



Prognostic Field Phenotyping: Zygi Experimental Station, DAF 2014, Cyprus

## ***COST FA1306* "The quest for tolerant varieties - Phenotyping at plant and cellular level"**

### **WG1 Meeting**

### **"Phenotyping: from the lab to the field"**



**Larnaca, 13-14 November 2014**

***Local Organizer: Agricultural Research Institute, Cyprus***

**COST FA1306 Plant Phenotyping****"The quest for tolerant varieties - Phenotyping at plant and cellular level"**

<b>Day 1</b>		
<b>8:00 9:00</b>	<b>Registration</b>	
<b>9:00 9:05</b>	<b>Opening of the meeting</b>	<b>Sebastien Carpentier</b>
<b>9:05 11:20</b>	<b>Oral presentations</b>	<b>Chair Sebastien Carpentier</b>
9:05 9:20	Matthew Reynolds	Using phenomics to develop new wheat lines adapted to heat and drought stress
9:20 9:35	Dionysia Fasoula	Factors controlling the Crop Yield Potential in whole-plant field phenotyping
9:35 9:50	Astrid Junker	High throughput plant phenotyping at IPK: facilities and applications
9:50 10:05	Carl-Otto Ottosen	3D laser triangulation, a simple and robust method for automated growth determination in challenging environments
10:05 10:20	Hannes Kollist	Stomatal regulation – from tissue level to intact plants and from model species to crops
10:20 10:35	Imre Vass	Characterization of biomass and grain yield responses to drought stress in wheat by using non-invasive plant phenotyping tools
<b>10:35 10:50</b>	<b>Coffee break</b>	
<b>10:50 11:20</b>	<b>Invited speaker</b>	
	<b>Christophe Salon</b>	
<b>11:20 12:20</b>	<b>Workshop – WG1 development</b>	<b>Three groups discuss: joint activities (chair C-O Ottosen), outputs (chair D Rubiales), training of young scientist (chair A Junker)</b>
<b>12:20 14:00</b>	<b>Lunch</b>	
<b>14:00 17:00</b>	<b>Oral presentations</b>	<b>Chair Astrid Junker</b>
<b>14:00 14:30</b>	<b>Invited speaker</b>	
	<b>Onno Muller</b>	Phenotyping photosynthesis and water relations from lab to field
14:30 14:45	Tiina M. H. Lynch	Detecting differences in stomatal conductance in C3 perennial ryegrass <i>Lolium perenne</i> and C4 <i>Miscanthus</i> spp. using thermal imaging
14:45 15:00	Lucio Conti	Defining the role of florigen genes in plant developmental reprogramming under drought stress
15:00 15:15	Gerrit Polder	Wageningen UR Phenomics Innovation and Demonstration Centre
15:15 15:30	Terezia Salaj	Potential of somatic embryogenesis for in vitro propagation of conifer trees
<b>15:30 16:00</b>	<b>coffee break</b>	
16:00 16:15	Miguel Costa	Assessing canopy temperature patterns in two grapevine genotypes subjected to deficit irrigation
16:15 16:30	Ismail Turkan	Investigation of the Interaction Between GA3, ROS and Antioxidants in the First Internode Elongation That Contributes to Drought Tolerance of <i>Triticum aestivum</i>
16:30 16:45	Ebe Merillo	Species-specific stomatal responses to two simultaneously applied conflicting abiotic factors
16:45 17:00	Pirjo Peltonen-Sainio	Phenotyping of disease resistance, phenology and nutrient use efficiency of a wide barley germplasm adapted to high latitudes
<b>17:00 18:00</b>	<b>Workshop – WG1 development</b>	<b>Three groups report: joint activities, outputs, training of young scientist</b>
<b>18:00 19:00</b>	<b>poster session</b>	
<b>20:00</b>	<b>Dinner</b>	

**COST FA1306 Plant Phenotyping****"The quest for tolerant varieties - Phenotyping at plant and cellular level"**

<b>Day 2</b>		
<b>09:00</b>	<b>Oral presentations</b>	<b>Chair D. Rubiales</b>
<b>9:00 9:30</b>	<b>Invited speaker</b>	
	<b>Jose Luis Araus</b>	Field phenotyping for improving abiotic stress resilience in crops
9:30 9:45	Sebastien Carpentier	The quest for drought tolerant varieties: Phenotyping the Musa Biodiversity with simple phenomic tools
9:45 10:00	Roland Pieruschka	Integrating controlled and field phenotyping
10:15 10:30	Jan Fila	Functional analysis of the selected phosphoprotein candidates identified in tobacco male gametophyte
<b>10:30 11:00</b>	<b>coffee break</b>	
11:00 11:15	Olga Grant	Imaging and isotopes: applications for phenotyping diverse species under optimal and sub-optimal conditions
11:15 11:30	Giuseppe Sciara	High-throughput phenotyping of a maize introgression library reveals drought-responsive QTLs for plant growth
11:30 11:45	Kristina Ložienė	Selection of blueberries genotypes tolerant for variation of climatic conditions
11:45 12:00	Eva Rosenqvist	Dynamic or static light and the influence on physiological phenotyping
12:00 12:15	Inger Martinussen	Phenotyping of Nordic wild berry species
<b>12:15 14:30</b>	<b>Lunch</b>	
<b>14:30 16:00</b>	<b>Oral presentations</b>	<b>Chair E Rosenqvist</b>
14:30 14:45	Marta de Vasconcelos	Is it a nutrient or is it a pest? High-throughput phenotyping can distinguish them best
14:45 15:00	Diego Rubiales	Major gaps in screening methodologies for parasitic weed resistance at the lab and field level
15:00 15:15	Simon Fraas	Hansa Trace - A high throughput imaging auxanometer for roots and hypocotyls of Arabidopsis
15:15 15:30	Andreas Voloudakis	DsRNA application for plant virus control and endogenous plant gene expression regulation. A new functional genomics tool?
<b>15:30 16:00</b>	<b>Invited speaker</b>	
	Christoph Germeier	Crop Information Systems as a Means to Establish Good Phenotyping Practice and to Collect Data for Trait Mining
<b>16:00 22:00</b>	<b>excursion Field + Nicosia</b>	

# **INVITED SPEAKERS**

## **Phenotyping plant root and their interaction with soil micro-organisms**

**Christophe Salon**

**<sup>1</sup>UMR Agroécologie, GEAPSI Group and High Throughput Phenotyping Platform, INRA, 17 rue Sully, BP86510 Dijon Cedex, France**

The plant interacts with numerous and diverse microorganisms, especially in the soil volume surrounding roots, called rhizosphere. Understanding plant-microorganism relationships in the rhizosphere is of great agronomical and ecological importance they can potentially lead to beneficial effects for the plant (nutrition, health etc).

However, to address these challenges, the main bottleneck is the development of high-throughput, non-invasive phenotyping systems/methods adapted to characterize the numerous phenotypic traits involved in these interactions. The talk will describe how the scientific community address this challenge and will then detail a novel high throughput phenotyping system (rhizotrons, rhizocab) used to phenotype root development and growth, and possibly functionality of nodulated roots.

## **Phenotyping photosynthesis and water relations from lab to field**

**Onno Muller, M.Pilar Cendrero Mateo, Hendrik Albrecht, Francisco Pinto, Anke Schickling, , Roland Pieruschka, Ulrich Schurr, Uwe Rascher**

Phenotyping in the field is an essential step in the phenotyping chain from well-defined and controlled conditions in the laboratory and greenhouse to the heterogeneous and fluctuating environment in the field. Field measurements represent a significant reference for the relevance of the laboratory and greenhouse approaches and an important source of information on potential mechanisms and constraints for plant performance to be tested at controlled conditions. Here we present a range of methods that are being deployed within the German Plant Phenotyping Network focusing on, photosynthesis and water relations. Specialized field platforms are established (a) to test innovative phenotyping technologies (b) to install semi-controlled field installation to support breeding approaches for future CO<sub>2</sub> – concentrations (breed-FACE) and (c) to study the transfer of phenotypic properties from controlled environments to stands in the field. The methods used showed thus far that; sun induced fluorescence and light induced fluorescence transients techniques allow for remote estimating of photosynthesis at canopy and leaf to plant level respectively and active thermography estimates leaf water content and transpiration rates. These methods will be further tested and incorporated in (semi-) automated systems positioning sensors in the field introducing a promising portfolio to measure plant traits for field phenotyping and to enhance our understanding of relevant traits under natural conditions.

## **Field phenotyping for improving abiotic stress resilience in crops**

**JOSE LUIS ARAUS ORTEGA <jaraus@ub.edu>**

Constraints in field phenotyping capability currently limit our ability to dissect the genetics of quantitative traits, especially those related to yield and stress tolerance. Development of effective field-based high-throughput phenotyping platforms (HTPP) remains a bottleneck for future advances in crop breeding. However, progress in sensors, aeronautics and high-performance computing are paving the way. Field HTPP will combine a high capacity for data recording/scoring and processing and non-invasive remote-sensing methods, together with automated environmental data collection, and all at an affordable cost. Laboratory analyses of key plant parts may complement direct phenotyping under field conditions. Examples of the performance of different phenotypical approaches in different crop species are provided

## **Crop Information Systems as a Means to Establish Good Phenotyping Practice and to Collect Data for Trait Mining**

**Christoph U. Germeier**

**1 Julius Kühn-Institut, Federal Research Centre for Cultivated Plants,  
Institute for Breeding Research on Agricultural Crops, Erwin-Baur-Str.  
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Lack of access to phenotyping information is seen as the most limiting factor for increased use of plant genetic resources in agriculture, horticulture, crop improvement and research. Phenotyping data are complex. This results from Genotype x Environment x Management interactions, and good phenotyping (documentation) practice requires background information, on experiment design, site, treatments, observation methodology, plant development, interference of stresses, pests and diseases. G x E x M interactions also call for multi-location studies to estimate stability or adaptability of traits in different environments and management practices. Highest variation, and representativeness for practice conditions, is faced in participatory on farm research. It requires sophisticated designs involving many actors (e.g. farms as replications), geo-statistical approaches, for each participant a well defined role implemented and descriptive data documented in a comparable way.

A European project on Avena Genetic Resources for Quality in Human Consumption (AVEQ) involved 15 partners and nine experiment sites in a geographical range from Estonia to Bulgaria. A web based management system has been developed to coordinate actions in experimental fields and analytical labs and to collect results into a central database. Types of data requested for documentation, modelling and design will be presented. A vision on an inclusive open source genetic resources phenotyping network will be given.

# **ORAL PRESENTATIONS**

## **Using phenomics to develop new wheat lines adapted to heat and drought stress**

**Matthew Reynolds and Maria Tattaris**

**International Maize and Wheat Improvement Centre (CIMMYT)**

*Germplasm Development.* Research networks involving national wheat programs in Asia and Africa have permitted development and testing of a number of physiological approaches to breeding. Infra-red thermometry -as a tool to screen for adaptation to hot, irrigated environments- was the first approach to be verified, through extensive field evaluation (Reynolds et al., 1994). As a result, a new crossing program based on physiological characterization of crossing block material was initiated. Outputs include a generation of drought adapted wheat lines based on strategic crossing of complementary physiological traits (PT) that have been included in CIMMYT's international distribution system since 2010 (e.g. lines of Semi Arid Wheat Yield Trials -SAWYT-). The new PT lines show superior performance over conventional material in most international environments (e.g. Pask et al., 2014; Reynolds et al., 2014). For example, in the 17<sup>th</sup> SAWYT the average yield of PT lines beat the group of conventionally bred lines at 75% of international sites. In 2013 the Pakistan National Program released 'Pak13' a new cultivar based on a PT cross that showed outstanding performance in national yield trials, as well as disease resistance. This ongoing effort has involved broadening the genetic base of conventional wheat gene pools through extensive use of genetic resources, including landraces and products of inter-specific hybridization with members of the Triticeae tribe (Reynolds et al., 2009).

*Phenotyping Tools and Platforms.* One of the prerequisites for successful application of phenomics in breeding is the establishment of reliable screening tools and platforms that can precisely measure expression of physiological traits in realistic field environments. Genetic gains associated with selection for canopy temperature have been precisely quantified in genetic populations (Pinto et al., 2010), and new spectral water indices have been developed that reliably estimate difficult-to-measure water relations traits, as well as yield and biomass under heat, drought and yield potential conditions (Gutierrez et al., 2010). The first aerial remote sensing platforms for large scale genetic resource screening was developed in Mexico and more than half of the accessions of the World Wheat Collection have been screened (Tattaris et al, 2013).

*Gene Discovery.* Rapid phenotyping tools and precision methodologies -as described above- have application in gene discovery. However, development of well-controlled genetic populations have also been prerequisite to QTL analysis to avoid confounding effects of agronomic traits like phenology and height (Reynolds et al., 2009; Pinto et al., 2010). Using these approaches, QTL for both drought and heat adaptive traits have been identified on 4 different chromosomes of the Seri/Babax RILs population, showing for the first time a

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common genetic basis for these key abiotic stresses (Pinto et al., 2010). Similarly the phenology-controlled ‘Wheat Association Mapping Initiative’ panel has been used for gene discovery work (Eade et al., 2014; Lopes et al., 2014; Sukumaran et al, 2015).

*Crop design.* To define the best constellation of traits for application in breeding - and determine priorities for genetic understanding- it is necessary to demonstrate the significance of key adaptive mechanisms, develop models of adaptive traits, and show where crop management interventions can ameliorate stress factors. Research in Mexico has demonstrated roles of the several physiological mechanisms explaining differential adaptation among modern wheat cultivars, information that has been used to develop conceptual models of adaptive traits that highlight wheat’s genetic limitations under water limitation (Reynolds and Tuberosa, 2008), heat stress (Cossani and Reynolds, 2012), and yield potential conditions (Reynolds et al., 2012).

- Cossani CM, Reynolds, MP (2102). Physiological traits for improving heat tolerance in wheat. *Plant Physiology* 160 1710-18.
- Eade EA, Patrick F. Byrne, Scott D. Haley, Marta S. Lopes, Matthew P. Reynolds, 2014. Genome wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes, *Theoretical and Applied Genetics (TAG)* DOI 10.1007/s00122-013-2257-8
- Gutierrez M, Reynolds MP, Klatt AR, 2010. Association of water spectral indices with plant and soil water relations in contrasting wheat genotypes. *Journal of Experimental Botany* 61 3291–3303
- Lopes MS, Dreisigacker S, Peña RJ, Sukumaran S, Reynolds MP, 2014. Genetic characterization of the Wheat Association Mapping Initiative (WAMI) panel for dissection of complex traits in spring wheat. *TAG* (accepted).
- Pask Alistair, A.K. Joshi, Y. Manes, I. Sharma, R. Chatrath, G.P. Singh, V.S. Sohu, G.S. Mavi, V.S.P. Sakuru, I.K. Kalappanavar, V.K.Mishra, B. Arun, M.Y. Mujahid, M. Hussain, N.R. Gautam, N.C.D. Barma, A. Hakim, W. Hoppitt, R. Trethowan, M.P. Reynolds (2014). A Wheat Phenotyping Network to Address Challenges To Wheat Productivity In South Asia. *Field Crops Research* (accepted for publication)
- Pinto RS, Reynolds MP, Mathews KL, McIntyre CL, Olivares-Villegas JJ, Chapman SC (2010). Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. *TAG* 121:1001–1021
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- Reynolds M.P. and Tuberosa R (2008). Translational research impacting on crop productivity in drought-prone environments. *Current Opinions in Plant Biology* 11: 171-179
- Reynolds MP, Manes Y, Izanloo A and Langridge P (2009) Phenotyping for physiological breeding and gene discovery in wheat. *Annals of Applied Biology*: 155: 309–320
- Reynolds MP, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, Parry M, Slafer G, 2012. Achieving yield gains in wheat. *Plant Cell and Environment* 35: 1799–1823.
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- Sukumaran S, Matthew P. Reynolds, Marta Lopes, and Jose Crossa. Adaptation to plant density: A component of high yield potential in spring wheat through GWAS, (2015). *TAG* (submitted)
- Tattaris, M., Reynolds, M., Pietragalla, J., Cossani, M.C. and Ellis, M. (2013), Airborne Remote Sensing for High Throughput Phenotyping of Wheat: *Proceedings of Workshop on UAV-based Remote Sensing Methods for Monitoring Vegetation*. Sept 9-10 2013, Cologne, Germany.

## **Factors controlling the Crop Yield Potential in whole-plant field phenotyping**

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**Plant Breeding and Molecular Genetics, Agricultural Research Institute,  
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Field phenotyping is the most challenging kind of phenotyping and at the same time, the most relevant for plant breeding and food security. The challenges relate to the highly heterogeneous and unpredictable environments in farmers' fields, as well as to the fact that phenotyping important quantitative traits, like yield and stability of performance, creates additional levels of complexity. In their beginning, phenotyping platforms were mostly accommodating plants grown in pots in controlled environments. Very recently, the necessity for accurate field phenotyping is becoming more and more evident to a wider scientific audience. To that end, prognostic breeding<sup>1</sup> is an integrated methodology that embodies field phenotyping for high and stable crop yield of plants and sibling lines grown at spacings that eliminate interplant competition, *i.e.*, eliminate the interference with the equal sharing of growth resources, including the physical interference through the root and canopy system. Two novel selection equations, based on innovative field experimental designs, assess concurrently the crop yield potential and stability of performance. Contrary to popular belief, plant density was shown<sup>2</sup> not to represent a component of crop yield potential. Thus, it is possible to phenotype individual plants grown in the absence of inter-plant competition predicting crop yield potential. In addition, this realization opened the way for the creation of density-neutral cultivars<sup>2,3</sup>, *i.e.*, cultivars yielding optimally over a wider range of plant densities. This also offers a novel explanation for the disastrous effects of the 2012 USA drought, where corn hybrids, being created specifically to yield optimally under high planting densities, failed to perform under drought.

1. Prognostic Breeding: a new paradigm for crop improvement. *Plant Breed. Rev.* 2013, 37, 297-347.

2. Honeycomb Breeding: Principles and Applications. *Plant Breed. Rev.*, 2000, 18, 177-250.

3. Nonstop selection for high and stable crop yield by two prognostic equations to reduce yield losses. *Agriculture* 2012, 2, 211-227. doi:10.3390/agriculture2030211.

## **High throughput plant phenotyping at the Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben: facilities and applications**

**Astrid Junker, Moses Mahagu Muraya, Kathleen Weigelt-Fischer, Christian Klukas and Thomas Altmann**

**Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)  
Gatersleben, Corrensstraße 3, D-06466 Stadt Seeland OT Gatersleben,  
Germany**

An important prerequisite for the identification of efficient and high-yielding plant lines is the development of sensitive and high-throughput-enabling concepts for the quantitative capturing of performance related plant traits.

IPK owns three whole plant phenotyping facilities for high-throughput imaging of small (such as *Arabidopsis thaliana*), medium (such as *Hordeum vulgare*) and large plants (such as *Zea mays*) that – according to the plant-to-sensor-principle – are used for imaging-based observation of plant performance and plant care (watering) in an automated fashion. Plant imaging in different wavelength bands of electromagnetic radiation (visible and near infrared, fluorescence imaging) in combination with a comprehensive image analysis pipeline (Klukas et al. 2014) allows for the quantification of a wide range of plant structural and functional traits. Detailed and standardized protocols for cultivation of several different model and crop plants have been implemented and adapted to the special requirements of high throughput plant phenotyping under environmentally controlled conditions (Junker et al., submitted). Novel sensors for 3D plant capturing and analysis of functional chlorophyll fluorescence as well as concepts for root phenotyping and environmental/soil monitoring are currently/will be established in existing platforms. Applying controlled conditions, these facilities have been used to dissect plant traits with good reproducibility, and were able to mimic variation of vegetative growth that has been observed under natural (field) conditions (Junker et al., submitted).

Phenomics facilities at the IPK will be introduced and future upgrades outlined. Using the example of different applications the potential of high throughput plant phenotyping as an integrative, multi-disciplinary approach will be highlighted.

Klukas, C., Chen, D., and Pape, J.M. (2014). Integrated Analysis Platform: An Open-Source Information System for High-Throughput Plant Phenotyping. *Plant Physiol* 165, 506-518.

Junker, A., Muraya, M.M., Weigelt-Fischer, K., Arana-Ceballos, F., Klukas, C., Melchinger, A.E., Meyer, R.C., Riewe, D., and Altmann, T. (2014) Optimizing experimental procedures for quantitative evaluation of crop plant performance in high throughput phenotyping systems. Submitted.

## **3D laser triangulation, a simple and robust method for automated growth determination in challenging environments**

**Katrine Heinsvig Kjær<sup>1\*</sup>, Carl-Otto Ottosen<sup>1</sup>**

**Aarhus University, Department of Food Science, Kirstinebjergvej 10, 5792 Aarslev, Denmark**

Advanced plant phenotyping platforms are expensive, both in terms of the market price and in terms management and running costs. Furthermore, several techniques and sensors developed to gather information from plants noninvasively are not robust under fluctuating climate conditions and in the field. If we want to use phenotyping platforms to improve the crop management and clarify genetic differences, simple and cost-efficient methods with high-throughput capacities should be considered. PlantEye (Phenospex B.V, Heerlen, NL) is a commercial 3D laser scanner using a near infrared laser to gather plants in 3D, and was designed to track daily changes in plant growth with high precision and in harsh environments. The sensor projects a laser line on plants and computes via triangulation height profiles from each image. These profiles are merged into a 3D data point cloud and several plant parameters are computed automatically. PlantEye uses an onboard filter to remove artefacts from sunlight reflections and it is resistant to environmental challenges allowing phenotyping in greenhouse or in the field under full sunlight and at high temperatures.

We present a validation of the PlantEye sensor to estimate phenotypic variation in *Brassica napus* L. (rapeseed) and demonstrate that the near infrared laser line has no negative effect on the photosynthetic performance of the plant. All studies were conducted *in situ* under greenhouse conditions with fluctuations in natural irradiance, temperature and humidity. Our results demonstrate a high potential of 3D laser scanning to be used for simple estimation of phenotypic variation in fluctuating climates at low cost and in high temporal resolution.

## **Stomatal regulation – from tissue level to intact plants and from model species to crops**

**Hannes Kollist, Triin Vahisalu, Ebe Merilo, Mikael Brosché, Hanna Hõrak,  
Liina Jakobson, Pirko Jalakas, Kristiina Laanemets, Maris Nuhkat**

**Plant Signal Research Group, Institute of Technology, University of  
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Stomatal closure is one of the first plant responses to unfavorable changes in the surrounding environment; it prevents loss of water and restricts entrance of pathogens and air pollutants. On the other hand, closed stomata limit plant growth by preventing CO<sub>2</sub> uptake. Thus, identification of mechanisms underlying stomatal regulation is important for designing strategies to breed crops with improved yield and stress tolerance. There has been a major progress in stomatal research within recent years, however, the prior challenges are not only "From the lab to the field", but also "From isolated guard cells and epidermal peels to real-time responses in intact plants" and "From Arabidopsis to crops". Within last years, we have established a set of custom-made semi-high-throughput gas exchange systems to study stomatal regulation in response to various stimuli in intact plants. This research was instrumental for identification of guard cell anion channel SLAC1 and its regulation by phosphorylation. We have also shown the importance of ABA signaling through its PYR/RCAR receptors for whole-plant stomatal conductance and regulation in response to various environmental factors. More recently we developed gas exchange devices suited for measuring larger and taller plants such as cereals or tree seedlings. Our first results show that in natural situations, when more than one environmental factor changes and guard cells need to integrate conflicting signals, the final outcome – stomatal opening or closure – is species-specific.

## **Characterization of biomass and grain yield responses to drought stress in wheat by using non-invasive plant phenotyping tools**

**Imre Vass<sup>1</sup>, Kenny Paul<sup>1</sup>, János Pauk<sup>2</sup>, Zsuzsanna Deák<sup>1</sup>, László Sass<sup>1</sup>,  
1Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, H-6726 Szeged, Temesvári krt. 62, Hungary  
2Department of Biotechnology, Cereal Research Non-Profit Ltd., H-6726 Szeged, Alsó kikötő sor 9, Hungary**

We studied the effect of drought on biomass accumulation and grain yield in two model wheat cultivars, which have been classified in the literature as drought sensitive (Cappelle Desprez) and tolerant (Plainsman). Our data show that biomass accumulation and grain yield respond differentially to water limitation in these cultivars, namely Cappelle Desprez has higher biomass, but lower grain yield, while the Plainsman cv. has lower biomass accompanied with higher grain yield under conditions of drought stress. Our data also show that changes in physiological parameters of leaves show different responses to drought stress depending on their developmental stage. Namely, the primary leaves, which support grain development in the spikes show similar response in their CO<sub>2</sub> fixation, drought factor index, leaf temperature, and electron transport parameters as the grain yield, whereas the secondary leaves, which support overall green biomass growth show similar responses as biomass accumulation. Therefore, the analysis of these two different leaf types could be used for the indication of biomass and grain yield responses, respectively. Our findings demonstrate that the agronomically highly important traits of biomass and grain yield are not necessarily correlated in wheat and possibly in other cereal crops. Although monitoring of physiological parameters of primary and secondary leaves can reflect differential responses of grain yield and biomass accumulation, respectively, phenotyping of green biomass growth alone is not sufficient for predictions of grain yield changes. Therefore, in cases when the aim of phenotyping is the optimization of grain yield under stress conditions the phenotyping protocol should include direct grain yield assessment.

## **Detecting differences in stomatal conductance in C<sub>3</sub> perennial ryegrass *Lolium perenne* and C<sub>4</sub> *Miscanthus* spp. using thermal imaging**

**Tiina M. H. Lynch<sup>1,2</sup>, Susanne Barth<sup>1</sup>, Philip J. Dix<sup>2</sup>, Olga M. Grant<sup>3</sup>**

<sup>1</sup>Teagasc Crops Environment and Land Use Programme, Oak Park Research Centre, Carlow, Ireland

<sup>2</sup>Biology Department, National University of Ireland Maynooth, Co. Kildare, Ireland

<sup>3</sup>School of Agriculture & Food Science, University College Dublin, Belfield, Dublin 4, Ireland

Lack of water is a major stress in crops and with increasing cost of water, efficient irrigation control is necessary for both food and biomass crop farming to be economic. Understanding grasses' physiological responses to drought is important for selecting suitable varieties for specific environments. Using thermal imaging to measure leaf temperature allows for the detection of a reduction in stomatal conductance to be observed before any changes in the plant's physical appearance become visible.  $I_G$ , an index proportional to the leaf conductance of water vapour, can be calculated from thermal images. In two separate experiments, 8 genotypes of perennial ryegrass *Lolium perenne*, an important C<sub>3</sub> pasture and forage grass, were subjected to 4 drought treatments in a temperature controlled greenhouse and 14 genotypes of *Miscanthus*, a highly productive perennial rhizomatous C<sub>4</sub> grass, were subjected to severe drought and control treatments. For *L. perenne*, treatment was found to have a significant impact on both  $I_G$  and Crop Water Stress Index (CWSI) throughout the experiment, and differences between genotypes were also observed during the 2<sup>nd</sup> and 4<sup>th</sup> weeks of the 7-week experiment.  $I_G$  had a strong positive correlation with biomass production while CWSI exhibited an equally strong but negative relationship. Both treatment and genotype had an effect on biomass production with a significant interaction between the factors: on average, a 40% decrease in irrigation led to c. 30% loss in biomass production. For *Miscanthus*, preliminary results indicate that both treatment and genotype have an effect on  $I_G$  and CWSI. Differences in biomass production are also likely.

## **Defining the role of florigen genes in plant developmental reprogramming under drought stress**

**Lucio Conti\*, Sara Castelletti\*, Claude Welcker#, François Tardieu# and Chiara Tonelli\***

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Plants adaptively respond to drought with different strategies including an early flowering response referred to as drought escape – DE –. DE allows plants to complete their life cycle and anticipate more detrimental drought consequences. Since early flowering constitutes the major attribute of DE, the manipulation of DE has direct practical implications, as longer vegetative growth duration is often associated with high yield potential in crops. Florigen genes are key floral regulators in several plant species and play a role in coordinating plant development according to external cues. We have recently found that drought – dependent variations in florigen levels largely accounts for the phenotypic variability of DE responses found in Arabidopsis mutants. However the mechanism underpinning DE via florigen transcriptional and post-transcriptional regulation as well as whether coordination between DE and other drought - survival tactics exists is still largely unknown. Indeed, whilst diversification in florigen expression enabled the expansion of maize cultivation to temperate latitudes it could also contribute to drought stress adaptation. We are addressing these questions by performing a high-throughput flowering phenotype screen utilizing 350 maize lines grown under different watering regimes. We will relate individual florigen eQTLs to the broader phenotypic datasets, thus linking florigen eQTLs to physiological QTLs. Our goal is to understand the mode and adaptive significance of flowering as a major DE strategy, thus providing insights into the mechanisms that enable plants to adapt to changing environments.

## **Wageningen UR Phenomics Innovation and Demonstration Centre**

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High throughput phenotyping facilities are rapidly becoming essential for significant advancements in plant breeding. The necessary integration of image analysis, pattern recognition, plant physiology including crop growth models, quantitative and molecular genetics, automation and robotics poses several scientific challenges. Wageningen UR combines all of these expertise's and is involved in several international phenotyping initiatives like EU projects (EU-SPICY), EU-COST actions (FA1306: 'The quest for tolerant varieties: Phenotyping at plant and cellular level') and phenotyping networks (EPPN, IPPN).

The expertise of all groups at Wageningen UR comes together in the Phenomics innovation and demonstration centre, which is the organization's entry point to facilities and tools for large-scale field and greenhouse phenotyping.

These enabling technologies include 2D and 3D plant reconstruction modules, hyperspectral cameras, X-ray tomography for root analysis, chlorophyll fluorescence setups and other novel sensors plus vision-guided robotics to automate manual labor, as well as recently developed software for modeling growth and yield in 3D, which facilitates predictions of plant performance based on the measured phenotypic features.

At the Wageningen University and Research Centre several research projects are currently exploring the potential of these technologies spanning the full spectrum from long-term academic research to industrial applications. To elaborate the future perspective of research and development in this field a number of examples will be discussed.

## **Potential of somatic embryogenesis for *in vitro* propagation of conifer trees**

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Somatic embryogenesis is differentiation of embryos without sexual fusion of gametes from somatic cells of a plant body under *in vitro* conditions. The differentiated structures are called somatic embryos. The process was first described for carrot in 1958 and later for numerous plant species, including conifer trees (1985). In conifers the process can be divided into several steps as initiation, maintenance of initiated structures, maturation of somatic embryos, plantlet (somatic seedlings) regeneration. Somatic embryogenesis for selected conifer species (*Pinus nigra* Arn., *Abies alba* Mill. and its hybrids) has been initiated from juvenile explants as immature and mature zygotic embryos as well as from seedlings cotyledons. The initiated embryogenic tissues were maintained on solid proliferation media or in liquid as suspension cultures. The conifer somatic embryos are bipolar structures consisted of embryonal part characterised by meristematic cells and suspensor composed of long vacuolised cells. Under appropriate conditions the bipolar structures developed passing the developmental stages of their counterparts in seeds (zygotic embryos). Finally their development was completed by whole plant (somatic seedling) regeneration. Using the *in vitro* approach somatic seedlings have been obtained in *Pinus nigra* and *Abies* hybrids and the regenerated plantlets have been transferred to soil.

The work was supported by Slovak Academy of Sciences as MVTs COST 1306.

## **Assessing canopy temperature patterns in two grapevine genotypes subjected to deficit irrigation**

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A better understanding of grapevine responses to drought and heat stress can help to optimize breeding and vineyard management for improved water use efficiency, yield and berry quality. Faster and robust field phenotyping and genotype characterization are needed in precision viticulture, in particular in dry and hot regions such as the Mediterranean. Canopy temperature has been used to monitor drought stress in plants/crops and to characterize stomatal physiology in different species including grapevine. Thermography permits the remote determination of surface temperature of individual leaves/canopy in field conditions and also to assess the range and distribution of temperatures from different parts of the canopies. We hypothesized that grapevine genotypes may show different canopy temperature patterns along the day as a result of different stomatal behaviour and heat dissipation strategies. We have monitored the diurnal and seasonal course of canopy temperature ( $T_c$ ) in two *V. vinifera* genotypes, Aragonez (syn. Tempranillo) and Touriga Nacional subjected to two irrigation regimes: 1) sustained deficit irrigation (SDI) and 2) regulated deficit irrigation (RDI). Thermography measurements were complemented by determination of the diurnal course of leaf water potential ( $\psi_{leaf}$ ) and gas exchange (stomatal conductance to water vapour and net  $CO_2$  assimilation). Measurements were done in 2013 and 2014 and took place at different phenological stages: i) mid-June (green berry stage), ii) mid-July (veraison), iii) early August (early ripening) and iv) before harvest (late ripening). Results are discussed over the use of thermal imagery to derive information on genotype physiology in response to changing environmental conditions and to mild water stress induced by deficit irrigation.

Key-words: thermal imaging, genotypes, diurnal and seasonal stress responses, phenotyping

## **Investigation of the Interaction Between Gibberellin (GA<sub>3</sub>), Reactive Oxygen Species (ROS) and Antioxidant Enzyme Activities in the First Internode Elongation That Contributes to Drought Tolerance of *Triticum aestivum* cv. Tir**

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Cultivation of grass plants is usually achieved by sowing the seeds a few centimeters deep or less shallower from the soil surface. In the semi-arid areas, however, the shallowly sown seeds cannot take up enough water for germination and seedlings suffer from severe drought stress. Drought is a very common environmental factor and limits growth and productivity of plants. Plants develop several mechanisms to cope with limited water conditions. One of these strategies is deep sowing tolerance which can be described as the ability of the elevation of the shoot apical meristem above the soil surface by elongation of mesocotyl or first internode. There are only a few reports concerning the first internode elongation in the literature. It was previously reported that the internode elongation of a deep sowing tolerant wheat cultivar was more sensitive to gibberellin (GA<sub>3</sub>). It was also suggested elongation of the first internode depended on the GA–K<sup>+</sup> interaction. More recent works revealed exogenous application of GA<sub>3</sub> induced the expansion of the epidermal cells and proliferation of the cortical cells and the internode elongation in a straight manner relied on the synchronization of these two processes. In the recent years, ROS have emerged as important regulators of plant growth and development. In *Arabidopsis thaliana* and *Zea mays*, it was suggested that the phosphorylation of microtubule associated protein MAP65 by reactive oxygen species (ROS) activated kinases was necessary for the establishment of the aforementioned synchronization. These findings indicate that ROS can play roles in the first internode elongation. Therefore, we tried to characterize the interaction between gibberellin (GA<sub>3</sub>), reactive oxygen species (ROS) and antioxidant machinery in the internode elongation of a deep sowing tolerant wheat variety cultivated in Turkey, namely *Triticum aestivum* cv. Tir. It was established that Tir wheats had 8.56-13 cm long coleoptiles even when they were sown to depths of 9-15 cm. Therefore, Tir wheat can be a good model for investigating the roles of ROS and gibberellin in the internode elongation. Wheat seeds were sown on the surface of approximately 2 cm thick of 1 % agar and covered with 5 cm, 10 cm, 15 cm, 20 cm and 25 cm of soil. To treat wheat seedlings with exogenous gibberellin, GA<sub>3</sub> dissolved in dimethyl sulfoxide (DMSO) was added to the 1 % agar medium at a concentration of  $2.89 \times 10^{-6}$  M. 13 days after planting shoot and first internode length of control and GA<sub>3</sub> treated plants were measured. Then, the first internodes of the seedlings were harvested and H<sub>2</sub>O<sub>2</sub> content, level of lipid peroxidation (in terms of thiobarbituric acid reactive substances = TBARS) and the activities of peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and NADPH oxidase (NOX) were determined. Also,

## **COST FA1306 Plant Phenotyping**

**"The quest for tolerant varieties - Phenotyping at plant and cellular level"**

superoxide dismutase (SOD) isoenzymes were identified. It was found that first internode length correlated with soil depth. Both control and GA<sub>3</sub>-treated plants that were sown deeper had longer shoots and first internodes. GA<sub>3</sub> stimulated the elongation of the shoots and the first internodes. The shoot length of control plants increased with increase in sowing depth. On the other hand, the shoot length of GA<sub>3</sub>-treated plants was not affected by soil depth. H<sub>2</sub>O<sub>2</sub> content in the first internodes of control plants did not change based on soil depth. On the contrary, H<sub>2</sub>O<sub>2</sub> level was slightly lower in the first internodes of GA<sub>3</sub> treated plants that emerged from deeper. TBARS levels were relatively low in all groups and did not differ with respect to neither soil depth nor GA<sub>3</sub> application. Soil depth and GA<sub>3</sub> application did not influence POX activity in the first internode. On the other hand, in control plants a negative correlation was determined between CAT activity and soil depth. GR activity of the control plants showed almost no difference regarding to sowing depth. To our knowledge this is the very first study that shed light on the first internode elongation in terms of ROS levels and activities of antioxidant enzymes.

## **Phenotyping of disease resistance, phenology and nutrient use efficiency of a wide barley germplasm adapted to high latitudes**

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Barley (*Hordeum vulgare* L.) is the most important crop at high latitude cropping systems. Diseases represent a serious threat for barley production as they cause yield losses and high yield variability associating with inefficient use of nitrogen (N). Such challenges are projected to increase in the future due to climate change. To cope with future risks of increased use of fungicides and N fertilizers in an environmentally and economically feasible way, improved resistance against barley diseases and enhanced nutrient use efficiency (NUE) are needed. The resistance of wide barley germplasm against barley net blotch, spot blotch and scald was phenotyped as single plants and populations both in the field and greenhouse. Furthermore, we screened nitrogen use efficiency and phenology traits at single plant and plant stand level in the field with two N rates. We found that Nordic landraces may carry resistance that has so far been overlooked as a potential resistance source in barley breeding programs. In general, current cultivars exhibited higher disease resistance, slightly later maturity, and better N uptake efficiency, but not as clearly N utilization efficiency, than their predecessors or landraces. Combining both of these NUE traits in the future breeds may, however, provide the means to further improve NUE. Disease resistance and improved NUE would be essential characteristics that reduce the environmental footprint of widely grown barley crop at high latitudes.

## **The quest for drought tolerant varieties: Phenotyping the Musa Biodiversity with simple phenomic tools.**

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KULeuven is responsible for the management and safe storage of the Bioversity International collection of banana (*Musa* spp). Our mandate is to secure the crop's gene pool and encourage its use. The latter, however, requires an in-depth knowledge of the variability among the accessions. The crop has its origins in the humid tropics and yield loss increases dramatically as drought intensifies. Circumstantial evidence suggests that the *Musa balbisiana* (B) genome confers greater drought tolerance to bananas and plantains than the *Musa acuminata* (A) genome. Hence the genetic makeup may affect the stress response. In attendance to have access to the recently established high throughput phenotyping platform Phenovision in Ghent (VIB), we developed a method to phenotype a selected set of *Musa* varieties belonging to different genomic groups. We apply different lab models (phytotron, greenhouse) to monitor off-line and on-line phenotypic plant variables in response to a changing water availability. Using a multivariate analysis (PCA/PLS), we were able to classify the different variables according to their contribution in explaining the observed variance between the genotypes and generated a classification of the genotypes to objectively assess the tolerance level.

## **Integrating controlled and field phenotyping**

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One of the central topics of modern phenotyping is the translation of phenotypic characterization from controlled and semi-controlled conditions in growth chambers and glasshouses to field conditions. The presentation will propose the simultaneous need for both approaches and will discuss options to link controlled and field approaches. Differences in targets of mechanistic, high-throughput and field phenotyping will be developed, options and limitations to link the different scales as well as concepts of emerging properties at stand level (canopy and rootopy) as well as proxy concepts will be discussed. The talk will include recent studies and models designed for translational research between lab and field within the institute as well as in various phenotyping networks.

## **Functional analysis of the selected phosphoprotein candidates identified in tobacco male gametophyte**

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Angiosperm mature pollen represents an extremely desiccated structure surrounded by a tough cell wall. On the other hand, the rehydrated pollen starts a rapid pollen tube growth. Such changes from quiescent to the rapidly growing structure are accompanied by changes in protein phosphorylation. Recently, we have published the phosphoproteomics data set of tobacco mature pollen and 30-min activated pollen. There were presented 139 phosphoprotein candidates with 52 unambiguous phosphorylation sites. Several of them were selected for subsequent functional studies.

Since tobacco has its limitations in genome annotation and since *Arabidopsis thaliana* offers a collection of T-DNA insertion lines, we selected some of the phosphoprotein candidates and found their Arabidopsis homologues. The seeds of the T-DNA insertion lines were cultivated and the phenotype of the whole plant as well as the phenotype of the pollen grains was observed. The candidates showing a phenotype will be subjected to subsequent experiments including complementation analysis, subcellular localization of the protein, and also Phos-Tag SDS-PAGE separating the forms according to their degree of phosphorylation.

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## **Imaging and isotopes: applications for phenotyping diverse species under optimal and sub-optimal conditions**

**Abstract submission for 1st meeting or work group 1 phenotyping at the plant level**

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Imaging of plants in different wavelengths is an effective means of obtaining information about both their morphology and physiology. It allows assessment of spatial variability, which can be exploited for better understanding of variation within or between leaves, or for rapid assessment over large numbers of individuals. Isotope composition analysis is also useful for assessment of certain physiological variables, and has the advantage of integrating information over time. Imaging (digital, thermal, and fluorescence) and isotope (carbon, oxygen, and nitrogen) composition analysis have been used in a range of experiments with horticultural, forage, and forestry species, as well as a model species (tobacco) under both optimal growing conditions and stress. These techniques have shown variation between genotypes in stomatal closure in response to drought, in photochemical efficiency in response to cold temperatures, in growth rate and persistency, and in transpiration and water use efficiency. Such variation is being used in selection of optimal genotypes for specific conditions and for enhanced understanding of the genetic determinants of plant phenotypes in specific environments. Combining imaging and isotope analysis holds potential for phenotyping at high resolution – both spatially and temporally.

## **HIGH-THROUGHPUT PHENOTYPING OF A MAIZE INTROGRESSION LIBRARY REVEALS DROUGHT-RESPONSIVE QTLs FOR PLANT GROWTH**

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Keywords: maize, drought tolerance, water use efficiency, high-throughput phenotyping, QTL

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A maize introgression library of 73 lines derived from the cross between Gaspé Flint (an early line) and B73 used as donor and recurrent genotypes, respectively (Salvi et al. 2011; BMC Plant Biology, 11:4), was tested in the high-throughput phenotyping platform PhenoArch (INRA, Montpellier), which automatically images and measures evapotranspiration of up to 1,860 plants. The lines were grown in pots with eight replicates (one plant/replicate) under well-watered and water-limited conditions at soil water potentials of -0.1 and -0.4 MPa, respectively. Biomass accumulation and leaf area expansion were estimated from 13 images per plant every second day, together with leaf appearance rate and transpiration. The final fresh biomass was also measured. Preliminary results show both positive and negative effects of the introgressed Gaspé chromosomal fragments on the B73 background. The analysis of the results will allow us to test: a) how previously characterized loci known to constitutively regulate vegetative growth affect growth plasticity under different water regimes; b) for the presence of (i) growth-related genes/QTLs previously identified by means of meta-QTL analysis and (ii) novel loci affecting the plasticity of vegetative growth under water-limited conditions.

## **Selection of blueberries genotypes tolerant for variation of climatic conditions**

**Kristina Ložienė, Giedrė Abrutienė, Juozas Labokas**

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Blueberry (*Vaccinium corymbosum*) fruits are significant sources of phenolic compounds in the human diet because these biologically active compounds have high antioxidative effect. Thus blueberries have become popular with consumers and are commercially produced as important crop over a wide geographic range both in the United States and Europe. On purpose to meet the requirements of consumers the more attention is giving to fruits production, however the quality (i. e. amount of phenolics) of fruits is very important too. Although the phenolic amount in genotypes of blueberries is affected by genetic differences, the seasonal variations of climatic factors also influence on variation of their quantity. Therefore the selection of genotypes, which synthesize the stable amounts of phenolics even under not favourable climatic conditions, is important.

The monitoring of total phenolic content (TPC) of seven blueberry genotypes growing in open ground of field collection (Institute of Botany, Nature Research Centre, Vilnius, Lithuania) continued three year. The influence of natural climatic factors (precipitation, temperature and photosynthetically active solar radiation) on TPC was investigated on purpose to select genotypes in which the variation of TPC would be least. A one way analysis of variance showed, that variation in TPC between growing seasons was significant ( $p < 0.05$ ) in many investigated genotypes, excluding cultivars 'Northblue' ( $F = 4.9$ ,  $p = 0.06$ ) and 'Gretha' ( $F = 3.3$ ,  $p = 0.1$ ).

## **Dynamic or static light and the influence on physiological phenotyping**

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Phenotyping often takes place under standard conditions in protected cultivation where most factors are maintained stable. The influence of individual abiotic parameter is often studied under tightly controlled conditions in growth chambers and greenhouses where stress is avoided. Plants usually encounter multiple (mild) stress conditions related to temperature, water and light leading to priming and natural acclimation, so what happens if the plants are exposed to a dynamic light reaction rather than a constant exposure. Two varieties of leafy lettuce (*Lactuca sativa* 'Batavia' and 'Lollo Rossa') were growth in a fully controlled climate chamber with constant temperature (21 +/- 0.5) and humidity (60 +/- 5) and a fixed Daily Light Integral of 22 mol m<sup>-1</sup> day<sup>-1</sup>. In one treatment the PFFD was maintained at 450 μmol m<sup>-2</sup> s<sup>-1</sup>, while the other varied from 0 to 800 μmol m<sup>-2</sup> s<sup>-1</sup>.

To evaluate the effects of two classic phenotyping parameters –net photosynthesis and several parameters of chlorophyll fluorescence we performed continuous measurements on fully developed leaves over approximately two weeks. We used two CIRAS-2 cuvettes and six Moni-PAM heads in the two treatments. Samples were harvested twice for fresh/dry weights of both varieties but only the reddish 'Lollo Rossa' was used for photosynthetic measurements.

There were no differences in the dry weight responses while there was some variation in net photosynthesis during the day and some fluorescence parameters differed between the treatments and time of days.

The results will be discussed and the potential effect of the measuring protocols in phenotyping evaluated.

## **Phenotyping of Nordic wild berry species**

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Wild berries have always been an essential part of Nordic culture and cuisine. The most harvested and also most commercial significant species are bilberry (European blueberry – *Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), cloudberry (*Rubus chamaemorus*), raspberry (*Rubus idaeus*) and cranberries (*Oxycoccus palustris* and *Oxycoccus microcarpus*). The wild berries are perfectly adapted to their habitats and represent a valuable genetic resource as an input of new traits/genetic variance to existing commercial berry varieties or to be exploited further as wild or cultivated resources. In addition, the wild berries, with all their genetic diversity, are excellent material for studies on genotype and environmental effects and interactions on traits like berry quality and plant performance. We have been phenotyping bilberry and cloudberry in their natural habitats, in test plots using clonal material and in experiments under controlled climatic conditions in a phytotrone. Traditional characters as flowering time, number of flowers, number of shoots, branching, height and berry weight have been recorded over years on clonal collections. These results have been combined with berry quality data obtained from metabolomics performed on berries produced under different climatic conditions (different light quality and quantity and temperatures). In addition, we have started sequencing of the cloudberry genome using the released female varieties 'Fjellgull' and 'Fjordgull' and F1 hybrids from crosses between 'Fjellgull' and the male variety 'Apollen' and a hermaphroditic variety 'Nyby'. With this we hope to determine the polyploidization mode in the octoploid cloudberry and check for collinearity of orthologous markers to raspberry (*R. idaeus*) and other *Roseaceous* genomes. Knowing whether cloudberry has an allo- or autopolyploid nature will be useful for breeding of cloudberry. Comparative genomics across the *Rubus* genome will be useful for breeding also for commercially more important crops such as raspberries.

## **Is it a nutrient or is it a pest? High-throughput phenotyping can distinguish them best.**

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Oftentimes forestry and agricultural producers face the dilemma of deciding if a given crop is lacking a specific nutrient or if is infected by a particular pest. E.g., iron deficiency chlorosis (IDC), which induces leaf chlorosis and stunting, can easily be confounded with drought stress or nematode attack. How can we screen in the laboratory for varieties which are well adapted to different stresses, and be sure that they will behave the same way in the field? Also, how can scientists in the field screen for these problems and implement best practices? Visual identification of plant diseases is a challenging task that requires laborious techniques and that oftentimes results in frustrating and wrongful assessments. In order to best define agricultural practices, including prevention, management and treatment, it is imperative to have fast, reliable and proper diagnostic methods. In this presentation, and using IDC as a model, the **laboratorial** phenotyping techniques which can be used to identify resistant genotypes will be presented (ionomics, transcriptomics and genomics, but also "anatomics"). Next, the most state of the art **field**-based technologies including satellites (SENTINEL2), drones, Loop Mediated Isothermal Amplification (LAMP), *Volatile organic compounds (VOCs)* and portable X-ray CTs, will also be presented. The presentation will conclude with the integration of these two types of data (lab and field) for optimal decision making and varietal selection.

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## **Major gaps in screening methodologies for parasitic weed resistance at the lab and field level**

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A number of parasitic plants have become weeds, posing severe constraints to major crops. Breeding for resistance is acknowledged as the major component of an integrated control strategy. However, resistance against most parasitic weeds is difficult to access. This has made selection more difficult and has slowed down the breeding process (Rubiales et al. 2006, 2009, 2014). Proper phenotyping remains today as a major bottleneck for parasitic weed resistance breeding.

Resistance is a multicomponent event, being the result of a battery of escape factors or resistance mechanisms acting at different levels of the infection process. Understanding these will help to detect existing genetic diversity for mechanisms that hamper infection and to combine different mechanisms into a single cultivar will provide durable resistance in the field. Here we will summarize and critically review current screening methods and existing gaps that could be filled with adoption of novel phenotyping methodologies.

Rubiales et al., 2006. Screening techniques and sources of resistance against parasitic weeds in grain legumes. *Euphytica* 147:187-199.

Rubiales et al., 2009. Breeding approaches for crenate broomrape (*Orobanche crenata* Forsk.) management in pea (*Pisum sativum* L.). *Pest Manag. Sci.* 65:553-559.

Rubiales et al., 2014. Identification and multi-environment validation of resistance against broomrapes (*Orobanche crenata* and *Orobanche foetida*) in faba bean (*Vicia faba*). *Field Crops Res.* 166:58-65

## **Hansa Trace - A high throughput imaging auxanometer for roots and hypocotyls of Arabidopsis**

**Fraas, S., Niehoff, V. and Lüthen, H**

In order to identify auxin response mutants at high throughput we developed an imaging-based system for recording growth responses at high time resolution. The HansaTrace system (published APR 2014\*) utilizes consumer grade flat-bed scanners as key-hardware-components. They are being used to scan primary roots of Arabidopsis seedlings grow on low-nutrient or excised hypocotyl segments. Scanning up to 120 roots simultaneously, in 4 petri-dishes per scanner, the system produces enough growth phenotypic data to potentially characterize a growth mutant within days. We developed ImageJ plugins for an automatic analysis of the scanner-images. , one for cropping the images, one for thresholding, skeletonizing and measuring the root/hypocotyl lengths (HansaTrace). We feel that this platform can be used for dissecting auxin related signaling chains and to identify other mechanisms involved in plant growth regulation.

\*Fraas, S., Niehoff, V. and Lüthen, H. (2014), A high-throughput imaging auxanometer for roots and hypocotyls of *Arabidopsis* using a 2D skeletonizing algorithm. *Physiologia Plantarum*, 151: 112–118. doi: 10.1111/ppl.12183

## **DsRNA application for plant virus control and endogenous plant gene expression regulation. A new functional genomics tool?**

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The well-established method of pathogen-derived resistance by employing transgenic plants expressing viral encoded genetic material, as well as cross protection using attenuated strains, have been shown to be effective approaches for virus control. However, these methods are not permitted, as yet, for the agricultural practice in EU. In both cases the molecular mechanism of resistance is based on RNA silencing (RNA interference, RNAi). RNAi is induced by dsRNA molecules and the protective effect of the exogenous application of dsRNA has been shown<sup>1,2</sup>. For example, dsRNA molecules, produced *in vitro* with commercially available reagents or *in vivo* in suitable bacterial cells<sup>3</sup>, derived from the *Cucumber mosaic virus* (CMV) capsid protein encoding gene (dsRNA-CMV-CP) or the CMV silencing suppressor 2b (dsRNA-CMV-2b) applied onto plants rendered plants resistant in high percentages. In particular, protection against CMV reached 35 to 55% or up to 75% in tobacco plants upon treatment with *in vitro* or *in vivo* produced dsRNA-CMV-2b, respectively). When dsRNA-CMV-2b was combined with dsRNA-CMV-CP, both produced *in vivo* and applied exogenously, protection reached 85%. This non-transgenic approach provides an alternative to plant genetic engineering control strategy that could be applied to any cultivar of choice and is compatible with high-throughput technological means for plant handling. The use of two sets of siRNAs that correspond to CMV sequences aggravated the symptoms exhibited by CMV in tobacco. The exploitation of this methodology for local RNAi in plants could be foreseen as a means of a functional genomics tool.

<sup>1</sup> Holeva MC, Sclavounos AP, Kyriakopoulou PE, Voloudakis AE (2006) *In vitro* or *in vivo* produced dsRNA induces tobacco and tomato resistance to Cucumber mosaic virus (CMV). 13th Hellenic Phytopathological Congress of the Greek Phytopathological Society, 15-19 October 2006, Athens.

<sup>2</sup> Holeva MC, Sclavounos, AP, Kyriakopoulou PE, Voloudakis AE (2007) External application of dsRNA of the capsid protein (CP) or 2b gene of CMV reduces the severity of CMV-infection in tobacco. XIII IS-MPMI Congress, 21-27 July 2007, Sorrento, Italy.

<sup>3</sup> Voloudakis A, Holeva M, Bamford D, Poranen M, Sarin P, Vargas M, Tenllado F (2014) Efficient Double-Stranded RNA Production Methods for Utilization in Plant Virus Control. *Methods in Molecular Biology* (in press).

# POSTERS

## **Phenotypic characterization of a banana field trial in Arusha (Tanzania): from lab models to the field.**

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**Theme:** Phenotyping at the plant level

Bananas and plantains (*Musa* spp.) are among the most important crops in the world in terms of production and exportation, and a major staple food for hundreds of millions of people in developing countries. However, it needs vast amounts of water and yield is significantly affected by drought. Unfortunately, little is known about the physiology of drought stress response in banana and the range of tolerance/susceptibility of different accessions. We aim at validating the results of earlier phenotypic characterizations in the lab and screen house by studying the phenotypic response under drought field conditions. Thus, an experimental field trial has been established in IITA-Arusha, where a tropical climate including two rainy seasons (from November to January and from March to May) and one dry season (from June to October) is suitable for banana growth and evaluation of drought stress response. We are characterizing the level of tolerance/susceptibility of 4 accessions, 2 presumed drought tolerant (ABB Cachaco; AA Pahang) and 2 drought sensitive (AAAh Nakitengwa; AA Guyod), which have been set in a Complete Randomized Block Design with 10 replicates per accession and 2 treatments (irrigated and not irrigated). Physiological parameters (e.g. leaf temperature measured with Infra-Red camera) and growth/yield-related parameters (e.g. earliness, plant height/girth at flowering or bunch weight and number of hands/fingers at harvest) will be evaluated during 2 growth cycles. In addition, root and leave tissues will be collected to test the expression level of a set of candidate genes responding to drought stress. Results will be compared to those obtained under laboratory/greenhouse drought-induced conditions at KU Leuven.

## **Chlorophyll fluorescence – measuring principles and protocols**

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Chlorophyll fluorescence (CF) is a versatile non-invasive method to monitor a variety of plant stresses. The simplest fluorescence parameter to measure are the quantum yield of PSII,  $\Phi_{\text{PSII}} = F_q'/F_m'$ , and electron transport (ETR). It only requires a saturating light pulse to measure in light. The most used parameter, though, is probably  $F_v/F_m$ , which estimates the maximum photochemical efficiency of PSII. It requires 20-30 minutes dark-adaptation of the leaf and can monitor stress created by e.g. light, low or high temperature, or nutrient deficiencies. If the leaf is dark-adapted first,  $\Phi_{\text{PSII}} = F_q'/F_m'$  measured in light can be complemented with a full quenching analysis where the non-photochemical quenching (NPQ) and the redox state of PSII ( $q_L$ ) are part the energy balance of PSII, all affected by stress.

The use of CF in high-throughput phenotyping by fluorescence cameras is limited by the technical difficulties to apply and detect a saturating pulse from a 3D plant and it will only give a snapshot of photosynthesis. Monitoring-PAM is a new tool for medium-throughput phenotyping, where CF can be measured on up to seven leaves simultaneously. It can follow the development of stress *in situ* and can be used to design measuring protocols for high-throughput phenotyping.

Even though CF is a non-invasive method the saturating pulses do affect photosynthesis and may contribute to down-regulation of PSII through increased NPQ. Therefore, the measuring protocol should be designed with care.

Examples will be shown on how several species respond to naturally fluctuating light and high temperature, and how different CF sampling frequencies affect the results. Both have implications for how high-throughput phenotyping by CF should be designed.

## **High throughput plant screening at the Campus Vienna Biocenter: Plant Sciences Facility (CSF GmbH)**

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The Campus Science Support Facilities GmbH (CSF) is a public funded non-profit research institute, situated at the Vienna Biocenter Campus (VBC). We offer access to state of the art research infrastructure and scientific services. CSF is organized in ten scientific core facilities equipped with cutting edge instruments and highly skilled technical and scientific personnel.

The CSF Plant Sciences Facility (PlantS) operates 22 high quality, highly specialized and state-of-the-art plant growth chambers and provides professional support to the outstanding "green research" at the VBC. Several chambers are capable of providing exceptional environmental conditions i.e. low temperature (frost), high temperature, different light intensities, different light spectra (LED: UV<sub>375</sub>, blue<sub>405</sub>, blue<sub>450</sub>, white and red<sub>660,730</sub>) and different gas conditions (e.g. CO<sub>2</sub>) allowing precise environmental simulation across different climate zones and the simulation of various environmental stress conditions.

For the objective, reproducible and high-throughput assessment of plant phenotypic traits we operate an automated, chamber-integrated (sensor-to-plant) screening system linked to LemnaTec GmbH image analysis software. Implementation of new sensors and increase of the phenotyping capacity are planned for the near future. In parallel and close collaboration with the CSF pcIMAG Facility we develop plant phenotyping methods based on a state-of-the-art 15.2 tesla MRI.

Moreover, PlantS focuses on non-GMP production of plant-derived proteins (molecular pharming) and small-scale protein production for basic research.

## **Dynamics of photosynthesis-related parameters of oak (*Quercus* spp.) and sweet chestnut (*C. sativa* Mill.) seedlings in the course of infection with three *Phytophthora* species**

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### **Abstract**

The oaks (*Quercus* spp.) are considered to be a key forest-forming species within the low forest vegetation zone in Bulgaria, covering about 35 % of the area occupied by broad-leaved forests (Lyubenova et al., 2009). The sweet chestnut (*C. sativa* Mill.) is narrowly distributed in the country and could be found in isolated localities, in most of them heavily suffering from the fungal pathogen *Cryphonectria parasitica* Murrill (Risteski et al., 2014). Three-month old container-grown seedlings from Turkey oak (*Q. cerris* L.), red oak (*Q. rubra* L.) and sweet chestnut (*C. sativa* Mill.) were infected with three different *Phytophthora* species, (*P. cactorum* (Lebert & Cohn) J. Schröt., *P. plurivora* T. Jung and T.I. Burgess and *P. quercina* T. Jung and T.I. Burgess), by adding 100 ml suspension obtained after 10 days cultivation of *Phytophthora* on oatmeal medium at 25 °C. Dynamics of net CO<sub>2</sub> assimilation, transpiration and stomatal conductance of young leaves were followed every odd week for two-month period of time. The gas-exchange measurements were performed by use of infrared gas analyzer Li 6400. Changes in the studied parameters were observed on inoculation of seedlings with *Phytophthora* spp. mycelium suspension. While sufficient decrease in photosynthetic activity occurred after 7 weeks, sharp reduction in both stomatal conductance and transpiration were registered one week after start of the experiment. Further on the values of both parameters in *Q. cerris* seedlings followed pronouncedly different pattern as compared to those in the other species regardless of the infection agent specificity, which might be a sign of a higher tolerance towards *Phytophthora* spp. infection.

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## **ASSOCIATION MAPPING FOR ROOT TRAITS IN WHEAT SEEDLINGS AS RELATED TO AGRONOMIC PERFORMANCE**

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Keywords: durum wheat, roots, yield, association mapping, QTL, drought tolerance

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In this study, seedlings of 183 durum wheat elite accessions were phenotyped in order to identify QTLs for root system architecture (RSA); the QTLs identified were compared with QTLs detected for grain yield and its component traits, plant height and peduncle length measured in a previous study where the same accessions were evaluated in 15 field trials with a broad range of soil-moisture availability and productivity (Maccaferri et al., 2011, JXB, 62:409-438). The following RSA traits were phenotyped as described in Sanguineti et al. (2007, AAB 151: 29-305) for seminal root angle, primary root length, total root length, average root length, root number, and shoot length. Highly significant differences among accessions were detected for all traits. The highest heritability ( $h^2 = 0.72$ ) was observed for seminal root angle. Out of the 48 QTLs detected for RSA, 15 overlapped with QTLs for agronomic traits and/or grain yield in two or more environments. The congruency of the effects of RSA traits and agronomic traits was evaluated. Seminal root angle appears the most promising trait for further studies on the adaptive role of RSA plasticity on field performance in environments differing for water availability. Our results provide novel insights on the genetic control of RSA and its implications on field performance of durum wheat.

## **Species-specific stomatal responses to two simultaneously applied conflicting abiotic factors**

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Plant stomatal responses to single environmental factors are well studied; in nature, however, guard cells have to integrate multiple, often conflicting signals and ensure the most appropriate response: stomatal opening or closure. We studied the stomatal responses to a simultaneous application of conflicting abiotic factors in six evolutionarily distant mono- and dicotyledonous herbs using custom-made gas exchange devices. We aimed to clarify whether the crosstalk between opening- and closure-inducing pathways leading to stomatal response is universal or species-specific.

The studied species responded similarly to changes in single environmental factors, but showed species-specific responses to two simultaneously applied conflicting factors. The cereal stomata tended to close if any of the closing factors (elevated CO<sub>2</sub> concentration, low air humidity, darkness) was present, whereas the stomata of *Arabidopsis* always opened in two-factor combinations. This indicates that the principal mechanisms to process environmental signals are probably conserved in angiosperms, whereas a species-specificity of stomatal regulation exists in more complex situations. Next, we were interested in whether previous growth conditions can fine-tune stomatal regulation pathways and whether these species-specific stomatal response patterns differ between well-watered and drought-grown plants of the same species.

## **Characterisation of *Pinus nigra* Arn. embryogenic tissues cultured in liquid media**

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Embryogenic suspension cultures of *Pinus nigra* have been established by resuspending of a defined amount of tissues growing on solid media and growth parameters, maturation capacity as well as structural aspects of somatic embryos have been investigated. The initial weight of tissue using for establishment seems to be an important factor for successful proliferation and the optimum inoculum weight was defined as 1-2.5g. The growth of tissues was evaluated as sedimented cell volume (SCV) and showed strong dependence on the cell line. The structural features of somatic embryos were very similar as in tissues growing on solid media. Bipolar somatic embryos of different developmental stages have been observed at any given time. The maturation of somatic embryos in liquid medium was limited to the precotyledonary developmental stage. For this reason the maturation has been tested using semi solid media of the same composition as the liquid one. This approach resulted in development of somatic embryos to the cotyledonary stage and after germination complete plantlets (somatic seedlings) have been obtained. The experiments have shown somatic embryos of *Pinus nigra* were able to grow in liquid media without the loss of structural features, contrary their development and regeneration into plantlets required semi solid media.

The work was supported by Slovak Academy of Sciences as MVTs COST 1306.

## **Screening pea (*Pisum sativum*) for aphid (*Acyrtosiphon pisum*) and weevil (*Bruchus pisorum*) resistance under field and under controlled conditions**

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Pea aphid (*Acyrtosiphon pisum*) and pea weevil (*Bruchus pisorum*) are serious insect pests affecting pea production worldwide. Little resistance has been reported to both insects. We started three years ago field screenings that allowed selection of promising accessions of *Pisum* spp. Results will be presented and discussed.

The more resistant accessions are currently being further studied under controlled conditions in dual-choice and no-choice tests to measure levels of antixenosis and/or antibiosis. Available screening methods and possibilities for adoption of novel phenotyping methodologies will be discussed.