra of their canopy. The proposed approach is based on the characteristic rosemary absorption features along the visible and shortwave infrared spectral regions at 550 nm, 1200 nm and 1690 nm, respectively attributed to leaves color, the oxygen-hydrogen bond bending in water molecules and distinctive carbon-hydrogen bond features typical for terpenes and phenolic compounds. Correlations between measured CA content by high performance liquid chromatography (HPLC) and latent components obtained by genetic algorithm-based partial least squares regression (GA-PLSR) were assessed using data collected from 79 rosemary cultivars. Randomly selected canopy spectra were used to calibrate and cross-validate 100 iterations, using the 'leave-k-out' approach. The root mean squared error (RMSE) obtained for calibration and cross-validation were 0.86% and 1.15% CA content from the dry leaf matter, respectively. The GA-PLSR model successfully predicted the CA content among the various cultivars, further providing evidence of high weightage to the above-mentioned absorption features also obtained from two best-wavelength combination selections. This work showed feasibility for in-vivo CA content estimation in field grown rosemary plants and can be beneficial in breeding projects as well as commercial fields.

## **DARE YOU IMAGINE? – CONSUMER AND STAKEHOLDER PERCEPTION AS PHENOTYPIC TRAITS**

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The New Zealand Institute for Plant and Food Research Limited

Every commercial approach that involves a radical departure from current and traditional methods requires sufficient acceptance from stakeholders and consumers to be successful. This trait is often ignored or its importance downplayed until the technique is entering active commercialisation.

Global population growth and increased urbanization generate a need for more efficient and sustainable production systems to supply fresh horticultural food products in areas with high population density. Our research is aimed at generating a controlled environment production system that allows rapid and flexible production of fresh produce through advanced grafting methods. This requires the development of novel Stimulated Grafting techniques which enable the combination of plant tissues and species that are currently incompatible. In addition, the integration of artificial structures into plants system to supplement or replace tissue function is being explored, with a focus on the plant's vascular system.

Whether such radical innovations would be adopted by the horticulture industry is so far unknown. To estimate the economic and social impact and acceptance of Stimulated Grafting, we conducted exploratory work to understand stakeholders' perceptions and attitudes towards the technology under development. We focussed on four cultural backgrounds (New Zealanders of Māori decent, New Zealanders of European decent, Chinese and European), to explore industry excitement, economic viability and practicality as well as socio-cultural acceptance. Insights gained from stakeholders will inform the research teams about any potential concerns and limitations around social and cultural licences, and the possible approaches to address these concerns.

# **PHENOTYPING OF PSOPHOCARPUS TETRAGONOLOBUS (L.) DC. ACCESSIONS FOR GENETIC DIVERSITY ASSESSMENT THROUGH AGRO-MORPHOLOGICAL TRAITS, SINGLE NUCLEOTIDE POLYMORPHIC MARKERS AND A MULTISPECTRAL IMAGING SYSTEM**

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Phenotyping of germplasm collections will speed up current breeding strategies, however, it could be time-consuming. Winged bean is a crop with exceptional nutritional potential but with very little research attention on its genetic improvement due to lack of accurate knowledge on the existing intra-specific diversity among the available winged bean germplasm. In addition, the seed morphometric characters are useful to distinguish between different accessions. Assessment of the genetic diversity of thirty-winged bean accessions collected from the Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria was carried out using seventeen agronomic traits obtained from field trials conducted at three different locations in Nigeria and seed traits obtained from multispectral imaging system using VideometerLab 4 instrument. Significant differences ( $p < 0.05$ ) were observed among the accessions, environments, and accessions x environment interaction for all agro-morphological traits. Accessions were grouped into three clusters based on the agro-morphological traits, six clusters based on the Single Nucleotide Polymorphic (SNP) markers while three clusters were obtained based on the multispectral image analysis. Accession TPT-7 was identified to be different from the other accessions and could serve as a good resource for future winged bean improvement programs. The result also confirmed that multispectral imaging system is an invaluable tool for discriminating among accessions of same crop species.

# **CIRCULAR BIO-ECONOMY IN AGRICULTURE SECTOR: A STEP TOWARDS SUSTAINABILITY**

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Moving towards advanced standards of sustainability, India has to shift towards the adoption of the Circular Bio-Economy (CBE) approach in the agriculture sector. However, to aid this transition, the determination of a sustainable waste management technology and supporting strategies is very much essential. In attempt to this, a hybrid methodology based on fuzzy Strength, Weakness, Opportunities, and Threats (SWOT) and fuzzy Technique for Order Preference by Similarity to the Ideal Solution (TOPSIS) was developed. The fuzzy approach used in both the cases was to address any sort of ambiguity during the decision-making process. From the fuzzy SWOT analysis, the decision-makers allocated the most importance to the "ease of adoption" criteria, among the list of internal factors. Whereas, from the list of external factors, the most importance was allocated to the "price competitiveness with respect to fossil feedstock" criteria. Further, composting and anaerobic digestion are considered to be the most sustainable technologies for enhancing the agricultural waste of the country. However, composting showcases more opportunities for its adoption as compared to anaerobic digestion. Furthermore, the results from fuzzy TOPSIS suggest the provision of financial support to both the local farmers and investors to be the top-ranked strategy for the successful implementation of the CBE approach.

## **PHENOLAB 4.0: A PROJECT TO EXPAND THE ITALIAN HTPP PLATFORMS**

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PhenoLab 4.0 project expands the technologies of the Research Infrastructure (RI) already present in Basilicata Region at the Research Center Metapontum Agrobios of ALSIA. The RI, in the current configuration, let high-throughput plant phenotyping (HTPP) in a semi-controlled environment and today it represents the main HTPP technological node in Italy, focal point of the national network PhenItaly (<http://www.phen-italy.it>), Italian node of EMPHASIS project approved in the ESFRI 2016 roadmap.

Phenolab 4.0 is a project supported by Basilicata Region EFRD 2014-2020, and it focuses on investments aimed at increasing both automation and the number of sensors for imaging, allowing to build new greenhouse areas and to acquire growing rooms equipped with automatic systems for detecting additional morpho-physiological parameters. Phenolab 4.0 will also implement plant phenotyping technologies in open field condition, consisting of a fleet of UAVs as a remote sensi platform, equipped with thermal camera, LIDAR, multispectral and hyperspectral cameras, integrated by a ground robot platform and multiple ground truth sensors.

The mission of the Phenolab 4.0 is to be a referring point at the international level, offering RI accesses to scientists and stakeholders to carry out research on plant development under a series of abiotic and biotic stresses in climate rooms, greenhouse and field.

## FIELD PHENOTYPING UNDER PLANT PHOTOVOLTAICS

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To meet the increasing demand for sustainable energy production photovoltaics are proposed in synergy with horticultural and agricultural fields. So-called plant- or horti- and agri-photovoltaics (PV) are PV systems that allow, and even can benefit, plant growth and production under or in between the photovoltaics. In addition, PV modules can also collect water and redistribute this under and between the panels when needed. We will present a new plant PV system that is installed in the brown coal area of North Rhine Westphalia end of 2021.

This plant photovoltaics system, built within the BioeconomieRevier project, consist of 4 different systems: 1) an open and 2) closed fixed south oriented standard system with rain water harvesting of approximately 3 m in height and 3) a system without and 4) with rain water with east-west tracker modules approximately 4 m in height. Under the 4 systems and in 3 open control fields a unique rails system is mounted that allows for high throughput phenotyping with a hanging self-driving battery powered vehicle. This vehicle has a universal sensor mounting place that will allow for sensors quantifying structural and functional plant traits.

We will present the results of the growth performance under the PV of cover crops such as mustard seed and *Phaecelia* and/or initial experiments with bread beans. These will be compared with previous results of ornamental plants grown under an existing horti-agri PV in north Germany. Similar to non-shade ornamental plants that showed a decrease in exposed leaf area, quantified from RGB images, a strong response to shade of the PV is hypothesized in these crops. Future target crops for this plant photovoltaics system are medicinal and berries that may rather benefit from the shade such as ornamental shade plants when water is available. High throughput field phenotyping is expected to quantify the response to shade under photovoltaics to help select and optimize plant production.

## **BREEDING FOR HIGH YIELDING FORAGE GRASSES USING HIGH-THROUGHPUT FIELD PHENOTYPING**

**JOANNA PRANGA**; DR. IRENE BORRA-SERRANO; DR. TOM DE SWAEF; MATHIAS COUGNON;  
AN GHESQUIERE; IVAN JANSSENS; GREET RUYSSCHAERT; PROF. DR. ISABEL ROLDÁN-RUIZ;  
DR. PETER LOOTENS

ILVO

Breeding programmes of forage grasses aim at improving their yield and quality while enhancing tolerance to biotic and abiotic stress conditions. Given the large numbers of plants and plots that need to be screened, the assessment of plant phenotypes remains a considerable challenge. Therefore, technological solutions for rapid, non-destructive, and accurate estimations of plant traits are needed. High-throughput field phenotyping (HTFP) offers great potential in this area. Studies conducted at ILVO (Belgium) focused on the development of HTFP protocols and procedures within the perennial ryegrass (*Lolium perenne* L.) breeding programme. To monitor canopy height and to build models for yield predictions, information from rising plate meters (RPM), artificial height references (AHR) and ground control points (GCP) were compared with drone-derived data (RGB imagery). We developed approaches capable of determining canopy height accurately and demonstrated that yield prediction models performed better when drone data were used instead of RPM data [1]. It was further shown that yield estimations improved when 'mean intensity' values were incorporated into the model [2]. In a recent study [3], HTFP procedures were further optimized by integrating multispectral (MS) data and machine learning techniques. The performance was enhanced by combining structural and spectral features (lowest rRMSE of 13.1%) while MS predictors slightly outperformed RGB ones. Each of these experiments were a single-field study. Thus, we are currently validating our models using new multi-seasonal data from several field trials across northern Belgium. The overall goal is to ensure the general applicability of advanced procedures (in different seasons and locations) and make them ready for practical breeding. Therefore, the improvement of the robustness of yield predictions will be explored in the future.

Reference: [1] Borra-Serrano et al. (2019); [2] Aper et al. (2019); [3] Pranga et al. (2021)

## LOW-COST ROOT PHENOTYPING SOLUTIONS TO SUPPORT WHEAT IMPROVEMENT

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To enhance the climate resilience of future crops, researchers around the world are exploring opportunities to improve root systems. Rhizoboxes provide a valuable tool to non-invasively phenotype root systems under controlled environmental conditions. However, modern rhizobox platforms can be quite expensive due to the cost of customised components, assembly, and maintenance. To help reduce the gap between laboratory and field experiments, we present a cost-effective 'DIY' rhizobox platform to phenotype root systems adaptable for different laboratories and greenhouses. The rhizoboxes can be assembled by hand and made from timber, where materials for each box costs only 22 AUD. To highlight the utility of the method, we evaluated a panel of wheat lines selected for divergent seedling root traits (seminal root angle and root biomass). Results demonstrate the method can be used to differentiate genotypic effects for root distribution, specifically root biomass at different soil levels, and also traits extracted from image analysis using RhizoVision Explorer. Furthermore, root traits captured using the low-cost rhizobox platform were correlated with field-based root phenotypes, which highlights the potential to use the low-cost platform to support advances in root research. This method could be applied to other crops, such as small-grained cereals or pulses, and could help support advances in root research, particularly for programs with a small budget or limited resources.

# **A SUMMARY OF SEVERAL YEARS WORK USING PHENOTYPING METHODS IN FIELD TRIALS: SUCCESS AND THE INEVITABILITY OF FAILURE—EXPERIENCE MAKES YOU BETTER**

**STEVE WEST**

RD4AG - Research Designed for Agriculture

RD4AG is an independent CRO in Yuma, Arizona, working with a broad range of horticultural, row and field crops in the Desert Southwest of the United States. Established in 1983, RD4AG has been utilizing various Field Based Phenotyping techniques over the last decade for the evaluation of a range of trials from simple plant pathology evaluations to canopy covers, NDVI, canopy coverage, to more complicated maturity evaluations of horticultural crops, plant health effects from Biostimulants grown under various abiotic stress situations, soil moisture affecting products, herbicides and so on. The list is ever expanding. As early adopters, we have had plenty of failures, as well and many successes. Working within the confines of lower budgets and needing performance in the short term, some of our techniques are not eloquent, but effective. And evolving. Always evolving.

This presentation can be from 15 to 45 minutes in length. It will cover our experiences with various technologies, important factors to consider when designing, maintaining, executing and evaluating out door, open field trials. It will also cover things which did not work well, which in our experience, is essential in achieving success.



# Sensors, Robotics & Automation

# PHENOTYPING ROBOTICS FOR MICRO-LABS: A LOW-COST INDOOR OPEN-SOURCE PLATFORM FOR PRECISE PHENOTYPING OF PLANT SHOOTS IN 3D

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JULIE CHARLAIX<sup>1</sup>; AURELE MACE<sup>1</sup>; DR. TIMOTHEE WINTZ<sup>2</sup>; ALIENOR LAHLOU<sup>2</sup>;  
MARIE TISSERAND<sup>1</sup>; OLIVIER RAZEBASSIA<sup>3</sup>; DR. TEVA VERNOUX<sup>1</sup>; DAVID COLLIAUX<sup>2</sup>;  
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Plant shoot architecture can be described by a huge collection of morphological traits of great value for agriculture, plant breeding or research. Automated image-based phenotyping platforms are essential to overcome time-consuming measurements. Yet, most available models are first driven by throughput efficacy on simple traits, resulting in closed-source, expensive and towering platforms, while the diversity and complexity of measurable traits is still limited. These solutions are inappropriate for small scientific teams, with a tight budget, limited requirement for throughput and frequent new needs related to their research. In return, the complicated access to such platforms hinders community-based modular contributions. Affordable, open-source solutions have emerged for 2D phenotyping. Here, we propose a comprehensive platform to automate the precise phenotyping of individual plant shoots of medium size in 3D. It consists in four modules: a robotic imaging box, a software suite for 3D-reconstruction, trait extraction and data analysis, a web app to explore results and a data management system. First, we favour the accessibility for plant researchers: it is low-cost, has a small lab-compatible footprint, all modules are provided as dockers (easy install, reproducible runs), and a workflow management system eases the run of complex pipelines. Second, our open-source design is also aimed at developers: the standard hardware components can be reused to adapt the set-up or to use other sensors, the modular code is amenable to integrate contributions to build specific and flexible pipelines. As a proof-of-concept, we showcase the first automated measure of fruit phyllotaxis in the model plant *Arabidopsis thaliana*, an obligate 3D precise measurement with a current manual standard only. By providing access to 3D plant phenotyping for a wider audience, our platform could hopefully nucleate a community of users and developers to boost open-source innovation in the field.

# **A LOW-COST MULTI-SENSOR SYSTEM FOR AUTOMATED IN SITU MONITORING OF PLANT IN VITRO CULTURE**

**HANS BETHGE**

Leibniz Universität Hannover

The current development of sensor technologies towards ever more cost-effective and powerful systems is steadily increasing their areas of application in different horticultural sectors. In terms of plant in vitro culture being the basis of many biotechnological methods of breeding and of propagation of disease-free plants limited research regarding automated sensor application was carried out up to now. However, manual visual monitoring for contaminations and malformations of the cultures is a costly and time-consuming repetitive work for commercial in vitro laboratories. With about 60 - 70% labour costs in particular represent the largest share in the in vitro production and thus offer great potential for increasing the efficiency of micropropagation laboratories through automation of work processes.

Due to the specific growth conditions of in vitro plants cultured in closed vessels, monitoring approaches face a number of challenges like condense water formation, opacity and total reflection of parts of plastic lids or media surfaces. Therefore, most evaluation methods used for determination of plant growth in vitro were limited to destructive and non-real-time methods. In addition, digital phenotyping of in vitro plants could offer advantages with regard to the objective and continuous quantification of plant characteristics.

This project focuses on the development of a phenotyping platform for an automated acquisition of multi-sensor data, including RGB, thermal, depth and spectral data. Hardware components were selected to be exclusively low-cost and the software design is based on common open-source frameworks like Docker, OpenCV and PlantCV. The automated XYZ-scanning system collects data since August 2021 of in vitro cultures, thus first time-lapse videos and growth curves have already been recorded and analysed. Furthermore time-lapse depth-images of in vitro cultures were successful acquired by sequential shifting of the point measurement-based distance sensor. Sensor application in plant tissue culture promises great potential for a non-destructive growth analysis.

Keywords: Plant tissue culture, phenotyping, image analysis, non-destructive growth analysis, Low cost

# **COST-EFFECTIVE HIGH-THROUGHPUT 3D MAPPING OF LIGHT IN PLANT RESEARCH**

**MICHELE BUTTURINI**

Wageningen University & Research

In plant research, photosynthetically active radiation (PAR, 400–700 nm) is one of the most important parameters to monitor. Even in highly controlled research environments (e.g. climate rooms and greenhouses), PAR can have undesired spatial and temporal variability. The spatial variability can be present both in the horizontal plane and light intensity can rapidly attenuate from top to bottom, even in absence of plants. The measurement of uniformity of PAR in the growing space is typically performed manually - or with the assistance of a data logger - notating the PAR measured by a PAR quantum sensor at a set of arbitrarily predetermined positions within the growing surface - typically in a grid pattern. Furthermore, due to the possibility of light source failure, such an operation must be repeated throughout the plant's growth in the case of an experiment with a relatively long duration. This is a tedious and time consuming task that is potentially prone to error.

Here, we present a design for a low-cost device for measuring and recording the spatial distribution of PAR using a simultaneous localization and mapping device (Intel® RealSense™ Tracking Camera T2653D) to continuously monitor the location of the PAR quantum sensor. The output of such a device is a set of xyz-data points accompanied by measurements of light intensity, allowing for the mapping of PAR intensity in three-dimensional space in the absence and presence of canopy.

A comparable method employing Spectroradiometers as opposed to a PAR quantum sensor could enable three-dimensional mapping of the light spectrum. To further automate the process of spatially and temporally mapping the light environment, the device described could be coupled with unmanned aerial and/or terrestrial vehicles that will remain operational throughout the entire duration of the plant growth cycle.

## **EARLY SUCCESSES AND LIMITATIONS IN A NEW HIGH-THROUGHPUT FIELD PHENOTYPING PLATFORM**

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Australian Plant Phenomics Facility, The University of Adelaide

High-throughput field phenotyping offers drastic improvements in speed and cost for measurement of key crop traits. These methods are objective, repeatable and non-destructive, enabling collection of data multiple times throughout a growth season. The Australian Plant Phenomics Facility have operated the world-first FieldExplorer (FE) field phenotyping platform – manufactured by CropTraits – for two years, collecting data over a range of crops to inform plant breeding and agronomic decision making. The FE is a self-propelled semi-automated field phenotyping system that collects LiDAR, RGB, and Hyperspectral (400 – 1700 nm) data, from which many crop traits can be estimated.

A LiDAR and hyperspectral data were used to investigate a range of biophysical variables for several crop species in the field (wheat, barley, oats and legumes). For LiDAR, biomass estimation was successful under some conditions ( $r^2 = 0.75 - 0.92$ ), though less so under windy conditions or for very closed canopies ( $r^2 = 0.07 - 0.39$ ). However, other biophysical variables were also found to covary with LiDAR structural measures, such as wheat yield ( $r^2 = 0.55 - 0.85$ ) and phosphorous uptake ( $r^2 = 0.74$ ) or forage legume vigour ( $r^2 = 0.72 - 0.93$ ). For hyperspectral data partial least squares regression (PLSR) was successfully used to predict several traits from full spectra, including forage legume vigour ( $r^2 = 0.79 - 0.91$ ), biomass ( $r^2 = 0.90$ ) and crude protein ( $r^2 = 0.53$ ); and wheat phosphorous uptake ( $r^2 = 0.84$ ), phosphorous content ( $r^2 = 0.60$ ) and yield ( $r^2 = 0.85$ ).

These results are promising, providing useful insights to aid plant breeders in understanding the relative influences of genotype, environment and management during line selection. Ongoing refinements to data collection and analysis are likely to increase the number and predictability of traits measurable with field phenotyping platforms, increasing rate of genetic gain by making large scale screening of breeding lines more efficient.

# **NON-INVASIVE MICROSCOPIC 3D IMAGING OF INTERNAL PLANT LEAF MORPHOLOGY WITH OPTICAL COHERENCE TOMOGRAPHY**

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PROF. DR. GUIDO VAN DEN ACKERVEKEN PHD<sup>2</sup>; PROF. JEROEN KALKMAN PHD<sup>1</sup>

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Optical coherence tomography (OCT) is a label-free non-invasive optical imaging technique, able to image 3D morphology of biological tissue with micro meter-scale resolution. While most developed for the field of ophthalmology and intravascular imaging, OCT has also been applied to plants for pathogen detection [1], [2] and morphological imaging [3], [4].

OCT has a much higher penetration depth than confocal microscopy, it does not rely on fluorescent labels, and it can be applied while the leaf is still attached to the plant, thus allowing for longitudinal studies. Compared to X-Ray CT, OCT is less expensive, it can create better contrast in soft tissue and the leaf only needs to be accessed from one side. Thus OCT can be a valuable tool for plant phenotyping at tissue level, for example to quantify local leaf thickness, thickness of the epidermal cell layer, cell volume, vascular bundle diameter and other morphological features.

We increase the penetration depth by infiltrating the leaf with water, resulting in a high quality 3D images of the internal morphology of the plant leaf, including cell walls, gas-exchange pockets, and vascular bundles with a resolution of 3 to 7 micro meter. We segment both the top and bottom surface of the leaf to obtain lateral resolved leaf thickness.

Based on our imaging results and experience, we will further discuss design choices that influence resolution and penetration depth, best practices for OCT imaging of plants and potentials and challenges for plant phenotyping with OCT.

The contribution is largely based on [4], complemented with new results and findings.

[1] Chow et al., J. Biomed. Opt., (2009)

[2] R. E. Wijesinghe et al., Appl. Opt., (2017)

[3] J. W. Hettinger et al., Plant Physiol., (2000)

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## **AN APP FOR NEAR REAL-TIME WEED DETECTION**

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Weeds are a major environmental burden on farm economy. Conventional approaches such as manual or chemical weed control are costly and labour-intensive. Additionally, chemicals in herbicides have negative health impacts for human beings and the environment. Tools for the automation of weed identification will enable precision and cost-effective weed control. Here we share a new app for automated near real-time identification. This online tool was developed to process Hyperspectral imaging (HSI) data using machine learning and Dash (an open-source python library for developing reactive web app) for accurate and simultaneous identification of up to four weed species from perennial ryegrass and white clover. The model can detect all six species. A near real-time predicted map of identified species (two forage species and four weeds) in different colours is displayed in the browser. The app could be used for fast and efficient detection and control of weeds even in the most complex environments.

## MOBILE AND RAPID IN-FIELD PASTURE BIOMASS MEASUREMENT

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The measurement of pasture biomass can be aided by mobile platforms and sensors such as LIDAR, RGB, and hyperspectral imaging. Among these tools, LIDAR has been recognised as the elementary sensor that has the capacity to contribute to the accurate and real-time measurement of this trait. LIDAR technology constitutes an efficient means of capturing 3D point cloud data, allowing for expression of depth and volume from the plant status. The aims of this study were to develop the 3D model of the field rows and plots of perennial ryegrass for rapid prototype development, and manufacturing of the mechanical parts of the rover. Subsequent to this, we aimed to accommodate motion and operation control software, interfaced with the main processing computer to integrate the LiDAR measurement data processing algorithms with RGB images to calculate the yield of white clover in a mixed sward with perennial ryegrass. Correlations found in this study include:  $R^2 > 82\%$  for multiple ryegrass plots/genotypes,  $R^2 > 90\%$  for a few clover plots/genotypes using LIDAR data and  $R^2 > 80\%$  bicultural forage composition using RGB data, which was boosted to  $R^2 > 90\%$  when it was combined with LIDAR data. Integrated RTK-GNSS data enabled the accurate mapping of the scanned areas. Our tool provides a precise, non-destructive, and cost-effective system for real-time in-field measurement of pasture biomass with highly anticipated scientific and commercial benefits.

## **USING STATISTICAL SHAPE SPACES FOR TEMPORAL REGISTRATION OF LEAVES IN 3D**

**KAROLINE HEIWOLT**; PROF. GRZEGORZ CIELNIAK

University of Lincoln

Plants are dynamic organisms. To capture the temporal variations in their morphology, non-destructive whole-of-lifecycle phenotyping is needed. New three-dimensional (3D) sensing technologies open up possibilities for automation of the quantitative analysis of plant structure, including temporal phenotypes such as emergence timing of new organs or growth rates.

However, associating point clouds obtained from repeated 3D scans across time is challenging. A non-rigid registration, which can locate correspondences between scans, while also allowing for growth and changes in appearance between scans is needed. A crucial step in this process is re-identifying and tracking the same individual plant components over time.

We present a landmark-free leaf shape compression algorithm, which allows for the extraction of 3D shape features from leaf point clouds, characterises leaf shape and curvature efficiently in few parameters, and makes the association of individual leaves in feature space possible.

The approach consists of three steps: (1) Surface triangulation of the input point cloud to obtain a concave triangle mesh, (2) extraction of a point set along the 3D mesh boundary, (3) and further compression of boundary coordinates using Principal Component Analysis. The resulting compressed features retain information about leaf shape, including outline and 3D curvature, scale, and global position.

This low-dimensional shape representation finds applications in storage and mapping of plant scans. Additionally, we demonstrate the ability to distinguish different leaf shapes and to perform temporal leaf-matching in feature space, using a small set of temporal scan sequences of Tomato plants taken from the public Pheno4D data set.

# **NOVEL METHODOLOGY BASED ON DRONE-BORNE RADAR SENSING FOR SUGARCANE SOIL MOISTURE ESTIMATION AND HARVEST DATE AND PRODUCTIVITY PREDICTION**

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Sugarcane is an important crop worldwide, especially in Brazil due to its multiple products such as sugar, ethanol, biodegradable products, energy generation, and food for animal production. This article presents a novel methodology for measuring the soil moisture through the sugarcane plantation and for predicting the sugarcane harvesting date and productivity using an imaging Synthetic Aperture Radar (SAR) system, operating in 3 bands: P, L, and C.

The soil moisture estimation proposed method merges two models. One is based on the use of an improved Dubois model in conjunction with the 3 bands, providing a root-mean-square error (RMSE) measurements lower than 4% for a sugarcane height up to 0.5 m. The other model, minimizes the vegetation effects by combining the L and P band SAR information and biophysical parameters through a neural network algorithm, achieving an RMSE lower than 5% for sugarcane height greater than 0.5 m. Merging the two models, for bare soil and soil under vegetation cases, the soil moisture can be measured with an RMSE of 4.39% for any sugarcane crop height.

Our sugarcane harvest prediction method is based on the above-ground biomass (AGB) estimation using the reflectivity information from the 3 bands, achieving an RMSE of 2 kg/m<sup>2</sup>. By correlating the field measurements of the ripening index (RI) with the AGB measurements by the SAR system, an indirect estimate of the RI by the radar is obtained. Observing that the AGB reaches its maximum approximately 280 days after planting and the maximum RI, which defines the harvesting date, approximately 360 days after planting for the species IACSP97-4039, and starting from an AGB map collected by the SAR system, it is possible to predict the harvesting date and the corresponding productivity with competitive average errors of 8 days and 10.7%, respectively, with three months in advance, whereas typical methods employed on a test site achieve an average error of 30 days with three months in advance.

## **A LOW-COST GAMING SENSOR-BASED PHENOTYPING PIPELINE FOR GREENHOUSE SYSTEM PRODUCTION**

**PHD. MONICA HERRERO PHD**; HUGO TARDY; ALBERTO MORCILLO; ENRIQUE GONZALEZ;  
DIEGO GONZALEZ-AGUILERA

University of Salamanca

Currently, plant phenomics is considered as the key to reduce the genotype-to-phenotype knowledge gap in plant breeding. For plant phenomics demand, 3D imaging systems have proved to be highly accurate. However, it is urgently required to implement high-throughput phenotyping procedures to increase the amount of data from high-dimensional imaging sensors in order to measure more complex phenotypic traits.

The aim of this project is to introduce imaging based-gaming sensors into greenhouse production systems for advanced monitoring and plant structural and functional analysis. We develop a workflow to 3D map plants in greenhouse production systems in an innovative way, ensuring flexibility and simplicity in data acquisition, automation in the process and high-quality results, using low-cost sensors. The methodology consumes Point Cloud Data from the MICROSOFT KINECT II SYSTEM (Microsoft Corporation, Redmond, WA, USA). This is constituted by an infra-red emitter and receptor, a RGB sensor and an accelerometer.

In this way, the plant community can easily access phenotyping tasks through low-cost imaging sensors that will help to face the limitation defined as the phenotyping bottleneck, increasing the efficiency in greenhouse production systems.

Keywords: phenotyping, point cloud, Kinect, greenhouse, low-cost.

# **THE ELECTRONIC NOSE AND MACHINE LEARNING USED IN THE DETECTION OF GAS EMANATING FROM SOYBEANS, DURING WATER STRESS, AND IN THE OBSERVATION OF THE EMISSION DURING THE DAY**

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The development of non-invasive methods and accessible tools for application to plant phenotyping is considered a breakthrough. This work presents the preliminary results using an electronic nose (E-Nose) and machine learning (ML) as affordable tools. An E-Nose is an electronic system used for smell global analysis, which emulate the human nose structure. The soybean (*Glycine Max*) was used to conduct this experiment under water stress. Commercial E-Nose was used, and a cuvette was designed and built to conduct the measurement of the gas sample from the soybean. This experiment was conducted for 45 days, observing the stages of plant growth during this period. This container is embedded with relative humidity (RH (%)), temperature (°C), and CO<sub>2</sub> (ppm) sensors, as well as the natural light intensity, which was monitored. These systems allowed intermittent monitoring of each parameter to create a database. The soil used was the red-yellow dystrophic type and was covered to avoid evapotranspiration effects.

The measurement with the electronic nose was done daily, during the morning and afternoon, and in two phenological situations of the plant (with the healthful soy irrigated with deionized water and underwater stress) until the growth V5 stage to obtain the plant gases emissions.

Data mining techniques were used, through the software "WekaTM" and the decision tree strategy, "K-nearest neighbors (KNN)," and linear discriminant analysis (LDA).

From the evaluation of the sensors database, a dynamic variation of plant respiration pattern was observed, with the two distinct behavior observed in the morning (~9:00 am) and afternoon (4:00 pm). With the initial results obtained with the E-Nose signals and ML, it was possible to distinguish the two situations, i.e., the irrigated plant standard and under water stress, and the influence of the two periods of daylight.

Future investigations should be carried out that enable early detection of the stress level.

# THE INFLUENCE OF STRIP TREATMENT AND BORDER-ROWS EFFECTS ON CABBAGE GROWTH USING TIME-SERIES OF UAV-IMAGERY IN A STRIP-INTERCROPPING FIELD

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Intercropping is a good alternative towards developing more sustainable agricultural systems. However, there is limited quantitative data available about plant growth and development in intercropping systems. To get such data, we used unmanned aerial vehicles (UAVs) to collect data on the growth of individual plants in intercropping field using aerial images. This allowed to study the effects of different treatments and plant location. A strip-cropping field was established where cabbage strips of different treatments alternated with wheat. On the STRIP\_VAR strips, cabbage cultivars Rivera and Christmas Drumhead were planted along with wheat cultivars Lennox and Lavette. The STRIP\_ADD strips were sown with the Rivera variety alongside Lennox strips with broad bean. STRIP strips, Rivera variety was sown next to the Lennox strips. A monoculture of Rivera was used as a comparison plot to compare the effects of monocropping to intercropping in REF\_TIME. The individual height of cabbage plants was estimated using RGB imagery and structure-from-motion analysis to create a digital surface model (DSM). The individual height was calculated from the DSM by estimating the height of the vegetation in a small radius around the plant location and the height of the soil in a larger radius. We look at two aspects: (1) investigating the accuracy of UAV-estimated individual height compared to manual measurements, (2) a spatial analysis of individual plant growth and investigation of treatment and border-row effects. Results showed that the overall correlation coefficient ( $r$ ) was 0.88 with overall root mean square error (RMSE) value of 4.68 cm. The UAV data was used to analyze spatial patterns in plant growth and to study treatment and border-row effects. In this study, UAV imagery was shown to provide accurate height estimations and allow a spatial analysis of plant growth. These results provide a better understanding of difference treatment in strip-cropping field and the functionality of border-row effects.

## **LOW-COST PLANT PHENOTYPING WITH SIMPLE CAMERA ARRAY AND AUTOMATED DATA PROCESSING**

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Plant phenotyping is currently widely used in plant research and agriculture applications to study plant behaviour in various conditions and environmental scenarios such as abiotic and biotic stresses and related plant adaptations. In the recent development of indoor plant phenotyping technology there are two prevailing directions of the setup: a) sensor to plant setup where a plant is stationary and various sensors are automatically positioned around the plants and b) plant to sensor setup where sensors are at a fixed location and plants are moved around using various conveyor systems Both these setups are mechanically complex and require sophisticated automation to achieve high-throughput performance. Contrary to these setups there is a third possibility so-called camera array where both plants and sensors (cameras) are stationary and all data acquisitions are done in a highly parallel manner. Within our OloPhen phenotyping platform, we built a camera array of 40 Raspberry pi coupled with camera module V2) in a growth chamber to perform simple plant phenotyping based on RGB images. This system has been developed and tested primarily for soil-based screening of crop emergence. In this assay, the first moment when the plant appears from the soil is detected. After image acquisition, the image data are processed and evaluated using in house developed Python scripts. To improve the data evaluation we are recently incorporating a neural network for determination of complex changes coupled with plant emergence on the ever changing background of the soil.

## **PHENOTYPING WITH DRONES**

**ING. HENK KRAMER;** DR. SANDER MÜCHER; JAPPE FRANKE; STAN LOS

Wageningen Environmental Research

From 2017 onward we have carried out several projects in which we have acquired imagery with a wide range of drone camera systems. The aim was and is to develop methods that enable phenotyping with drones for different crops. Experiments were done on quinoa, maize, winter wheat, spinach and potatoes. Field measurements were taken during these experiments. Next to RGB cameras, also Multi-Spectral, Hyper-Spectral, Thermal and LiDAR cameras have been used. Determined plant traits were for example vegetation structure (height, volume), lodging, ripening and diseases.

The poster will show results from these experiments with attention for the pros and cons of using drones. Results will include as well a comparison between imagery from drones and the field robot NPEC TraitSeeker.

# **PHENOCAVE: AN AUTOMATED, STANDALONE, AND AFFORDABLE PHENOTYPING SYSTEM FOR CONTROLLED GROWTH CONDITIONS**

**FERNANDA LEIVA**<sup>1</sup>; DR. PERNILLA VALLENBACK PHD<sup>2</sup>; DR. TOBIAS EKBLAD PHD<sup>3</sup>;  
PROF. DR. EVA JOHANSSON PHD<sup>1</sup>; DR. AAKASH CHAWADE PHD<sup>1</sup>

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Plant growth facilities offer control over certain environmental conditions such as light quality, temperature, and humidity influencing plant growth. These functional and practical spaces permit inducing biotic and abiotic stresses in a controlled manner avoiding the climate variability found in nature. Nevertheless, measuring different features of plant growth non-destructively remains challenging. Despite several high-throughput phenotyping facilities that have been set up around the world, smaller custom-made affordable systems for particular needs still demand further work. Thus, we present “Phenocave” an affordable, standalone, and automated phenotyping system for controlled growth facilities. This non-invasive phenotyping approach supports consumer-grade digital cameras and multispectral cameras. Cameras are attached to a gantry with two linear motor drives allowing XY motions and individual top-view images of the complete workspace of Phenocave. In order to test Phenocave, two cases of studies are presented using sugar beet and wheat cultivars. The wheat plants were exposed to different irrigation conditions at different growth stages, thus influencing their growth rate and biomass accumulation. The correlation was significant between conventional measurements and the projected green area of the plants at different growth stages. In addition, post-harvest analysis of grain protein composition and content agreed well with those of previous studies. For the sugar beet plants case, seeds were treated prior to germination with different agents, hence affecting germination rates. Therefore, the results disclose that seed treatment(s) alter germination rates. Phenocave allows automated phenotyping of plants in controlled conditions, the blueprint and the results presented in this study will enable others to construct similar phenotyping approaches customized to their own needs with the protocols and results from Phenocave.

## **HIGH-THROUGHPUT ROOT AND SHOOT PHENOTYPING IN "GROWSCREEN-RHIZO III"**

**DR. HENNING LENZ PHD**; ALEXANDER PUTZ; JENS WILHELM; SIMON HÜNING; OLAF MÜCK;  
SASCHA ADELS; ANNA GALINSKI; DR. KERSTIN NAGEL PHD

Forschungszentrum Jülich GmbH

One half of most plants is hidden below ground, but providing water, nutrients, anchorage and interaction with the rhizosphere this half is very important for survival and productivity. One option for making roots accessible are rectangular containers with a transparent side called "rhizotrons" which allow non-invasive measurements of roots grown in substrate. Using this window into the rhizosphere, root development can be followed over time and analyzed under various conditions.

Selection of crop varieties adapted e.g. to future climate scenarios requires phenotyping of large numbers of plants which will only be feasible using automated high-throughput platforms. Automation further enables handling of heavy and bulky growth containers such as rhizotrons. Based on our experience with automated low- to medium-throughput rhizotron systems (Nagel et al., 2012, Nabel et al., 2018) we further developed some previous concepts to build our newest non-invasive high-throughput phenotyping facility "GrowScreen-Rhizo III" with almost 900 rhizotrons covering 700 m<sup>2</sup> greenhouse space. In the course of the redesign we opted for industry state-of-the-art transport using autonomous guided vehicles (AGVs) to move around small groups of rhizotrons in a flexible manner regarding space and time. For maintaining throughput in plant measurements and irrigation multiple imaging stations became mandatory which are operating in parallel and independently from each other. In addition, industry-like infrastructure for substrate handling and specially designed machinery for rhizotron filling was integrated into the process.

Validation of the new platform as well as further extension and optimizing on hard- and software side are ongoing to facilitate high-throughput phenotyping experimental workflows. The different functionalities as well as the potential of the novel platform will be highlighted in the presentation.

## DIGITAL ASSESSMENT STATION FOR HERBICIDE SCREENING

**DR BO LI**; DR. ROB LIND; NICHOLAS SMITH; BOB COLLISON; GEORGE ARCHIBALD;  
ANNE SEVILLE; DR. SEBASTIAAN SPEETJENS

Syngenta

In the early stages of lead generation in crop protection research, molecules are tested for herbicidal activity. After spraying the molecules of interest onto a panel of test crops and weeds, the effect of the molecules tested is evaluated by a human assessor, to determine which molecules will be progressed for further characterisation. At Syngenta we aim to establish a digital image analysis and deep learning pipeline to accurately assess herbicide glasshouse screens and replace 'by eye' herbicide screening.

To enable the transition, to digital assessment, an imaging box was constructed to image the plant samples from multiple angles with high resolution cameras and a rotating stage to create a full set of images that record all details of canopies. An in-house image analysis software and deep learning algorithms was developed to implement automated plant species recognition and canopies segmentation. Several phenotypic parameters can be generated including canopy size, affected canopy area, colour score, plant height and plant vitality. With future automation on glasshouse logistics, we believe this system can provide many benefits over current plant screening process:

- Improve the quality and consistence of data obtained.
- Assist understanding the herbicide activity and decision making with generating more detailed phenotypic data.
- Implement time-lapse experiments for multiple assessments.
- Store historical data for future revisit.
- Reduce ergonomic risks with moving plants.
- Reduce test cycle time and improve glasshouse use efficiency.

# **MULTIPLEXLAB: A HIGH-THROUGHPUT CNC MICROSCOPE POWERED BY MACHINE LEARNING TO ASSIST IN PHENOTYPING IMPORTANT PLANT SEED AND ROOT TRAITS**

**VINICIUS LUBE MSC<sup>1</sup>**; DR. MEHMET ALICAN NOYAN PHD<sup>2</sup>; ALEXANDER PRZYBYSZ<sup>1</sup>;  
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Monitoring, tracking, and analyzing the dynamic growth of living roots is essential to understanding their responses to changes in the surrounding environment. Despite the advantages of recent technologies developed for non-invasive phenotyping, these devices remain costly, not easily accessible to the user, and most require extensive training and specialized knowledge for maintenance. To date, none of the developed tools allow the analysis of minute phenotypic variations because of their limitations in resolution and field-of-view (FOV) at high-throughput, which is a common bottleneck in plant phenomics. We designed and manufactured the state-of-the-art high-throughput platform to monitor plant seed germination and perform root phenotyping. MultipleXLab is a modular, low-cost, highly versatile setup that allows users to acquire high-resolution images with a large FOV automatically, a capability highly desired but still lacking for high-throughput phenotyping of root systems. The system can constantly monitor the germination of thousands of seeds and their growth into seedlings using a motorized 3D-printed plate holder with LED lighting. In addition to its lightweight and multiple configurations, MultipleXLab can be used to visualize plant growth and developmental plasticity in roots and perform profilometry from depth map reconstruction of the 2.5D root surface, permitting the setup to analyze shape deformation during stress conditions. It can also capture dynamic processes like insects feeding on flowers and roots, movement of soil-living microorganisms like worms, and free-living nematodes. We tested MultipleXLab to monitor seed germination and measure root growth of Arabidopsis developmental, cell cycle, and auxin transport mutants non-invasively hourly for several days. We show that MultipleXLab is a cost-effective tool that can serve multiple applications desired by plant biologists, the seed industry, crop scientists, and breeding companies.

## **VERTICAL PHENOTYPER: SMALL FOOTPRINT DEVICE FOR HIGH-THROUGHPUT PHENOTYPING OF SMALL PLANTS AND SEEDLINGS**

**DR. PAVEL MAZURA PHD**; DR. PAVEL KLIMEŠ PHD; PROF. DR. LUKÁŠ SPÍCHAL PHD

Palacký University Olomouc

Currently big instalments, infrastructure, buildings with controlled environments and big investments are needed for building and running large phenotyping systems. Unlike these devices, our target phenotyping area is high-throughput tests on large sets of small plants and seedlings in standardized formats e.g. microtiter plates. These setups are currently used preferentially for compound library screenings of bioactive substances and studies of effects of diverse environmental conditions on plant growth and development. New device fulfils important plant phenotyping community needs in the innovation, standardization, affordability and scalability of required screening technology.

Inspiration for new device comes from published results of phenotyping in small footprint setups:

De Diego et al 2017, An Automated Method for High-Throughput Screening of Arabidopsis Rosette Growth in Multi-Well Plates and Its Validation in Stress Conditions. *Front Plant Sci.* doi: 10.3389/fpls.2017.01702.

Rodriguez-Furlán C et al 2016, High throughput selection of novel plant growth regulators: Assessing the translatability of small bioactive molecules from Arabidopsis to crops. *Plant Sci.* doi: 10.1016/j.plantsci.2016.01.001.

## IN-FIELD DYNAMICS OF WHEAT ORGANS BY MULTIMODAL MACHINE VISION

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Field phenotyping is less advanced than in greenhouses or controlled facilities. Instead of bringing potted-plants to the sensors, the sensors must be carried above the crop, dealing with the wind and the changing sunlight for image capture. It constraints the sensor types, the scanning possibilities and the choice of the view point. The signal comes from a whole cover instead of individuals or organs. That signal is influenced by the background and does not provide traits separately for the organs (leaves, stems, ears, ...). This research aimed to develop and evaluate methods to characterise the wheat morphology and physiology at the organ scale, and study their dynamics in the field.

Nadir images were acquired in six field trials by two RGB cameras, a multispectral camera array and a thermal camera. The height of the wheat organs was computed by stereovision from the RGB images. The mean height of ear tops was retrieved with an accuracy of 96,6%. Using deep learning, the ears were counted with a F1 score of 0.93 and segmented with a F1 score of 0.86, then the plant height was used to compute the image footprint at ear height and measure the ear density. Regarding the multispectral images, the data from an incident light sensor were used to convert digital values to reflectance factor. To fuse the information from the multiple cameras, the RGB, thermal and multispectral images were registered using a two-steps approach: a global transformation based on the relative position of the sensors followed by a local transformation based on the mutual image content. The registration error was 2 mm. In the end, the image fusion provided the dynamics of vegetation indices and other traits at the organ scale. Those traits were exploited to derive nitrogen, water or disease stress indices. PLS regressions estimated dry matter and leaf area index, respectively with external validation RMSE of 2.19 t/ha and 0.95 m<sup>2</sup>/m<sup>2</sup>. Yield was estimated with a cross-validation RMSE of 0.32 t/ha.

# **EVALUATION OF THE SENSITIVITY OF PRECISION AG TECHNOLOGIES IN PROVIDING ACCURATE MEASURES OF BIOMASS YIELD IN PERENNIAL FORAGE CROPS**

**DR. ALI MISSAOUI PHD<sup>1</sup>; DR. SERGIO BERNARDES PHD<sup>2</sup>**

<sup>1</sup> University of Georgia;

<sup>2</sup> University of Georgia

Unlike row crops that need to be harvested once, cool-season perennial forage crops are managed for biomass yield on multiple harvests. Multiple harvests create an operational bottleneck for breeders and limit the number of entries and testing locations. Substantial investments are being made in the development of automated technological solutions for precision Ag for row crops. The adoption of these platforms to forage crops rests on their accuracy in estimating biomass yield with growth stages. The objective of this work is to evaluate the sensitivity of popular unmanned aerial vehicles (UAVs), imaging strategies and machine learning algorithms for optimal real-time biomass estimate in grass and legume forage crops. Experimental plots consisting of single plants, row plots, and sward plots were used in a survey/monitoring hybrid data collection approach including direct measurements and remote sensing. Two UAV platforms were equipped with a 42-megapixel RGB camera (Sony Alpha 7Rii), a five-band multispectral system (MicaSense RedEdge MX), a hyperspectral sensor (Resonon-Pika L), and a LiDAR (LiDARUSA Revolution 120). Data collections occurred at multiple growth stages and calibration targets were used to account for changes in irradiance during and between collections. Images were used to generate 3D canopy models of vegetation in the field and to compute morphometric and spectral indices descriptive of vegetation coverage, health and vigor. Following data collection by UAVs, plants are harvested and processed for DM computation. A feature-rich multivariate dataset is then compiled using a Geographic Information System to support the sampling of plots and metric extraction. Sampled metrics are used to evaluate relationships between direct yield measurements and values derived from UAVs for different species at different growth stages. The sensitivity and accuracy of these models is validated with physical biomass harvests at multiple locations.

## SEED-TO-PLANT, PLANT-TO-SEED PHENOTYPING

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ADÉLA KOLOUCHOVÁ<sup>1</sup>; KLÁRA PROCHÁZKOVÁ<sup>3</sup>; ALEŠ PEČINKA PHD<sup>3</sup>;  
**DR. MARKÉTA PERNISOVÁ PHD<sup>4</sup>**

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<sup>4</sup> Masaryk University

Many of the Arabidopsis research programs are focused on the detailed characterization of early developmental growth, followed by costly and laborious omics data processing. To get maximum of these datasets it is crucial to reduce any source of variability. One of the most challenging sources of variability are the micro-sized seeds and their manual handling.

Here, we introduce Boxeed, the state-of-the-art technology intended for the non-invasive, dry-seeds phenotyping, sorting, counting and precise seeding to growth media. Boxeed is designed for 80 µm – 3 mm seeds reaching the average working speed 600 seeds/hour. Phenotyping is based on 2D image analysis of individual seeds from multiple projections. The seed morphometric and fluorescence parameters are calculated in a real time, making it ideal for a seed selection and for single or multiple parameterized seed sorting directly from stocks.

In Arabidopsis thaliana ecotype Col, we have identified that just introducing proper seed-to-seed positioning and seed phenotyping is a key factor responsible for developmentally important impact manifested during early postembryonic growth has root growth variability. The described variability is quite considerable, and its understanding opens a bottleneck towards delicate characterization of the plant developmental processes.

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## **DEEP LEARNING FOR IMPROVED PHENOTYPING THAT ENABLES GENOME WIDE ASSOCIATION STUDIES OF SUDDEN DEATH SYNDROME IN SOYBEAN**

**ASHLYN RAIRDIN**; FATEME FOTOUHI; JIAOPING ZHANG; DAREN MUELLER;  
PROF. DR. BASKAR GANAPATHYSUBRAMIAN PHD; PROF. DR. ASHEESH SINGH PHD;  
SOMAK DUTTA; PROF. DR. SOUMIK SARKAR PHD; DR. ARTI SINGH PHD

Iowa State University

Sudden death syndrome (SDS) caused by *Fusarium virguliforme* is one of the most yield-limiting diseases to affect soybean, an important crop, impacting both farmers and consumers. Conventional phenotyping methods for rating sudden death syndrome (SDS) can be very laborious, time-consuming, and subjective. To further understand the genetic architecture of SDS, machine learning-enabled image-based phenotyping was used in this study. RGB Images were taken of 2 replications of 479 accessions in up to two different locations over two years. Accessions used in this study were previously genotyped with the Illumina SoySNP50K Chip. To quickly and accurately rate SDS symptoms in field, object detection and classification via machine learning was used to detect and rate SDS severity on plant canopy and assign a severity score on a scale of 1-4. These scores were then used to conduct a genome-wide association study (GWAS) to help understand the genetic architecture of SDS in soybeans. There were 46 significant SNPs found associated with various traits relating to SDS infection severity. Within these, a SNP was found that is 131 kbp from a previously reported candidate gene associated with SDS, SIK1. A trait classifying the severity class of majority of the canopy image was found associated with the SNP ss715615734, this SNP is near two potential candidate genes, Glyma.13g257100 and Glyma.13g256500. Both of these genes have been reported previously in association with biotic defense response.

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## **APPLICATION OF DRONES FOR SEED PRODUCTION: LEARNINGS FROM MONITORING BRASSICA CROPS IN SOUTH AFRICA**

**DR. GUSTAVO RAMIREZ PHD**

Syngenta Seeds

Since their appearance drones have been seen as game-changing technology for high throughput crop phenotyping and monitoring; there has been a lot of excitement about it. Some of their advantages include easiness of deployment, fast coverage or large areas, and ability to bear different types of digital sensors. Although the benefits of this technology for breeding trials where the need for high resolution phenotyping is obvious, implementation of drone-based platforms in commercial seed production is still challenging due to high cost. To assess the benefits of drones in vegetable seed production we monitored the development of two brassicas crops: broccoli and cauliflower in one of our production areas in South Africa. We collected high resolution images with drones (2.2mm GSD) as well as satellite images from Skysat, Planet and Sentinel (resolutions of 0.5, 3.5 and 10m GSP respectively) during flowering and seed development. Despite the good correlation between manual assessment and drones-derived images for flower cover, the high number of flights ( $\geq 5$ ) required to generate reliable flowering curves makes its application economically unviable. As the alternative to drones, high resolution satellite images of 0.5mGSD were not sensitive enough to discriminate between female-male adjacent rows. However, images of this resolution were sufficient to measure biomass cover and in conjunction with yield values could potentially be used for yield forecasting. Our findings indicate that there is still not an economically viable remote sensing solution for commercial seed production and at this moment nothing replaces a good pair of experienced eyes.

## **SATELLITE DATA IN THE WORLD OF PLANT BREEDING**

**DRIES RAYMAEKERS**; NICK GUTKIN; SAM OSWALD; DR. STEPHANIE DELALIEUX;  
DR. LAURENT TITS

VITO (Flemish Institute for Technological Research)

Remote sensing has a long track record of providing insightful information for agricultural applications. However the uptake of satellite based information in a breeding context has not yet been fully exploited. In this presentation we will demonstrate the use of different satellite products and how they complement with other data sources. Sentinel2 data from ESA's Copernicus program can be used to map the soil's heterogeneity of an experimental field over many growing seasons. This can help in selecting the location of a new trial or improve the spatial corrections of important traits like yield. Also, during the growing season, high resolution satellite imagery - from e.g., the Planet constellation - can provide interesting information on plot level. Just like drones, satellites carry multispectral cameras which can assess plant biomass and health status on individual plot level. While currently the location accuracy of such high resolution is limited, the temporal resolution and consistency surpasses that of any drone service. Satellite imagery can also monitor the performance of commercial varieties on farmers' fields which typically have a large variety of soil, climate and management practices. When integrated into farm management systems like <https://watchitgrow.be/> direct feedback loops can be created back into the breeding cycle. Even specific services can be offered to the farmer to manage their fields based on satellite and weather data combined with in-depth knowledge of the plant variety.

## **A COMPACT, LOW-COST AND PORTABLE HARDWARE/SOFTWARE SYSTEM FOR 3D PLANT PHENOTYPING**

**VITO RENÒ PHD**<sup>1</sup>; DR. ANGELO PETROZZA<sup>2</sup>; DR. STEPHAN SUMMERER<sup>2</sup>;  
DR. FRANCESCO CELLINI<sup>2</sup>; DR. FIROZEH SOLIMANI PHD<sup>3</sup>; PROF. GIOVANNI DIMAURO<sup>4</sup>;  
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<sup>3</sup> Politecnico di Bari;

<sup>4</sup> Università degli studi di Bari Aldo Moro

This abstract describes a trial for evaluating the use of a compact, low-cost and portable stereo camera system to quickly perform the 3D plant reconstruction avoiding calibration steps. The setup consists of two Intel Realsense D455 stereo cameras constrained on a vertical bar and inclined towards the plant to be framed. The plant is placed in front of the sensors and is rotated during the acquisitions. The plant is acquired under natural light. In each acquisition there is a tag of the apriltag family, which the purpose of acting as a reference and registration point for all the 3D models acquired by the camera.

A preliminary data acquisition was set up at ALSIA facilities, then the software for reconstruction and analysis was developed in order to evaluate the quality of the data that can be acquired with this system. The software first acquires a video formed by 2D images and the 3D model corresponding to each pair of frames. Subsequently, 3D models are registered using the geometric parameters automatically computed using the apriltag, which provides the pose that must be applied to register the 3D models one to the other. Finally, the overall model obtained from the union of the captured frames is saved and post processed.

Further post processing algorithms can be applied to identify relevant plant parts and then calculate plant traits. In this experiment, a clustering algorithm of the points of the three-dimensional model based on color was applied, resulting in an effective separation of the relevant parts of the plant. The results obtained suggest that this system may be useful for lean phenotyping purposes, e.g. qualitative analysis of the phenotype, as it is possible to obtain global information with a certain degree of accuracy about the 3D occupation of plant biomass.

# **A GROUND MOBILE ROBOT FOR AUTONOMOUS LIDAR-BASED FIELD PHENOTYPING**

**JAVIER RODRIGUEZ-SANCHEZ**; PROF. CHANGYING LI PHD

University of Georgia

Plant phenotyping has become an essential component of breeding programs and the development of new crop varieties. However, linking crop response with environmental and genotype interactions in the field has traditionally relied on manual measurements, which has contributed to the phenotyping bottleneck. Automated sensing techniques and robotics in combination with advanced data analytics can increase the capacity, repeatability, and cost-effectiveness of plant trait measurements over time, allowing researchers and plant scientists to improve the efficiency and throughput of the phenotyping process. In this work we present an autonomous robotic system and a data processing pipeline for efficient, high-throughput field phenotyping of cotton crops. We integrated an unmanned ground vehicle (UGV) with a high resolution 3D LiDAR to perform in-field terrestrial laser scanning (TLS) measurements without human intervention. The information gathered from the navigation sensors was effectively used to register the point clouds without artificial targets. The system provided high quality 3D data that was processed using a customized pipeline to characterize plant traits at the plot level—plant architecture, height and volume, and cotton boll mapping. This work demonstrates an effective and repeatable workflow for autonomous and efficient in-field TLS data collection, evaluation and interpretation for the assessment of cotton plant traits. The generalization and standardization of the workflow and the expansion of remote sensing and automation technologies to other species and field layouts can advance the rapid development of in-field phenotype-genotype analysis for complex traits, which ultimately will contribute to the advancement of crop breeding and genetics.

## **MAIZE PLASTICITY CHARACTERIZATION THROUGH 2D/3D PHENOTYPING IN FIELDS**

**MARIO SEROUART PHD<sup>1</sup>**; DR. RAUL LOPEZ LOZANO; BENOÎT DE SOLAN<sup>2</sup>;  
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<sup>1</sup> INRAE;

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Cultural practices have a significant impact on crop architecture and though on light interception efficiency by altering shoot components display within the canopy. Maize plants have the capacity of adapting their architecture in response to light environment. Indeed, the relationship between plant density and yield is determined by the ability of the plant to minimize the effect of intra-specific competition while maximizing light interception. These modifications are based on shade avoidance reaction theory, detected by low red/far-red ratios, and directly drives plasticity mechanism. Architectural plasticity is therefore an essential trait issue for breeders to develop maize cultivars capable of maximizing yields under high density conditions. The existing works on maize architectural traits are limited to one or two genotypes per study, often former varieties considering the breeding turn over, which makes difficult to disentangle the genotype x environment x management interactions behind them. Traditionally, in situ manual measurements of maize architectural traits, necessary to describe morphological plasticity are time-consuming and error-prone, which constraints experimental studies to a small number of treatments. Additionally, other limitations related to the realistic simulation of light environment make studies in controlled environment not optimal, considering the isotropic solar radiation assumption. Nevertheless, the development in recent years of instruments and methods for plant phenomics have paved the way to collect high-throughput observations of architectural traits with a relatively smaller cost, when compared to manual or destructive measurements. This study presents methods to extract features from RGB images or LiDAR point clouds, providing a promising way to obtain in-field phenotype traits with high ability of recording accurate traits, while improving the processing rate and computation time.

# **SELF SUPERVISED ML FOR INSECT PEST CLASSIFICATION**

**DR. ARTI SINGH PHD**

Iowa State University

Globally, crop insect pests lead to 10–40% yield loss. However, crop insect pest detection and mitigation remain an extremely challenging task for the farmers, due to several factors. While supervised learning has achieved a remarkable feat in insect detection, it requires significant human intervention in labeling the input data, thereby making the downstream tasks tedious and sometimes infeasible. This is particularly the case for identifying insects in the field, where labeling is tedious. In this work, we use the recent advances in self-supervised learning (SSL) to minimize the labeling requirements for the insect classification problem. Self-supervised learning methods leverage unlabeled data to produce pre-trained models. These pre-trained models can be fine-tuned with labeled data for downstream classification applications. Here, we compared three different self-supervised pre-training methods (Barlow Twins, Bootstrap Your Own Latent (BYOL) and Nearest-Neighbor Contrastive Learning of visual Representations (NNCLR)) to classify 22 types of agricultural insect pests. We pre-trained the ResNet-18 and ResNet-50 models using these three SSL methods on the original RGB images and foreground segmented images. The performance of SSL pre-training methods was evaluated using linear probing of SSL representations and end-to-end fine-tuning approaches. Our experiments show that SSL pre-trained CNN models were able to perform annotation efficient insect classification.

# **INSECT CLASSIFICATION AND DETECTION USING SELF-SUPERVISED LEARNING METHODS**

**DR. ARTI SINGH PHD**

Iowa State University

Annually many insects affect crops over different parts of the world and incur huge losses due to the crop damage. Timely classification and detection are crucial to mitigate the risks associated with the pest infestation. Creating a robust classification and detection framework – deployable as an app – for agriculturally relevant pests will have tangible benefits to farmers. However, this task is made difficult due to several challenges including (a) the large number of ag relevant insect classes, (b) the lack of carefully annotated images, and (c) issues of robustness, and uncertainty associated with predictions. Traditional object classification and detection requires large, annotated datasets. Annotating and labeling objects is tedious and expensive. Here, we develop a pipeline that performs insect classification and detection using self-supervised methods. In particular, we utilize a very large dataset of insects from a freely available source (iNaturalist). Self-supervised object detectors achieve the same on fine-tuning on a small percentage of the annotated samples. We deploy an object detector with a backbone pretrained on an unsupervised pretext task. These representations were shown to perform better for downstream tasks like localization than backbones pre-trained for classification.

# **IPOTS: INTERNET OF THINGS-BASED POT SYSTEM FOR CONTROLLING OPTIONAL TREATMENT OF SOIL WATER CONDITION FOR PLANT PHENOTYPING UNDER DROUGHT STRESS**

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Drought is one of the severely damaging abiotic stresses in agricultural production. Clarifying plant responses to drought stress is an important research topic for the genetic improvement of drought resistance. Hence, a cultivation platform that can assist users in controlling the soil water condition is required for accurately phenotyping plants under drought stress in an artificial environment. Thus, we developed an automatic irrigation system that simulates the drought condition in a growth chamber (iPOTS: Internet of Things [IoT]-based pot system for controlling optional treatment of soil water condition). The Wi-Fi-enabled iPOTS allows water supply from the bottom of the pot, based on the soil water level set by the user, and automatically controls the soil water level at the desired depth. Furthermore, the iPOTS allows the users to monitor environmental parameters, such as soil temperature, air temperature, humidity, and light intensity, in each pot. To verify whether the iPOTS mimics the drought condition, we conducted a drought stress test on rice varieties and near-isogenic lines, with diverse root system architecture (RSA), using the iPOTS installed in a growth chamber. Similar to the results of a previous drought-stress field trial, the growth of shallow-RSA rice was affected by drought stress compared with that of deep-RSA rice. The microclimate data obtained using the iPOTS improved the accuracy of plant growth evaluation. Overall, these results suggest that the iPOTS represents a reliable platform for phenotyping plants under drought stress. This work was supported by JST CREST Grant Number JPMJCR17O1, Japan.

# **NOVEL X-RAY-BASED TECHNOLOGIES FOR THE HIGH-THROUGHOUT 4D-PHENOTYPING OF GERMINATING SEEDS AND SEEDLINGS**

**DR. ANTJE WOLFF**

phenoLytics GmbH

Current morphological phenotyping technology for germinating seeds and seedlings is limited to 2D-image analysis and often relies on manual and subjective measurements. Here we present two technologies which allow, for the first time, to follow the development from seed to seedling in 3D over time at high throughput.

The X-ray based "phenoCheck" allows to analyze and automatically measure all external structures and internal organs in dry seeds in 3D, uncovering developmental defects and damages, currently at a rate of 200 seeds per minute. The phenoCheck can thus be applied for research uses, but also to optimize seed quality and guide processing.

A crucial question is which implications these seed quality features have for plant development and germination capacity. The "phenoTest" is a X-ray-based system to phenotype germinating seedlings non-destructively in 3D over time. Seeds are grown in containers in vertical filter paper, and via X-raying across time points, 3D-image volumes of each growing individual seedling are captured without opening the container. An analysis algorithm segments the different plant organs and fully-automatically measures their length, volume and orientation, currently at 50 seedlings per 2 minutes. Thus, the phenoTest provides unprecedented standardized big data on the morphologic development of seedlings.

Following the axiom that we can only optimize what can be measured, the technology allows to research the impact of stress conditions, seed treatments, seed production, ageing and storage etc on seed quality and on plant development. Germination capacity, vigor and dormancy can also be reproducibly quantified, and its determinants probed. The 4D phenotypic traits may also serve as early predictive markers for breeding and screening, potentially reducing the need for soil and field tests. We will present the technologies, its scientific uses and findings on the effect of diverse impact factors on seed quality and plant development.

## **TRAINING INSTANCE SEGMENTATION NETWORK WITH SYNTHETIC DATASETS FOR SOYBEAN PHENOTYPING**

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Soybean crop yield highly depends on three major aspects which are the number of pods per plant, the number of seeds per pod, and the seed size. Quick and precise segmentation of individual soybean organs is the prerequisite for the parameters acquisition. Although deep learning-based algorithms can achieve accurate training, it requires a large amount of labeled data. To facilitate high throughput soybean precise phenotype data extraction (including soybean seed, soybean pod, and mature soybean plant), we show a novel synthetic image generation method based on domain randomization to prepare plenty of labeled image datasets automatically. Then, we propose to train an instance segmentation network with our synthetic datasets. In the process of our model training, we propose a transfer learning method. Firstly, we train the instance segmentation network with the synthetic soybean seed dataset. Secondly, we fine-tune the trained soybean seed model on the synthesized soybean pod dataset. Finally, we fine-tune the trained soybean pod model on a small amount of soybean plant images to realize in-situ pod segmentation of mature soybean plants. After each segmentation, we can automatically obtain the morphological phenotypic information of each seed (seed area, seed length, seed width, seed color, and so on), the parameters of each pod (pod length, pod width, seed-per-pod, pod color, and so on), and the count of soybean pods per plant. We analyzed the results of the three trained models on the real-world datasets respectively (including soybean seed, soybean pod, and mature soybean plant). All of them show high segmentation accuracy. The results proved that the instance segmentation network can be trained by a synthetic image dataset to achieve a good performance. Constructing and utilizing such synthetic datasets can reduce the labor cost of manual annotation, and can promote the wide application of deep learning in the agricultural field.

## **CONTROLLED-ENVIRONMENT AND FIELD-BASED PHENOTYPING FACILITIES AT PURDUE UNIVERSITY**

**DR. YANG YANG PHD<sup>1</sup>**; PROF. MITCH TUINSTRA<sup>1</sup>; CHRIS HOAGLAND; JASON ADAMS

<sup>1</sup> Purdue University

Purdue University has established controlled-environment and field-based phenotyping facilities (The Ag Alumni Seed Controlled Environment Phenotyping Facility (AAPF) and The Indiana Corn and Soybean Innovation Center (ICSC)) to enable high-throughput assessment of complex plant traits with advanced machine vision other sensing technologies.

The AAPF features two costumer-designed growth chambers and fully automated fertigation systems. These growth chamber provide consistent environmental conditions (temperature, humidity, light intensity, CO<sub>2</sub> concentration) to accommodate growing in total 624 plants up to three meters in height. An automated conveyor belt system carries the plants to three imaging stations for image-based plant measurements. An RGB imaging system acquires color images of the plants and extract traits such as color, size, number of leaves, length of each leaf, etc of each plant. The plants can also be transferred to a hyperspectral camera system which provides spectral information in the Visible and Near-infrared (VNIR) as well as shortwave infrared (SWIR): 400 – 25,00 nm waveband. The HSI cameras can be used to determine relative water content of leaves, nutrient composition, as well as herbicide damage, and foliar pathogen progression. The 4D images provided by CT-Xray system offer the opportunity to non-destructively quantify changes in root architecture. Research carried out in the AAPF can provide insights that bridge the gap to help explain gene function, provide insight in nutrient use efficiency, and how water stresses impact plant growth.

The ICSC facilitates the development of ground-based and UAV-based sensing platforms for high-throughput assessment and quantification of key crop traits, such as crop cover, crop height, biomass estimation etc. Multimode sensing capabilities (hyperspectral imaging, LiDAR, RGB imaging) and a robust data pipeline enable advanced in-field research in breeding, crop protection, and basic.

# **IMPACT OF SENSOR BASED AUTOMATED IRRIGATION SYSTEM ON EFFICIENCY OF GROUND WATER USE FOR AGRICULTURE**

**DR. YASMEEN YASMEEN PHD**

Institute for social and economic change

In India, agriculture plays an important role in the economy and development of the country. Of the total irrigated area in the country, nearly 72% uses ground water as primary source of irrigation. But, substantial groundwater depletion is likely to worsen in future. This problem was compounded further with an unlimited power supply policy for irrigation. As a consequence, farmers have shifted towards water intensive crops which in turn is leading to significant depletion of ground water tables. Though free electricity is cited as one of the reasons for groundwater depletion, but, it is not empirically proven. Thus, an investigation was conducted to standardize soil depth for soil moisture estimation using an automated irrigation system and in turn to develop an irrigation schedule in collaboration with ICRISAT, Hyderabad during 2018-19. Sensors were installed in farmers' field at different locations according to soil textures for soil moisture estimation in two seasons for scheduled and automated irrigation systems. Factors like evapotranspiration, plant biomass, seed yield, and harvest index were captured. There was a significant variation ( $P < 0.05$ ) for studied traits and based on their per se performance, thus act as indicator traits for irrigation schedule in any IoT based irrigation system. Water use efficiency in the irrigation system was found higher (>200%) than the scheduled. Cost benefit analysis results in higher efficiency (>45%) than scheduled irrigation systems. Hence, sensor based automated irrigation systems are best proven technology to sustainably enhance WUE and prevent groundwater depletion and supply of appropriate irrigation also lead to good quality crops and higher productivity to the farmers.

# Informatics, Data & Analytics

## THE SHAPE OF THINGS: USING TOPOLOGICAL DATA ANALYSIS TO QUANTIFY SHAPE IN PLANT BIOLOGY

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Shape is foundational to biology. Observing and documenting shape has fueled biological understanding as the shape of biomolecules, cells, tissues, and organisms arise from the effects of genetics, development, and the environment. Less often we do consider that data itself has shape and structure, or that it is possible to measure the shape of data and analyze it. Often times, shared landmarks and common outlines may not be enough to capture the rich morphology we observe with our eyes. A new strategy is needed. We focus thus on Topological Data Analysis (TDA), an emerging mathematical discipline that uses principles from algebraic topology to comprehensively measure shape in a broad scope of different datasets. Using a function that relates the similarity of data points to each other, we can monitor the evolution of core mathematical shape features —connected components, loops, and voids. This evolution, a shape signature, concisely summarizes large, complex datasets.

To study the use of TDA, we compute both traditional and topological shape descriptors to quantify the morphology of a large collection of barley seeds scanned with X-ray Computed Tomography (CT) technology. We then successfully train a support vector machine (SVM) to classify 28 different accessions of barley based exclusively on the shape of their grains. While traditional shape descriptors can cluster the seeds based on their accession, topological shape descriptors can cluster them further based on their spike. We observe that combining both traditional and topological descriptors classifies barley seeds better than using just traditional descriptors alone. This improvement suggests that TDA is thus a powerful complement to traditional morphometrics to comprehensively describe a multitude of “hidden” shape nuances which are otherwise not detected and offers an exciting new path to link phenotype with genotype.

## **AUTOMATIC TRACKING OF GRAPE CLUSTERS AND EARLY PHENOTYPING FROM UAV VIDEO SEQUENCES**

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Phenotyping is recognised as a powerful tool in plant breeding. The phenotype of a plant includes morphological and physiological characteristics, and is a consequence of the interaction between genotype and environment. Phenotyping traits are related to several characteristics, like leaf characteristics, plant phenology or canopy architecture. These traits have been studied for a long time in viticulture through ampelography, which is the science that studies the identification and classification of grapevines (*Vitis*). Hence, most of these traits are recognised by the International Organisation of Vine (OIV) and Wine and the International Plant Genetic Resources Institute (IPGRI) as descriptors for grape varieties and *Vitis* species. Bunch length and width are OIV codes 202 and 203, respectively.

Traditionally, this information is achieved manually through visual surveys, which is time-consuming, expensive, and laborious. Moreover, its measurement is subjective since it depends on the technician in charge. Therefore, remote sensing is a promising tool to obtain phenotyping traits faster, cheaper, and more objectively. Object detection is a computer vision technique that is used to identify and locate the object in an image or video. Another functionality that can be built on top of object detection is object tracking, which consists of tracking the movement of an object as it moves through the video frames. Object tracking permits counting the number of objects in a video since it assigns a unique ID to each object, while storing important information of each object. This work aims to identify and track grapevine clusters and estimate their dimensions (OIV codes 202 and 203) in an early development stage using UAV RGB videos and Deep Learning. For this purpose, the PointTrack algorithm has been successfully implemented on 29 videos, allowing multi-object tracking, cluster segmentation, and permitting the estimation of the OIV descriptors.

## USE OF DEEP LEARNING FOR VEGETATION SEGMENTATION AND DISEASE QUANTIFICATION ON RGB IMAGES

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Semantic image segmentation is the task of classifying each pixel in an image from a predefined set of classes. This is often a preliminary step, very similar to visual analysis, before higher level of processing. Deep learning now offers powerful methods for this task. However, compared to image classification or object detection the constitution of training datasets and especially the labeling task is very complex and tedious as each pixel needs to be attributed to a single class. Therefore, in agriculture, there is a lack of publicly available datasets.

Our first step was the constitution of a large and diversified dataset of 4000 high resolution RGB images (provided by INRAE, Arvalis and partner Universities) labeled in two classes: vegetation and background. The diversity encompasses different sensors, crops, resolution and sensing configuration. This dataset will be made publicly available in 2022.

From it, we trained and evaluated a baseline model, using a U-Net architecture reaching a F-Score of 81.3%. Optimizations of the backbone, using self-learning technics and data augmentation allowed a slight improvement up to 83.5%.

To illustrate the use of this model we present an application to quantify foliar disease (here: Septoria symptoms) on wheat microplots. For this task, we complemented the general model by other more specific models to segment different parts of the crops: heads, stems and a green / senescent model has been trained to quantify the affected areas within the vegetative parts. From this, we were able to quantify and monitor the dynamics of the senescence in field (Trials of 2021). The results show very good agreement between automatic and manual notations and rankings.

As perspectives, we clearly identify the need of multi-classes models to provide a panoptic description of each imaged scene. Last, we hope that the provided dataset will be widely used and complemented by the community to help improving the methods and performances.

## DESCRIBING WOODY PLANT TRAITS - WOODY PLANT ONTOLOGY

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Woody plants are a major natural resource in Europe, with a huge ecological and economical impact, supporting millions of jobs across diverse industries (e.g. wine, fruit, olive oil, coffee, paper, timber and cork) and strongly contributing to the European GDP and therefore are a central research domain in academia and industry.

The annotation of data and metadata from woody plants is of utmost importance due to the duration of the experiments considering the lifespan of perennial woody plant species. Thus, the annotation of different traits, which can be continuous or discrete, taken for a long period of time regardless of the data collector, as well as of the methods or devices used, need to be taken into consideration.

Woody plants lateral meristems lead to extensive production of secondary growth tissues (wood/secondary xylem and cork/phellem). As a result, a set of characteristic traits related to secondary tissues can be described in these plants. Woody Plant Ontology (WPO, <https://doi.org/10.15454/JB2WCE>), describes variables used during woody plant observations. This ontology covers several domains of knowledge in woody plant biology, from the agronomic down to the biochemical level, with the goal of standardizing and organizing trait information. This will facilitate the integration, management and analysis of woody plant data, thus promoting its FAIRness.

The WPO is integrated in the Crop Ontology (CO) framework, which includes species specific ontologies describing traits, methods and scales (<http://www.croponontology.org/>). As an example, Morphological traits include 'Bark Roughness', 'Tree Ring Width', 'Wood Density' while Biochemical traits include 'Cellulose Wood Composition', 'Syringaldehyde/Coniferaldehyde Wood Contents'. All the traits were curated considering several aspects such as synonyms, the context of use, the scale, among others.

# **IMPROVE WHEAT LAI ESTIMATION FROM HIGH SPATIAL RESOLUTION MULTISPECTRAL IMAGES VIA SOIL REFLECTANCE CALIBRATION AND BACKGROUND CORRECTION**

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The leaf area index (LAI) is a key crop trait that can indicate crop health conditions and contribute to crop growth and development. The rapid and accurate LAI estimation in field conditions can optimize the in-season crop management in precise agriculture and accelerate breeding by providing high-throughput LAI estimates. This study aims to apply a hybrid method to develop a predictive model to rapidly estimate plot-scale LAI from wheat canopy reflectance collected with proximal (UAV-borne) multi-spectral cameras (visible to near-infrared range) under varying growing conditions. This predictive model was developed based on random forest regression (RFR) and trained over synthetic data generated by a radiative transfer model, which means the estimation accuracy of LAI retrieved with this RFR model on experimental data largely depends on the similarity between simulated multi-spectral data and observed multi-spectral data. In response to increasing data similarity, we proposed a soil reflectance calibration method to adjust simulations towards observations as well as a background correction method to adjust observations towards simulations. The application of soil reflectance calibration on synthetic data and background correction on original reflectance map can effectively improve RFR model's estimation accuracy of LAI especially for sparse canopy with apparent exposure of soil background. For this developed hybrid method, the RFR model can be built ahead of time as (i) all input parameters used to generate synthetic data can be determined based on prior knowledge and (ii) the realization of soil reflectance calibration and background correction can be achieved based on multispectral images without additional ancillary data. Therefore, this developed hybrid method shows potential to allow rapid, accurate, in-season phenotyping of LAI dynamics at early stages prior to head emergence.

# **FLOWERPHENONET: AUTOMATED FLOWER DETECTION FROM MULTI-VIEW IMAGE SEQUENCES USING DEEP NEURAL NETWORKS FOR 2D AND 3D TEMPORAL PLANT PHENOTYPING ANALYSIS**

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A phenotype is the composite of an observable expression of a genome for traits in a given environment. We introduce a novel method called FlowerPhenoNet which uses deep neural networks for detecting flowers in a multiview image sequence for application in temporal plant phenotyping analysis. Temporal phenotypes are broadly classified into two categories, e.g., trajectory-based and event-based. A set of phenotypes computed from a time series imagery is called a trajectory-based phenotype. Trajectory-based phenotypes are often represented graphically for visualization. Event-based phenotype reports the timing (i.e, the day of imaging) of the significant events of a plant's life, i.e., timing of transition from the vegetative stage to the reproductive stage by the emergence of first flower. After flowers are detected in the plant images, FlowerPhenoNet generates a flower status report consisting of the following temporal phenotypes, e.g., the day of emergence of first flower in a plant's life cycle, the total number of flowers present in the plant at a given time, the highest number of flowers bloomed, growth rate of a flower and blooming rate. The flower size (measured as the area of the bounding box enclosing the detected flower) as a function of time is represented as the flower growth trajectory. However, frequent change in orientation of flowers and their partial occlusions by leaves pose challenges in accurate estimation of flower size based on area measurement from a 2D image. Thus, we use iterative space curving algorithm to construct the 3D voxel-grid model of the plant using multiview image sequences to compute the flower volume for accurate measurement of flower growth trajectory. We introduce a benchmark dataset called FlowerPheno which contains image sequences of sunflower, canna and coleus plants captured by a visible light camera in a high throughput plant phenotyping platform from multiple view angles to demonstrate the efficacy of FlowerPhenoNet.

## **IMPACT OF INDIVIDUAL PLANT PHENOTYPING WITH UAV FOR SUNFLOWER: A STUDY CASE ON THE AGROPHEN PLATFORM**

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Contemporary innovations in plant breeding are improving rapidly: while massive genotyping and sequencing of long DNA fragments open precise point descriptions of genotype panels, high-throughput phenotyping (HTP) provides a detailed plant description used to link plant functions to a precise area of the genome. However, HTP is often used instead of manual measurements, while it could be used to obtain individual plant-scale and geo-localized measurements, especially for plants that are individually seeded such as sunflower, maize, sorghum or sugar beet.

Joint work between INRA Toulouse and Hiphen Avignon studied the feasibility of individual measurements to monitor early developmental and senescence stages in sunflower. The growth rate in the early stages was estimated for each of 142,924 plants from the drone's high-resolution RGB image using a deep learning approach (Faster-RCNN) to identify and extract the individual plant with the neighboring area to determine the area of influence of each plant, their relative green covering microplot at each date. After eliminating outliers within plots, phenotypic averages were calculated and correlated to different traits (senescence, yield, flowering date, maturity date).

Studying at the plant rather than the plot level reveals 1) that the median of traits per plant is equivalent to the median of traits per plot, but 2) that there are many more outliers in the distribution of trait values. The study of this diversity is used to characterize the intrinsic variability of a trait's genotype without the need for more replications. In addition, the elimination of outliers (irregular emerged plants / weeds) allowed for a more accurate correlation of yield. In the future, it opens a better assessment of the inherent variability of a trait genotype and to understand its genetic basis.

# 3D RECONSTRUCTION AND SHAPE ANALYSIS OF TUBERS OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) USING SPHERICAL HARMONICS

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Plant morphological data, especially in 3D, is of high interest to plant breeders and researchers. Morphological traits can be measured using a variety of methods including photogrammetry and laser scanning. Genomic data has become widely available, however, phenotypic data remains challenging to obtain at high throughput. Phenotypic traits must still be extracted from 3D measurements. Some traits, such as shape, are difficult to quantify. Geometric morphometrics provides methods to extract quantitative shape data, which can be used for germplasm selection.

We propose a pipeline to quantify shapes in 3D and analyze them using spherical harmonics. We apply it to tubers of *Helianthus tuberosus* as an example case. By quantifying morphological descriptors and mapping shapes into a morphospace, phenomic data may be better mapped to genomic data, accelerating the selection of individuals with desired traits for breeding. The pipeline generates 3D model mesh from a set of 2D images using Agisoft Metashape Professional software. Then, the models are automatically processed with Meshlab to correct any topological errors. Next, SPHARM-PDM analysis is conducted by SlicerSALT software to generate coefficient data. Lastly, the coefficient data is analyzed using principal component analysis (PCA) to discover relationships between shapes and plot them along PCA axes.

We analyzed 62 individuals representing 3 cultivars of *H. tuberosus* (n=19,23,20) and successfully mapped their 3D contour shapes to a morphospace. However, the generated shapes do not accurately reflect the real shapes. Based on prior experience, we suspect that the parametrization step may have failed in some cases. Manual registration of the models might be required to improve the correspondences of the models.

## **SPECIES COMPOSITION RATIO IN MIXED SWARDS INFERRED FROM HYPERSPECTRAL IMAGING DATA**

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Traditional ways of measuring the ratio of species-specific biomass in a pasture mix are limited by current manual harvesting and visual scoring techniques, which are time-consuming and/or inaccurate. Automation and proximal sensing for high throughput phenotyping has been used in recent years as a viable solution to this bottleneck. This study aims to gauge whether the hyperspectral reflectance data could enable high resolution and unambiguous species composition estimation in mixed swards. Here, we focus on using hyperspectral imaging and machine learning methods for white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) segmentation in a mixed sward. We used H2O AutoML package to build machine learning models for clover and ryegrass segmentation. On our data, acquired from two trials, we obtained a mean accuracy of 92.4% for segmenting clover, ryegrass and background soil, only using spectral information. Here, we conclude that different species of forages can be identified with reflectance-enabled imaging, which can augment field visual monitoring methods.

## DEEP LEARNING INSTANCE LEVEL SEGMENTATION OF TOMATO SEPALS ON HYPERSPECTRAL IMAGES

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Tomatoes are extremely prone to pathogenic fungi infections. During product storage, inadequate temperature and humidity conditions can create ideal settings for the fungi to germinate. The most fragile parts of the tomatoes are the sepals, especially on their tips, which are the main entrance spots for fungal spores. Segmentation of tomato sepals in hyperspectral images is an essential step in designing automated systems for fungal infection sensitivity assessment. Precise segmentation of sepals could contribute to predictive models for early assessment of the risk of undesired fungal occurrence.

In this study, we analyze the state-of-the-art deep learning architectures for instance segmentation to identify which architecture provides the most precise tomato sepal segmentation in hyperspectral images. The dataset consists of 36 spectral images, captured using SPECIM FX10 camera (Specim, Finland) with 224 channels, covering the spectral range from 900-1700nm (Near Infrared Range) with a resolution step of 3.46 nm.

The dimensionality of the spectral images was reduced to 3 channels using Principal Component Analysis (PCA). Three mask R-CNN baselines are tested on sepal segmentation task on these 3 channel pseudo images a: ResNet + FPN (Feature Pyramid Network), ResNet + C4 (the original Faster R-CNN baseline) and DC5 (Dilated ConvNet) with variations in the number of epochs on which the Common Objects in Context (COCO), a large-scale object detection and segmentation dataset is previously trained.

For each model, the same training procedure was adopted with 15 images used to fine-tune the pre-trained models. The segmentation performance of the models is evaluated using the standard mAP (mean average precision) metric. The most precise segmentation is achieved using mask RCNN, ResNet+C4, reaching a mAP of 92.6% on the independent test set.

## **MAPEO - AN OPERATIONAL DRONE ANALYTICS PLATFORM FOR PHENOTYPING**

**NICK GUTKIN**; DRIES RAYMAEKERS; SAM OSWALD; JURGEN DECLOEDT

VITO (Flemish Institute for Technological Research)

Drone based, or drone assisted phenotyping has been described in the literature for many crops and traits with generally high accuracies in comparison to field based methods. Also the drone industry has pointed towards agriculture as one of the leading industries for drone use. However, the practical implementation into the experimental fields of plant breeders or public research facilities has been limited to demonstrations and publicly funded research programs. The step towards fully exploiting the potential of drone based phenotyping requires (i) the simplification of data acquisition and data handling, (ii) data analytics which are focused to extract relevant agrometrics on micro-plot level and (iii) the integration within day-to-day workflows. Over the past 5 years, we have collaborated with plant breeders and public research facilities to develop MAPEO, a drone image processing workflow dedicated to experimental fields. Crop emergence for wheat and corn plots, diseases in sugar beets and wheat trials, fruit detection for pumpkins and strawberry are just a few traits for which image analytics were provided and which resulted in agrometrics which reaches or even surpassed field based measurements. Technologies like MAPEO are there to help make plant breeders to fully adopt and integrate drones into their day to day lives.

## **A SIMPLE METHOD FOR EXTRACTING LEAF VEIN IMAGES AND QUANTITATIVE VEIN NETWORK FEATURES**

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Kyushu Univeristy

Leaf veins are crucial anatomical structures for both transpiration and translocation in plants. In particular, the angiosperms have been known to have species-specific hierarchical vein networks. The complex network is considered to increase photosynthetic efficiency through resource transportation and improve robustness against leaf damages. A method to quantitatively evaluate the leaf vein structures is required for understanding their evolution, development, and physiology. However, most studies have focused on naive measurements (e.g., the length and angle of veins). In this study, we propose a simple yet effective method for the quantitative representation of vein networks. The proposed method involves three major processes: (1) extracts the vein image from a raw leaf image; (2) converts the vein image into a graph; (3) extracts the network features from the graph. In (1), U-Net, a convolutional neural network model for semantic segmentation, was trained on stained leaf images with mask data created by a conventional image processing pipeline and extracted vein images with high accuracy. The network features captured the species-specific vein network structures enough for classifying, some of which corresponded to the looping structure of higher-order veins. The proposed method generated high-quality vein images even without ground truth images and extracted quantitative descriptors focused on the leaf vein network. The simple method will contribute to understanding the diversity of the vein networks and their physiological correspondences.

## **HYPERSPECTRAL REFLECTANCE AND DRONE IMAGING TO DETECT AND DECODE DROUGHT RESPONSE IN SOYBEAN**

**SARAH JONES MSC**; PROF. DR. ASHEESH SINGH PHD<sup>1</sup>; DR. ARTI SINGH PHD<sup>1</sup>;  
PROF. DR. BASKAR GANAPATHYSUBRAMIAN PHD<sup>1</sup>; PROF. DR. SOUMIK SARKAR PHD<sup>1</sup>

<sup>1</sup> Iowa State University

According to the US Drought Monitor as of June 2021, over 27% of the Midwestern United States region suffered from drought, with 57% of Iowa experiencing severe drought. Because the Midwest region is the highest row-crop producer in the United States, it is important to develop soybean lines that can withstand periods of drought to maintain security and resource efficiency of United States agriculture production. Three reps of a 450 member mini-core collection representing the genetic diversity of soybean germplasm was screened for drought tolerance in sandy soil in Muscatine, Iowa over 3 years. This project aims to use novel drought phenotyping methods to collect a broader range of information compared to visual drought scores alone and utilize these deep phenotypes to explore genetic architecture of drought response in soybean. Drought symptoms were quantified by various methods including visual wilt score, RGB drone imagery, and hyperspectral reflectance. Drone imagery increases the speed of phenotyping to improve detection of drought stress across increased breeding population size or wide geographic areas opening routes of drought stress monitoring. Hyperspectral imaging captures spectral reflectance from regions of the electromagnetic spectrum beyond human vision, and therefore has the potential for detecting symptoms not yet visible to the human eye. These results can be used for increased information input into GWAS to decode genetic control of drought tolerance in soybean.

## **IDENTIFICATION OF WATERLOGGING ASSOCIATED QTLS IN A TWO-ROW SPRING BARLEY COLLECTION USING UAV- IMAGING**

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Barley (*Hordeum vulgare* L.) is a top producing cereal and a key crop for food security. Barley is grown in a range of environments and geographical areas where no other crop thrives. Due to climate change, periods of extreme rainfall are becoming more common and waterlogging stress is threatening barley production. Thus, selection and breeding of resistant crops with improved yield under abiotic stresses such as waterlogging is crucial for food security. However, phenotyping under extensive field trials is costly and laborious and is often considered a bottleneck in plant breeding programs. Fast and accurate methods/tools are required for continuous measurements of phenotypic traits for field experiments. The use of Unmanned aerial vehicles (UAV) is a cost effective and powerful tool for plant phenotyping, in particularly under waterlogging conditions. Herein, a core collection of a European Heritage Barley (ExHIBiT) consisting of 230 two-row spring barley landraces and old cultivars from Northern Europe was cultivated at UCD

Lyons Research Farm field in randomized plots with two replicates under waterlogging stress and control conditions in 2021. Two types of UAVs (DJI MATRICE 300 RTK and DJI PHANTOM 4PRO MULTISPECTRAL) with two different sensors systems, RGB (flown at

120m) and multispectral (flown at 80m) were used for image-based phenotyping. The experiment will be repeated in 2022 and all images will be analysed using Agisoft Metashape and MATABLAB-MathWorks software. Phenotypic data will be obtained using R based

package, FIELDimageR. To identify QTLS, Genome wide association study (GWAS) will be performed using GAPIT and BLINK and candidate genes will be identified based on linkage disequilibrium (LD). Results from the current study, will not only address the molecular mechanisms underlying the waterlogging response in barley, but will also provide an optimized UAV-based phenotyping and image analysing under extensive field trials for plant breeding programs.

## **IMPROVING GENOMIC PREDICTION ACCURACY USING HIGH-DIMENSIONAL SECONDARY PHENOTYPES**

**DR. WILLEM KRUIJER;** DR. CAREL F. W. PEETERS

Wageningen University & Research

Plant breeding aims to improve a small number of target traits, while modern phenotyping platforms can often measure hundreds or thousands of additional (secondary) traits. A central question is how such secondary traits can improve genomic prediction for the target trait(s) on the test set. Current approaches include multi-kernel methods (e.g. Riedelsheimer et al 2012), bivariate gBLUP applied to phenotypic dimension reductions (Arouisse et al 2021), and sparse Bayesian factor analysis (MegaLMM, Runcie et al 2021). These methods generally have poor accuracy in case secondary traits are measured only on the training set (the most common scenario), and are often computationally demanding for larger number of traits.

To overcome these limitations we propose a fast and flexible two-step approach: gfBLUP (genetic factor BLUP). First, we obtain a regularized estimate of the genetic covariance matrix between target and secondary traits, on which we subsequently perform a regularized factor analysis. Second, the factor projection allows for usage of standard multivariate gBLUP approaches. This two-step approach outperforms, in terms of accuracy and computational efficiency, existing methods in both simulated and real data.

Arouisse B., Theeuwen, T., van Eeuwijk, F. et al

Improving Genomic Prediction Using High-Dimensional Secondary Phenotypes  
Frontiers in Genetics 12 (2021)

Riedelsheimer, C., Czedik-Eysenberg, A., Grieder, C. et al.

Genomic and metabolic prediction of complex heterotic traits in hybrid maize  
Nature Genetics 44 (2), 217-220 (2012).

Runcie, D.E., Qu, J., Cheng, H. et al.

MegaLMM: Mega-scale linear mixed models for genomic predictions with thousands of traits.  
Genome Biol 22, 213 (2021).

# RESEARCH DATA MANAGEMENT TOOLKIT (RDMKIT) : PLANT PHENOTYPING DATA MANAGEMENT AND SHARING GUIDELINES

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Efficient sharing of plant phenotyping data has been a challenge for some years. It has been addressed by European infrastructures (ELIXIR, EMPHASIS) and international communities (CGIAR) that allowed to coordinate the effort of major institutes. They produced several solutions acting together, including data standards (Minimum Information About Plant Phenotyping Experiment, MIAPPE or the Breeding API, BrAPI) implemented in databases (PIPPA, PHIS, GnpIS, e!DAL-PGP among others) and exchange file format (MIAPPE Template, ISA-Tab). Those systems and standards have been designed to be as close as possible to researchers and experimenters needs. As a consequence, important efforts have been made to ease their adoption through documentations and trainings. But those information remain dispersed, and it can be complicated for new users to know where to get the right information. Therefore, several European research infrastructures gathered and build the Research Data Management toolkit (RDMkit, <https://rdmkit.elixir-europe.org/>) to guide the users, plant biologist and agronomists, among the available solutions and material. RDMkit is a one stop portal that gives a general overview and understanding of the complementarity of the solutions and link back to the extended documentation and training materials maintained for each of them. In the RDMkit, the plant community has built a set of pages and in particular plant phenotyping guidelines. The present poster gives an overview of the RDMkit logic and of the solutions presented in the Plant Pheno assembly page, including the procedures and contact to submit enrichment and additional solutions.

# **PHENOCAVE: AN AUTOMATED, STANDALONE, AND AFFORDABLE PHENOTYPING SYSTEM FOR CONTROLLED GROWTH CONDITIONS**

**FERNANDA LEIVA**<sup>1</sup>; DR. PERNILLA VALLENBACK PHD<sup>2</sup>; DR. TOBIAS EKBLAD PHD<sup>3</sup>;  
PROF. DR. EVA JOHANSSON PHD<sup>1</sup>; DR. AAKASH CHAWADE PHD<sup>1</sup>

<sup>1</sup> SLU - Swedish University of Agricultural Sciences;

<sup>2</sup> Lantmännen Lantbruk;

<sup>3</sup> DLF Beet Seed AB

Plant growth facilities offer control over certain environmental conditions such as light quality, temperature, and humidity influencing plant growth. These functional and practical spaces permit inducing biotic and abiotic stresses in a controlled manner avoiding the climate variability found in nature. Nevertheless, measuring different features of plant growth non-destructively remains challenging. Despite several high-throughput phenotyping facilities that have been set up around the world, smaller custom-made affordable systems for particular needs still demand further work. Thus, we present “Phenocave” an affordable, standalone, and automated phenotyping system for controlled growth facilities. This non-invasive phenotyping approach supports consumer-grade digital cameras and multispectral cameras. Cameras are attached to a gantry with two linear motor drives allowing XY motions and individual top-view images of the complete workspace of Phenocave. In order to test Phenocave, two cases of studies are presented using sugar beet and wheat cultivars. The wheat plants were exposed to different irrigation conditions at different growth stages, thus influencing their growth rate and biomass accumulation. The correlation was significant between conventional measurements and the projected green area of the plants at different growth stages. In addition, post-harvest analysis of grain protein composition and content agreed well with those of previous studies. For the sugar beet plants case, seeds were treated prior to germination with different agents, hence affecting germination rates. Therefore, the results disclose that seed treatment(s) alter germination rates. Phenocave allows automated phenotyping of plants in controlled conditions, the blueprint and the results presented in this study will enable others to construct similar phenotyping approaches customized to their own needs with the protocols and results from Phenocave.

# **CHARACTERIZATION OF FOLIAGE STRUCTURES USING PERSISTENT HOMOLOGY ANALYSIS**

**PHD KOJI NOSHITA**

Kyushu University

Recently, topological data analysis (TDA), which refers to mathematical methods for characterizing structures from data using topological concepts, has been widely available for the quantitative representation of morphological properties in several fields. In particular, persistent homology (PH) analysis captures topological features in data on a multi-scale. PH analysis enables the quantification of abstract topological features.

In this study, I show an example of quantifying different foliage structures using the persistence diagrams (PDs) and the persistence images. PDs depict the birth-death profiles of multi-dimensional holes during filtration. For practical use, I adopted PIs as a vectorized representation derived from the PD. 30 point cloud data per each foliage structure were generated based on the probability distribution of leaf occurrence on the radial plane. The point cloud data were quantified as the PDs and the PIs. The PCA was conducted for the PIs, and different distribution patterns were recognized among the four structures in the data space. Finally, a practical scenario by using the PH analysis on the foliage structures under field conditions will be discussed.

## **TIME-SERIES ANALYSIS OF UAV MULTISPECTRAL INDICES AS A TOOL FOR PREDICTING WHEAT PHENOLOGY**

**PROF. NICOLA PECCHIONI**<sup>1</sup>; CLEBER VINICIUS GIARETTA AZEVEDO<sup>1</sup>; JUSSI GILLBERG<sup>2</sup>; CRAIG BRELSFORD<sup>2</sup>; DR. FABIO FANIA<sup>1</sup>; DR. PATRIZIO SPADANUDA<sup>1</sup>; DR. IVANO PECORELLA PHD<sup>1</sup>; PAOLO VITALE<sup>1</sup>; DR. PASQUALE DE VITA PHD<sup>1</sup>

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A major challenge of genetic improvement remains to accurately predict heading date for maximizing yield adaptation to the environment. Crop modeling provides a valuable approach for estimating flowering time of wheat and other crops at an early stage. However, model calibration for each variety is time consuming and requires many ground observations. The advent of remote sensing platforms with high temporal and spatial resolution and the deployment of cloud computing and machine learning techniques (ML), should allow meeting the long-lasting expectations for predicting accurately wheat phenological stages. In the present study, ML techniques were applied on high resolution remote sensing data to predict flowering and maturity date in a large panel of durum and bread wheat genotypes growth under irrigated and rainfed conditions. Several vegetation indices (VIs) were collected during the entire wheat growing cycle using an unmanned aerial vehicle (UAV) mounted with a RGB and multispectral camera. Ground-truth heading date was used as a proxy for flowering time (FT) while the maturity date (MD) was identified at physiological maturity stage, both stages presented as the thermal time required to reach those growth stages during 2020-21 growing season. The results indicated that Gaussian Process Regression (GPR) ML model provided the best performance for identifying phenological prediction using different VIs. Notably, the highest overall accuracy values for FT and MD were 70% and 96%, respectively. The time-series analysis of MCARI1 (modified chlorophyll absorption in reflective index) showed the best FT prediction while the GNDVI (green normalized difference vegetation index) was more informative for the MD. No significant differences in accuracy were observed using the durum and bread wheat panel, as well as between irrigated and rainfed treatment. The proposed method demonstrated great potential in improving the accuracy of phenology detection in wheat breeding.

## **USING ONTOLOGIES TO FACILITATE APPLE ORCHARD DATA AND MODEL INTEGRATION**

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DR. FRANCISCO ROJO PHD; DR. XIUMEI YANG PHD

The New Zealand Institute for Plant and Food Research Limited

An apple production and supply chain digital twin (DT) is being developed in Plant and Food Research, New Zealand. This DT will provide a virtual environment for researchers, policy-makers and apple growers to investigate the management, environment and biotic and abiotic challenges on apple production. Challenges persist in harmonising a large volume of heterogeneous experimental and simulated data to facilitate the holistic generation of models and decisions. The DT needs to integrate historical and new data collected (or simulated) in multiple domains such as soil, plant (phenology, physiology and morphology), climate, management and orchard ecosystem. We addressed the integration challenge by prioritising ontology development in essential domains, including experimental conditions (climate and soil), apple tree phenology, morphology and physiology. We will develop a generic apple orchard ontology (AOO) to integrate existing ontologies such as soil ontology and Plant Experimental Conditions Ontology (PECO). To the best of the author's knowledge, no publicly orchard ontology was available to support a DT. Therefore, we aim to develop an AOO consisting of generic classes spanning multiple domains. For example, tree location in AOO can help merge soil and plant trait data by querying a given tree's geolocation. To constrain the complexity of the development, we started with the AOO development on an established research apple orchard that was managed under a planar training system. WebProtege will be used to facilitate the AOO development.

## **EXPLORING SELF-SUPERVISED MULTIMODAL DATA FUSION STRATEGIES FOR CROP CANOPY REFLECTANCE**

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SARAH JONES MSC; LIZA VAN DER LAAN MSC; ASHLYN RAIRDIN; DR. ARTI SINGH PHD;  
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Iowa State University

Today, ubiquitous sensors can collect a high volume of crop canopy reflectance data to improve performance in monitoring, forecasting, and prediction tasks. However, the collected data is heterogeneous in terms of data collection frequency, resolution, type, and fidelity. Fusing such multimodal data to generate an informative representation by utilizing inter-modal and cross-modal information synergistically to provide better performance in the downstream tasks is an open research area. In this study, we explored a self-supervised deep learning architecture, Multi-modal Multi-task Masked Autoencoders (MultiMAE), to fuse RGB, multispectral, thermal, hyperspectral, and radar images of the soybean canopy captured by ground-based, UAV-based, and satellite-based sensors. MultiMAE is based on a vision transformer, which captures long-range relationships within an image, not just low-level image statistics, and is suitable for multimodal data processing. To train MultiMAE, we randomly sample a small number of patches from various input modalities, encode them with a shared vision transformer encoder, and reconstruct the masked-out patches with modality/input specific decoders. Here we train MultiMAE on three different sets of inputs: (1) using a single modality, (2) using multiple modalities, and (3) using multiple modalities and pseudo-labeled (e.g., semantic segmentation) images of the modalities generated by off-the-shelf models. We compare the performance of the trained models on a specific downstream task and generate missing data points in any modalities using the information from the other available modalities. We particularly assess the impact of cross-modal data fusion on plant science specific downstream tasks.

## **PHENOTYPING ACROSS MULTIPLE PLATFORMS - CALIBRATING IMAGE-BASED SHOOT PHENOTYPING METHODS**

**PROF. TONY PRIDMORE PHD<sup>1</sup>; PROF. DR. XAVIER DRAYE PHD<sup>2</sup>; DR. JONATHON GIBBS<sup>3</sup>**

<sup>1</sup> University of Nottingham;

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<sup>3</sup> The University of Nottingham

The ability to perform cross-platform experiments is vital if maximum scientific benefit is to be obtained from the networks of phenotyping facilities emerging in individual nations and across Europe. Sound methods for the integration of data from multiple sites would support production of richer data sets and exponentially increase the experiments accessible to those networks. Central to the integration of data across platforms is the availability of effective calibration procedures. Methods are required of ensuring that e.g. leaf area measurements taken in one country can be safely used alongside those obtained from plants of the same genotype grown in another.

Plants are complex, highly variable objects often measured using image-based tools. Variations in camera settings, position and orientation, and the image analysis methods used can significantly affect the data these systems produce. Such variations must be considered when comparing data from different sites using different hardware and software.

It is common within phenotyping facilities to verify results obtained from image analysis tools by maintaining a set of 'calibration plants' which are also measured manually, and often destructively. Comparison of the output of manual and automatic methods allows scale factors to be computed which can correct for any systematic errors in image-based measurements. This approach does not, however, scale to distributed phenotyping infrastructures: it has proved difficult to share physical plants between sites to provide the common calibration plants needed to compute relative scale factors.

We describe an approach based on the exchange not of plants but of simple camera calibration objects and digital images produced using camera parameters obtained automatically from images of those objects. The approach was successfully piloted by the EPPN2020 consortium and will be developed into a community service within the recently announced EMPHASIS-GO project.

# **JOINT IMAGE ACQUISITION AND ANNOTATION PROTOCOL FOR VARIETY TESTING PHENOTYPING BASED ON MACHINE LEARNING**

MOUAD ZINE EL ABIDINE; DR. HELIN DUTAGACI; **PROF. DR. DAVID ROUSSEAU PHD**<sup>1</sup>

<sup>1</sup> INRAE

Manual phenotyping is known for being time-consuming, labor-intensive and subjective as it depends on the human visual perception. Machine learning and, lately, deep learning, have opened new perspectives to digital phenotyping. Using a set of images associated with the ground truth, automatic and high-throughput variety testing protocols can be developed. Although the accuracy of predictions through model-based classification can be very high, training such models demands a considerable cost: the time to create the ground truth, i.e., annotation. Annotation is known for being currently the bottleneck in deep learning problems., We aim to target the annotation problem and reduce the time-cost of this mandatory step. In this communication, we exploit camera motion during image acquisition to speed up the annotation. Instead of acquiring a large set of images and pass them to an expert for annotation of the target trait, we aim to perform the annotation during the acquisition by slowing down the camera when the region of interest appears in the camera field of view and speed up when nothing important is visible. From videos acquired in this manner, we extract target frames using motion features such as optical flow. We demonstrate the gain in annotation time obtained by motion-based annotation in comparison with the classical approach, and highlight the results on two applications: detection of russeting on apples and detection of scab on leaves.

## **ENHANCING WHEAT AND SOYBEAN BREEDERS' SELECTION DECISIONS WITH HIGH-THROUGHPUT FIELD PHENOTYPING BASED IDEOTYPE FORMULATIONS**

**DR. LUKAS ROTH**; PROF. DR. ACHIM WALTER; DR. ANDREAS HUND PD

ETH Zurich

Describing crop growth dynamics and responses to environmental factors becomes key in breeding—integrating such insights in selection strategies may allow mitigating adverse effects of climate change. While dynamics are difficult to capture by human eye, high-throughput field phenotyping (HTFP) can enhance breeders' perceptions on site.

Processing HTFP data is an interdisciplinary task. In a corresponding workflow, one needs to perform phenotyping campaigns (i.e., collecting raw data), achieve comparability between raw data (i.e., extracting low-level traits), describe the temporal dynamics of growth (i.e., defining intermediate traits), and finally use these 'dynamical traits' to gain insights on physiological and environmental drivers of crop performance.

To get an overview of sources of variation in HTFP, we develop a general plot-level model for repeated measurements. We further describe the extraction of three intermediate trait categories; (1) timing of key stages, (2) quantities at defined time points or periods, and (3) temperature dose-response curves. Using a wheat canopy growth simulation, we show that traits of all three categories can be extracted with high precision from HTFP data.

Switching to real data, we demonstrate that image-derived traits based on canopy cover, leaf area and plant height allow predicting yield and protein content from the dynamics of vegetative growth in soybean. The results exemplify that HTFP can be used to derive an optimal genotype, a so-called ideotype, for a given mega-environment. In a second example of winter wheat stem elongation monitoring with terrestrial laser scanning and drones we show that traits of all three categories and in particular temperature response parameters are heritable traits with a strong physiological basis. We provide indications that applications of these techniques in breeding may increase the selection efficiency.

## **AI-POWERED TECHNOLOGIES FOR NEXT-GENERATION CROP PHENOTYPING: AN UPDATE AND FUTURE PERSPECTIVES**

**DR. MICHAEL GOMEZ SELVARAJ PHD**

INTERNATIONAL CENTER FOR TROPICAL ag

Breeding for crop improvement counts completely on gene banks located worldwide. Gene banks, which contain novel genes and alleles, reinforce the selection and enhance the genetic gain in crops for characteristics such as yield, quality, and biotic and abiotic stresses. Acquisition of rapid and precise time series phenotypic information from large germplasm and breeding populations is critical for dissecting the underlying genetic traits. In recent years, high-throughput phenotyping technology has benefited immensely through remote sensing, artificial intelligence (AI), and robotics. Notably, integrating AI-driven applications through machine learning and robotics facilitates robust field data collection and image analysis. Hence, the Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) prioritizes next-generation phenotyping as one of the research priorities under One-CGIAR initiatives. Therefore, this presentation focuses on the one-CGIAR strategic initiative through a public-private partnership to strengthen the current phenotyping capacity. The research discussed here is broadly categorized to be mindful of future dynamics such as (1) water- and nitrogen-use efficiency traits in rice and *Brachiaria* grasses; (2) breeding for disease resistance in rice and bananas; (3) yield- and disease-prediction models in common beans; (4) storage root biomass prediction in cassava. Finally, we propose future directions and opportunities to promote in-field crop phenotyping.

# **HYBRID DEEP LEARNING AND SWIR HYPERSPECTRAL IMAGING FOR YELLOW RUST AND LEAF BLOTCH DISCRIMINATION IN WHEAT**

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Yellow rust, caused by *Puccinia striiformis* f.sp. *tritici*, and *Septoria nodorum* leaf blotch, caused by *Parastagonospora nodorum*, are common types of wheat leaf diseases that severely affect grain yield. Some studies have tried to detect and classify rust or leaf blotch using RGB and hyperspectral imaging. However, implementing leaf disease detection remains difficult since multiple diseases might occur on the same leaf and the size of disease spots on the leaves vary among diseases. This study investigates a reliable deep learning method with minimum data preparation and annotation for leaf disease classification. Hyperspectral images of healthy leaves and leaves infected by rust or leaf blotch, were recorded using a hyperspectral camera (HySpex SWIR-384) sensitive in the Short Wavelength Infrared (SWIR) region (1000-2500nm) with a predefined setup using halogen lamps as light source. The images were corrected with white and dark references. Areas representing different disease classes including yellow rust, leaf blotch chlorosis and leaf blotch necrosis were selected for each leaf. The classification was done using three classifiers including a shallow deep learning, hybrid deep learning, and Random Forest (RF) model. For hybrid deep learning model, a one-dimensional Convolutional Neural Network (Conv1D) model in combination with multiple layer perceptron applied. The hybrid deep learning model outperformed the shallow deep learning and RF, with total accuracy of 100%. The producer's accuracy was 100% for all classes and the kappa value was equal to one. These results show the potential of SWIR imaging in combination with hybrid deep learning for multiple disease distinguishing and percentage estimation, although further research needs to be done to investigate the model applicability under field condition with a bigger and more diverse dataset.

# **SPATIO-TEMPORAL GENERATION OF MORPHOLOGICAL FEATURES FOR PLANT GROWTH PREDICTION USING PROGRESSIVELY GROWING GANS**

**DHRUV SHETH**

Harvard Medical School

Recent Innovations in Precision Agriculture (PA) are driven by Computer Vision and Data Processing systems to quantify plant parameters. Quantitative analysis of Plant Phenotyping in PA and monitoring morphological traits is a protracting process, hindering the objective and phenotyping process. Generative Adversarial Networks (GAN)'s used for Generating features offer a catalytic approach to the time-consuming process to accelerate phenotyping and predict plant growth under different environmental factors before harvest. This research proposes a concept of using time-series plant growth datasets to understand Spatio-temporal features in plants and correlate the visual morphological features such as foliage or leaf density affected by external environmental variables and predict the growth and pattern specific to its environment. Environment-specific growth pattern prediction for each plant help in understanding the suitable environment for optimum growth of the plant and to maximize yield. This approach reduces the dependence over an experimental process to deduce the optimum growth environment through observational analysis and decreases cost over resources and labour. To achieve this process autonomously, an approach in Machine Learning, GANs are used. Spatio-Temporal 3D convolutions based Progressively Growing GANs are proposed to iteratively learn features in plants and generate future plant growth frames through image input frames of initial stages. After a thorough subjective and objective analysis of the generated frames, it can be understood that the Results showed high correspondence with Ground Truth frames and realistic generation of plant traits in images which made it visually interpretable and accessible.

# **THE RIPE AERIAL PHENOTYPING PLATFORM: ADVANCING TRANSLATION OF RESILIENT AND PRODUCTIVE CROPS TO MEET GLOBAL DEMANDS**

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Presently, environmental pressures threaten crop production and food security globally. Climate change induced by anthropogenic activities will increase the variability in growth conditions and the extreme conditions that threaten crop production. Coupled with these challenges are increasing global demands for agricultural outputs to meet needs of a growing population and for other agricultural demands including fiber and biofuels. Therefore, more productive and resilient crops are required to overcome these challenges, and this effort necessitates advances in phenotyping at multiple scales. Measuring crop responses to environmental variation and genetic improvements are critical under field conditions where environmental factors and canopy dynamics reflect true agronomic growing conditions. Similarly, measuring key agronomic traits in diversity panels or structured populations that represent hundreds to thousands of plots requires the complexity of plant canopies grown under field conditions. The need for high intensity data collection for a range of crop phenotypes such experiments require high throughput field phenotyping capabilities that blend non-invasive sensor technologies with advanced statistical analyses to determine growth, physiological, and nutritional traits under experimental treatments, environmental variability, and genotypic diversity. Here, we describe the world's largest, newly constructed cable-mounted aerial phenotyping platform that includes high-end sensor sensors including hyperspectral imaging cameras, multi-channel LIDAR, visible and thermal cameras with high spectral, spatial, and temporal resolution and the ability to maneuver over a 4 hectare area with measurement heights ranging from 0 to 10 meters above ground. The system we describe will increase, relative to our current capabilities, measurement area from dozens to thousands of plots and reduces measurement time from minutes to seconds per plot.

# ENHANCING CANOPY TEMPERATURE MEASUREMENT PRECISION WITH A DRONE-BASED UNCALIBRATED THERMAL CAMERA

**TREIER SIMON MSC.**<sup>1</sup>; PROF. DR. ACHIM WALTER<sup>2</sup>; DR. LUKAS ROTH<sup>2</sup>;  
DR. ANDREAS HUND PD<sup>2</sup>; DR. HELGE AASEN<sup>1</sup>; DR JUAN HERRERA<sup>3</sup>

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<sup>2</sup> ETHZ;

<sup>3</sup> Agroscope

Low canopy temperature (CT) is an integrative trait "being associated with yield in a range of conditions". It is indicative of the relative fitness of a genotype to the environment. Therefore, CT can be used as an indirect selection criterion for yield. Thermal measurements with handheld thermometers have their shortcomings, especially because genotypes should be measured at the same time to avoid distortions by changing environmental conditions. Main sources of short-term variability include wind, sunlight, clouds, and air temperature. Thus, thermal cameras mounted on drones are an interesting option to measure many plots in a relatively short time which reduces the variability between measurements.

To get absolute CT measurements, calibrated thermal cameras must be used. Cooled thermal cameras are precise but heavy and cannot be mounted on a lightweight drone. For uncooled calibrated thermal cameras, a calibration must be done prior to use to get accurate absolute CT values. This takes specific system knowledge, and these cameras are still relatively heavy. To our knowledge, there are no off-the-shelf ready-to-use calibrated thermal cameras that could be operated on a drone without significant integration efforts. However, there are uncalibrated thermal cameras that can be operated with standard drones and standard software.

This study sought to improve the quality of CT measurements on wheat with an uncalibrated off-the-shelf thermal camera by analysing multiple georeferenced original images for each plot in a ray tracing approach. We exploited spatial information on plot location, temporal information on measurements and geometric information on the sun-plot-camera relations to correct for short-term-variability. Preliminary analysis shows that Cullis broad sense heritabilities of CT as high as 0.95 can be reached by this approach.

# **AUTOMATED ANALYSIS OF SPIKE IN CEREAL CROPS WITH IMAGE AUGMENTATION IN GREENHOUSE AND FIELD ENVIRONMENT**

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Masarykova Univerzita CEITEC MU

In greenhouse and field environment, an automated detection and segmentation of grain spikes in cereal crops is a highly demanded task for extracting phenotypes, such as yield biomass, time stamping of plant life cycle from images and other associated agronomics traits. To address the challenge of spike localization, we trained greenhouse wheat images on two deep neural networks (DNNs): Faster-RCNN, Faster-RCNN with attention module (FRCNN-A) and for segmentation U-Net and Deeplabv3+. The experimental results show an average precision of 90.50% for Faster-RCNN and FRCNN-A 91.10% on combined wheat cultivars grown in two different greenhouses. The predictive power of trained deep neural networks changes for a spike depends on its spatial location in plant canopy. We also observed that the trained model on wheat can perform with high accuracy (>80%) on unseen barley and rye images captured in the same photo chamber. The image augmentation improves the AP of detection DNNs by 3%. On segmentation DNNs, the average dice coefficient of Deeplabv3+ is 93.5% and U-Net 90.6%. In the field environment having low illumination condition a cascaded of ensemble DNNs of Faster-RCNN and FRCNN-A with prior Lenet classifier on light and dark frame have an AP of 56% that outperforms single trained DNNs AP 50.5%. Our implementation is also available as a GUI-based tool SpikeApp which shows the application of detection and segmentation that extract 90 other spike phenotyping of greenhouse grown wheat plants.

# 3D CONVOLUTIONAL NEURAL NETWORK FOR IMAGE ANALYSIS AND PHENOTYPING OF PLANT ROOTS IN SITU

**CAMILO VALDES PHD**; PROF. DR. JENNIFER CLARKE PHD<sup>1</sup>

<sup>1</sup> University of Nebraska Lincoln (UNL)

Critical factors that determine crop yields for food, feed, and bioenergy are located underground, making them difficult to characterize and quantify. Traditionally, these factors have been measured by "shovelomics" or by growing plants in clear media and measuring traits by visible imaging. Modern phenomics technologies use one or several imaging modalities (e.g., visible, infrared, multi-spectral) to capture traits that reflect plant physiology or performance.

Analytical techniques for plant phenomics, or procedures to evaluate plant characteristics, are a crucial part of approaches to achieving desirable agronomic and biological traits. Advances in sensor technologies have paved the way for faster and more efficient plant phenotyping, with methods adapted from disciplines such as high-resolution 3D X-Ray computed tomography (CT). 3D CT root phenotyping datasets contain noise created by the capturing instrument, and a crucial step in their analysis is segmentation - the identification and classification of the scan's voxels as "root" or "non-root". Unlike roots in transparent mediums, roots in non-transparent mediums are difficult to segment from their surrounding materials as root and non-root voxels have overlapping CT values.

The challenge we address is the development of machine learning approaches for the segmentation of roots in 3D CT scans, and discuss subsequent trait extraction methods that enable the quantification of root systems and their traits in several agriculturally important crop species. Our approach learns the unique intensity distribution of CT values for each root scan to create a segmentation map that partitions the voxels along the putative root system boundaries, providing high quality data for subsequent trait extraction.

Our goal is to develop analytical strategies that enable phenotyping of critical plant species to further the development of crops with improved food, feed, and bioenergy performance under sustainable agricultural practices.

## **ESTIMATE OPTIMAL HARVEST TIME BY CROSS-SCALE ASSIMILATED DIGITAL BROCCOLI FARMLAND**

**HAOZHOU WANG<sup>1</sup>**; TANG LI<sup>1</sup>; ERIKA NISHIDA<sup>1</sup>; PROF. YUYA FUKANO<sup>2</sup>;  
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The broccoli head is an important component in the vegetable market. But harvesting at a non-proper time will lead to non-standard size for market shipping and be wasted. Compared to the one-time mechanical harvest, the conventional method selectively harvests by hand several times to decrease such waste, but the heavy labor cost eliminates the profit. Hence, obtaining the full field broccoli head development promptly to estimate the optimal harvest time, has emerged as an interesting research area. Several studies have already applied the close-range remote sensing techniques by drones and ground vehicles to calculate broccoli head sizes by 3D reconstruction. But the complex light condition, leave occlusion, and wind movement in the field, present limitations to achieving high-quality 3D head models. In this study, the high-quality in-field 3D models were generated by cross-scale data assimilation and template matching. Three scales of data were collected and aligned during the growing season. The 3D models of the aerial scale (full field), the ground scale (small sample region), and the indoor scale (individual broccoli), were reconstructed by drone, smartphone, and indoor semi-automatic rotating platform, respectively. Then, the geometry traits on three scales of the same broccoli were linked to find the mapping relationship from field to indoor high-quality 3D models. All the broccoli head 3D models in the field were replaced by the linked indoor 3D model with a slight shape transformation. Finally, the time-series full-field head size results and marketing price were used to build a prediction statistical model for harvesting time. The prediction statistical model was evaluated on the next round of planting.

## MAIZE PLANT DETECTION IN UAV IMAGES USING YOLOV5

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Computer Vision (CV) has made enormous progress in recent years and brought more possibilities in analyzing images for the purpose of object detection, especially with the aid of machine learning (ML). Unmanned Aerial Vehicle (UAV-) based high resolution images opens a perfect playground to apply CV and ML methods for the detection of plants or plant organs of interest. This study proposes a practical workflow based on YOLOv5 (You Only Look Once) and UAV images to detect and count maize plants in contrasting development stages. Additionally, we proposed a semi-auto-labeling method to reduce the pressure of labeling. Results showed that our trained model achieved the mean average precision (mAP) of 58.5%, 60.0%, 67.5% and 73.1% for the 3-leaf stage, rotation augmented 3-leaf stage, 7-leaf stage and rotation augmented 7-leaf stage, respectively. Results also showed that the image-rotation-based augmentation improved the model accuracy for low resolution images and high leaf occlusion. This work provides a reference for applying advanced ML and deep learning methods to UAV images for automated object detection and characterization of plant growth response to the environment.

## **3D DESCRIPTION OF VINEYARD CHARACTERISTICS BASED ON UAV-IMAGES**

**LAURA ZABAWA MSC.;** MELTEM CANTÜRK MSC; DR. LASSE KLINGBEIL;  
PROF. DR. HEINER KUHLMANN

Universität Bonn

Information regarding the macro structure of vineyards are important to optimize the management decisions of winegrowers. These include plant positions and canopy characteristics for guided decision making, supporting decision on pesticide applications or yield optimization. We propose an automatic pipeline to extract the plant height, canopy width, as well as trunk and post positions from point clouds, which are derived from image data, collected with an unmanned aerial vehicle (UAV).

The extraction of single rows is performed automatically by extraction Hough lines from binary images. For each row we derive the plant and post positions as well as detailed row parameters, including the plant height and canopy width. The whole pipeline is independent of the terrain slope and does not require assumptions like plant or row spacing.

In contrast to many other approaches, we provide detailed information and visualisations of the height and width in forms of diagrams and histograms, giving important clues on the distribution of these factors. Since the comparison with manual measurements depends highly on the sample positions and is therefore not meaningful, we focus on the comparison between the results for two training systems, the vertical shoot positioned (VSP) system and the semi minimal pruned hedge (SMPH). Furthermore, we are also able to identify the trunk positions with a precision of 78%.

## **IMPROVED INFIELD SOYBEAN SEED COUNTING AND LOCALIZATION WITH FEATURE LEVEL CONSIDERED**

**DR. JIANGSAN ZHAO PHD<sup>1</sup>**; DR. AKITO KAGA<sup>2</sup>; PROF. DR. MASAYUKI HIRAFUJI<sup>1</sup>;  
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Effective soybean seed counting tools can be used for its yield prediction directly in the field ahead of the final harvest. An integrated approach that can manage both counting and localization will be ideal for subsequent analysis. The traditional way of object counting through predicting bounding boxes or density map over instances are significantly limited by either the labor-intensive and error-prone labeling procedure or low localization accuracy. In order to quantify the soybean seed directly rather than in a sequential manner, we propose P2PNet-Soy, which is built upon the point-based framework for joint crowd counting and individual localization, P2PNet. Several strategies are considered to adjust the architecture and subsequent post processing to maximize the model performance in soybean seed counting and localization. First, two task-specific modules are applied: one module collects lower-level features for localization while another one extracts higher-level features for classification, respectively; the outcomes of two modules are used jointly to further improve the final performance of the model. Second, astrous convolution with different kernel sizes are used to extract scale invariant features to deal with soybean of different sizes. Thirdly, spatial attention module is used to separate the foreground and background for easier soybean counting and localization. At last, subsequent thresholding method is applied to merge very closely located predictions. Through training the model on infield images of cropped individual soybean plant taken from one side and testing on images taken from the opposite one with all the strategies above considered, the superiority of proposed P2PNet-Soy in soybean seed counting and localization over the original P2PNet is validated with a reduction of MAE from 48.06 to 10.25.

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# **FROM SEED TO SEED - COMBINING MULTI-SCALE PHENOTYPING, AI-POWERED ANALYSIS WITH GENETIC MAPPING TO BRIDGE THE GAP BETWEEN LAB-BASED PLANT RESEARCH AND IN-FIELD CROP IMPROVEMENT**

**PROF. JI ZHOU**

Cambridge Crop Research, NIAB

Recent advances in imaging and sensing, machine learning, computer vision and big-data analytics open new opportunities for plant research. Complex traits such as flowering, canopy structure and yield formation can be dynamically characterised and predicted, helping plant researchers understand growth patterns of key agronomic traits under varied environmental conditions. These advances can also enable biological discoveries to unravel the genetics behind target traits with evidence at the cell, organ, tissue, plant, and population levels. In this talk, the speaker will introduce with NIAB-Cambridge's seed phenotyping lab (i.e. SeedGerm 2.0 and hyperspectral seed imaging using VideometerLab), then link lab-based discoveries to crop early establishment in the field through aerial phenotyping and the AirMeasurer platform. He will demonstrate how to quantify dynamic traits that are agriculturally important, as well as the use of these phenotypic traits in modelling plant growth and development in varied environments. Furthermore, the speaker will demonstrate the usefulness of dynamic phenotyping in genetic mapping studies for rice and wheat, a joint UK-China collaborative research carried out by his China-UK lab. The speaker will also introduce aerial phenotyping of orchard fruits, 3D trait analysis for screening resource use efficiency wheat varieties, and embedded AI techniques for yield prediction in wheat and rice, demonstrating the great potential of AI and plant phenomics in addressing challenging biological questions.



# Sponsor Profiles

## Sponsor Profiles (Listed alphabetically)

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At Hiphen we have assembled a team of 20+ agronomists, image processing engineers, software engineers and data scientists who are laser focused on making high-throughput plant phenotyping accessible to the agricultural research community. We design plant assessment solutions that are actioned routinely by our clients, and we also develop custom-made image analytics applications that are tailor-made to tackle very specific agronomic challenges. We have committed our careers to data excellency and to uncover new phenotypic traits by innovating together with the scientific research community with whom we continuously create, test, and validate new methods to help solve the current agronomic challenges and those that are yet to come."



## Hoogendoorn Growth Management

Hoogendoorn Growth Management is one of the world's most innovators in the horticultural sector with more than 50 years' of experience. Our roots lie in the Netherlands, but we are present around the world.

Hoogendoorn has been developing various systems to control any greenhouse climate. Their latest innovation IIVO constantly and consistently monitors every aspect of your greenhouse environment including climate conditions, Co2 levels and water usage; but the system is capable of so much more. The systems collect and archive data to create a complete and holistic overview of the conditions inside of a greenhouse.

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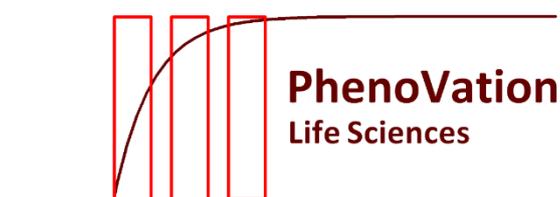
Millions of researchers, ag companies, and farmers around the world are working hard to develop new solutions to reduce the impact of farming on our planet. We believe digitalization will be the major driver in this process. That's why we develop digital solutions that capture crop data and translate it into actionable information, amplifying the impact of millions of people and accelerating the transition towards sustainable agriculture

We empower seed companies, crop protection companies, and indoor farms around the world in transforming their phenotyping processes into digital, agile and efficient development pipelines. Combining our expertise in sensors, automation, and data analysis with our clients' know-how, we're driving transformation.

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PhenoVation is based in Wageningen, the Netherlands, but our operations span the globe. We make our mark by delivering the best camera systems and keeping our customers' needs in mind when designing cutting-edge solutions. By constantly investing in extensive R&D, we have been able to create a wide array of products, each specifically made for its own ground-breaking applications. With anything from stand-alone camera systems to fully automated plant phenotyping systems, we can help you design your own tailor-made research facility. PhenoVation has developed imaging systems that are capable of measuring photosynthesis efficiency as well as chlorophyll and anthocyanin absorption. Moreover, our systems measure morphological parameters, allowing you to assess plants' growth and development.



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## Plant-DiTech

Plant-DiTech is enhancing researchers' ability to secure agriculture growth by introducing the PlantArray platform which enables researchers to efficiently screen simultaneously many plants' responses to different stresses and assess their yield potential within days. As physiology phenotyping is a direct way to immediately capture & quantify plants' responses, PlantArray detects plants' reactions long before they can be recognized visually. This results in achieving meaningful results for many plants within days, increasing quality, demonstrating a high correlation to field yield, and saving costs & time. The use of the technology holds a track record of over 40 scientific articles, and customers such as Wageningen University, University of Florida, Mars Wrigley etc. [www.plant-ditech.com](http://www.plant-ditech.com)



## PSI spol. s.r.o.

**PSI (Photon Systems Instruments)** is the world leader in development and manufacturing of instrumentation for imaging and monitoring of biological signals in plants and algae. We were the first company to offer a **chlorophyll fluorescence imaging system**, and we remain at the forefront of technology for monitoring growth and physiological activity in samples that range from individual cells to large crop and tree species.

As part of our mission to serve the scientific community, and to prove that we deliver the best possible quality and value, PSI has established its own **PSI Research Center** where clients can work with our devices prior to sale. Here, our clients may conduct proof of concept experiments and obtain training in the latest technology and software for plant science research. [www.psi.cz](http://www.psi.cz)



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SMO is a dynamic company that offers innovative solutions for various industries.

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Syngenta Seeds is part of Syngenta Group, one of the world's leading agriculture companies with 53,000 employees in more than 100 countries. Our ambition is to help safely feed the world while taking care of the planet — providing leading seeds, traits, crop protection, and precision and farm management innovations to help farmers be successful. Our technologies enable millions of farmers around the world to make better use of limited land and water resources, protect their crop yield and remain profitable. Innovation is the lifeblood of Syngenta, and the focus of our nearly 5,000 R&D employees throughout the world. With our investment of \$1.4 billion each year in R&D, we are accelerating innovation to help farmers increase their productivity in a sustainable way. To learn more, visit [www.syngenta.com](http://www.syngenta.com)



## University of Nebraska - Lincoln

UNL Phenotyping Services offers image based phenotyping on a fee for service basis. The University of Nebraska has invested in 3 facilities: The LemnaTec HTS Scanalyzer for small plants especially suited for Arabidopsis thaliana, the LemnaTec High Throughput 3D greenhouse platform for plants up to 2.5 m tall, and the 1 acre Spidercam Field Phenotyping platform. Our systems utilize visible (RGB), fluorescent, infra-red (IR),near infra-red (NIR) and hyperspectral cameras. The Greenhouse and field scale systems offer precise irrigation control to facilitate drought studies. We strive to tailor experiments to provide the best phenotyping data possible.

For further information please visit <https://ard.unl.edu/phenotyping/phenotyping-facility-inquiry>



**INSTITUTE OF AGRICULTURE  
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### About WPS

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