Plant Phenotyping Symposium
Next generation plant phenotyping for trait discovery, breeding and beyond: transnational access to European platforms

Barcelona, 11–12 November 2015
Organizers

Supported by:

Grant Number: 031A053A/B/C
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Ulrich Schurr, Forschungszentrum Jülich
Jose Luis Araus, University of Barcelona
Roland Pieruschka, Forschungszentrum Jülich
## Welcome by:

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<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution and Location</th>
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<tr>
<td>9:00–9:10</td>
<td>Dr. Jordi Alberch, Deputy President for Research, University of Barcelona, Spain</td>
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<td>Dr. Teresa Sauras, Vice Dean for International Cooperation of the Faculty of Biology, University of Barcelona, Spain</td>
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<td>Jose Luis Araus, University of Barcelona, Spain</td>
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<td>Uli Schurr, Forschungszentrum Jülich, Germany</td>
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<td>9:20–9:40</td>
<td>Roland Pieruschka, Forschungszentrum Jülich, Germany</td>
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### Abiotic Stress Part 1 – Chair: Imre Vass

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<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution and Location</th>
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<tr>
<td>9:40–10:05</td>
<td>Roberto Tuberosa, University Bologna, Italy</td>
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<td></td>
<td>A maize introgression library reveals ample genetic variability for root architecture, water use efficiency and grain yield under different water regimes</td>
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<td>10:05–10:30</td>
<td>Anetta Kuczyńska, IGR, Poznan, Poland</td>
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<td></td>
<td>Developmental aspects of barley drought tolerance</td>
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<td>10:30–10:45</td>
<td>Elisabeth Becker, Technische Universität München, Munich, Germany</td>
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<td>High-throughput phenotyping of hybrid wheat under drought and heat stress</td>
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<td>10:45–11:00</td>
<td>Rut Sanchez, University of Barcelona, Spain</td>
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<td>Comparative performance of δ^{13}C, δ^{18}O and δ^{2}H for phenotyping durum wheat adaptation to different water and nitrogen conditions</td>
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<td>11:00–11:30</td>
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### Abiotic Stress Part 2 – Chair: Fabio Fiorani

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<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution and Location</th>
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<tr>
<td>11:30–11:55</td>
<td>Josh Klein, Volcani Center, Bet Dagan, Israel</td>
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<td></td>
<td>Destructive and non-destructive quantification of drought resistance in isogenic tall and dwarf wheat</td>
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<td>11:55–12:20</td>
<td>Ernesto Igartua, CSIC, Zaragoza, Spain</td>
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<td>Early growth of barley cultivars and landraces in rhizotrons</td>
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<td>12:20–12:45</td>
<td>Gernot Bodner, BOKU, Vienna, Austria</td>
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<td>Whole plant vs. functional water saving: Different drought response of modern cultivars and landraces of durum wheat</td>
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<td>12:45–13:00</td>
<td>Karin Köhl, Max Planck Institute of Molecular Plant Physiology, Germany</td>
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<td>Identification of drought tolerance markers in potato</td>
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<td>13:00–13:15</td>
<td>Fred Baret, CAPTE, INRA-EMMAH, Avignon, France</td>
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<td>Leaf rolling in maize crops: Contrasting behavior among cultivars evidenced from ground and UAV observations</td>
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<td>13:15–14:15</td>
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<td>14:15 – 15:45</td>
<td>Poster Session</td>
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<tr>
<td>15:45 – 16:30</td>
<td>Key Lecture</td>
<td>Jose Luis Araus, University of Barcelona, Spain</td>
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<tr>
<td>16:30 – 17:00</td>
<td>Coffee break</td>
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<tr>
<td>17:00 – 17:25</td>
<td>Abiotic Stress Part 3 – Chair: John Doonan</td>
<td>Ankica Kondić-Špika, Institute of Field and Vegetable Crops, Novi Sad, Serbia</td>
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<td>17:25 – 17:50</td>
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<td>Roberto Papa, Università Politecnica delle Marche, Ancona, Italy</td>
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<td>17:50 – 18:15</td>
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<td>Michael Dingkuhn, IRRI, Manila, Philippines / CIRAD, Montpellier, France</td>
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<td>18:15 – 18:30</td>
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<td>Pervez Zaidi, Global Maize Program, CIMMYT-Asia, India</td>
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<td>18:30 – 18:45</td>
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<td>Sundeep Kumar, Division of Genomic Resources, ICAR-NBPGR, India</td>
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<td>20:30</td>
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<td>Conference Dinner</td>
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</tbody>
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### Standards in Phenotyping – Chair: Rick van de Zedde

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
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<tbody>
<tr>
<td>9:00 – 9:25</td>
<td>Francois Tardieu, INRA Montpellier, France</td>
<td>Combining information from multiple experiments in platforms and field</td>
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<tr>
<td>9:25 – 9:50</td>
<td>Björn Usadel, RWTH Aachen, Germany</td>
<td>Caring about standards? Does phenotyping need this?</td>
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<tr>
<td>9:50 – 10:15</td>
<td>Georgios Gkoutos, Aberystwyth University, United Kingdom</td>
<td>Exploring the phenome</td>
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<tr>
<td>10:15 – 10:30</td>
<td>Stijn Dhondt, VIB Ghent, Belgium</td>
<td>Plant phenotyping results at your fingertips: Design, manage, analyse, and visualize with PIPPA.</td>
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<td>10:30 – 11:00</td>
<td>Coffee break</td>
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### Root phenotyping Chair: Malcolm Bennett

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<tr>
<th>Time</th>
<th>Speaker</th>
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<tr>
<td>11:00 – 11:25</td>
<td>Michelle Watt, Forschungszentrum Jülich, Germany</td>
<td>Deeper root systems during grain development for future farming systems: Selection by combining field and laboratory phenotyping</td>
</tr>
<tr>
<td>11:25 – 11:50</td>
<td>Boris Rewald, BOKU, Vienna, Austria</td>
<td>Random forest models and Support Vector Machine analysis enhance trait selection and cultivar differentiation of mature pea root systems</td>
</tr>
<tr>
<td>11:50 – 12:15</td>
<td>Sixtine Passot, IRD Montpellier, France</td>
<td>Exploring root development and architecture in one of the most heat and drought tolerant cereals, pearl millet</td>
</tr>
<tr>
<td>12:15 – 12:30</td>
<td>Stefan Gerth, Fraunhofer Institute, Fürth Germany</td>
<td>X-ray CT for growth tracking of belowground organs and root systems</td>
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<tr>
<td>12:30 – 12:45</td>
<td>Alain Audebert, CIRAD Montpellier, France</td>
<td>Fine phenotyping of root and shoot dynamics on drought conditions in rice</td>
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<tr>
<td>12:30 – 13:45</td>
<td>Lunch</td>
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### Key lecture

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<th>Time</th>
<th>Speaker</th>
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<tr>
<td>13:45 – 14:30</td>
<td>Richard Richards</td>
<td>Phenotyping – How can we value add for plant breeders?</td>
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<td>14:30 – 14:55</td>
<td>Jaakko Kangasjärvi, University of Helsinki, Finland</td>
<td>Large-scale genetic screens with O3 to reveal components involved in ROS signaling</td>
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<tr>
<td>14:55 – 15:20</td>
<td>Konstantinos Vlachonasios, Aristotle University of Thessaloniki, Greece</td>
<td>Phenomics analysis of <em>Arabidopsis thaliana</em> chromatin remodeling mutants during drought stress</td>
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<tr>
<td>15:20 – 15:45</td>
<td>Kris Vissenberg, University of Antwerp, Belgium</td>
<td>Identifying novel regulators of the auxin dependent gravitropic response in <em>Arabidopsis thaliana</em> roots using RootTrace and a modified vertical confocal</td>
</tr>
<tr>
<td>15:45 – 16:00</td>
<td>Brian Forde, Lancaster University, UK</td>
<td>The Microphenotron: A new high-throughput platform for chemical biology applications</td>
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<td>16:00 – 16:30</td>
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<td>Coffee break</td>
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<tr>
<td>16:30 – 16:50</td>
<td>Uli Schurr, Forschungszentrum Jülich, Germany</td>
<td>EMPHASIS – European Multi-environment Plant pHenetyping And Simulation InfraStructure</td>
</tr>
<tr>
<td>16:50 – 17:10</td>
<td>Gemma Molero, CIMMYT, Mexico</td>
<td>Increasing yield potential and improving crop adaptation to climate change: Strategies and genetic gains</td>
</tr>
<tr>
<td>17:10 – 17:30</td>
<td>Peter Wenzl, Global Crop Diversity Trust, Bonn, Germany</td>
<td>Diversity Seek (DivSeek): An international partnership to harness the genetic potential of crop diversity</td>
</tr>
<tr>
<td>17:50 – 18:05</td>
<td>Tapio Palva, University of Helsinki, Finland</td>
<td>The Finnish Plant Phenotyping Infrastructure – The road to high throughput and high precision</td>
</tr>
<tr>
<td>18:20</td>
<td></td>
<td>Conclusion</td>
</tr>
</tbody>
</table>
Abstracts:
Oral Presentations
Field phenotyping: Affordable alternatives and novel avenues

Jose L. Araus¹, Shawn C. Kefauver², Omar Vergara¹, Susan Medina¹, Rut Sanchez-Bragado¹, Maria D. Serret¹, Jordi Bort¹, Nieto-Taladriz Maria T², Aparicio Nieves³, Marín Natalia³

¹ Department of Vegetal Biology, University of Barcelona, Spain
² National Institute for Agricultural and Food Research and Technology, Madrid, Spain
³ Instituto Tecnológico Agrario de Castilla y León, Spain

Contact: jaraus@ub.edu

Whereas efficient field phenotyping is a keystone for crop breeding, the cost of this technology may represent a bottleneck our ability to fully exploit these avenues. Such limitation is particularly evident for small to medium seed companies and national agricultural research institutions from developing countries, where a lack of resources limits the adoption of new technologies that are often very fashionable but expensive. Given their versatility, remote sensing techniques at the canopy level have become valuable tools for high throughput phenotyping. Among these approaches, conventional 2-D digital RGB imaging represents an affordable and flexible, alternative. Examples will be given on the use of RGB to assess genotypic resilience to diverse abiotic and biotic stresses compared to multispectral approaches. More frontier applications, such as the use of RGB images for the automatic counting of agronomical yield components, the recognition of foliar diseases, or the evaluation of plant height and lodging, will be discussed. The last part of the presentation will be devoted to introducing novel traits, particularly those derived from laboratory analyses of stable isotopes. Within this category we may include new fractions (e.g. plant water and water soluble fractions), applications (e.g. assessment of spike photosynthesis, root deep), and completely novel (δ²H) stable isotopes.

Phenotyping – How can we value add for plant breeders?

Richard Richards¹

¹ CSIRO Agriculture Flagship, Australia

Contact: Richard.Richards@csiro.au

We use phenotyping approaches and platforms for many reasons. One of our main goals is to provide tools and ideas for the development of new breeding methods for plant improvement. This is for biotic and abiotic factors as well as for the selection of yield potential. In many cases the phenotyping approaches will also provide links with genomic technologies for use in breeding.

Unfortunately, phenotyping methods for abiotic stress and for yield potential have rarely been adopted by breeding programs. To address this and in an attempt to bridge the gap between breeders and ‘phenotypers’ I shall discuss the language of breeders so that we can develop a better dialogue. The language involves such topics as the value proposition of the trait, target environments, trade-offs, selection methodologies, heritabilities, correlated responses and genetic complexity. I shall also discuss typical phenotyping procedures undertaken by plant breeders so that we understand where phenotyping fits into breeding programs and what actually works. I shall also give examples of phenotyping approaches that have been incorporated into breeding programs. Finally, I shall discuss high throughput field phenotyping approaches and what breeders may be seeking from these.
A maize introgression library reveals ample genetic variability for root architecture, water use efficiency and grain yield under different water regimes

S. Salvi, S. Giuliani, M. Cani, G. Sciarra, R. Bonina, C. Welcker, L. Cabrera, A. Grauling, F. Tardieu, P. Meriggi, R. Tuberosa

1 Department of Agricultural Sciences, University of Bologna, Viale Fanin, 44, 40127 Bologna, Italy
2 Laboratory of Plant Ecophysiologica Responses to Environmental Stresses, INRA, Place P. Viala 3, 34060 Montpellier, France
3 Horta srl, Via E. Gorda 55, 29122 Piacenza, Italy
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The genetic dissection of root system architecture (RSA) provides valuable opportunities towards a better understanding of its role in determining yield under different water regimes. To this end, a maize introgression library comprised of 75 BCS lines derived from the cross between Gaspé Flint (an early line; donor parent) and B73 (an elite line; recurrent parent) were evaluated in two experiments conducted under well-watered and water-deficit conditions (WW and WD, respectively) in order to identify QTLs for RSA, biomass accumulation (BA), water use efficiency (WUE), flowering time (FT), grain yield (GY) and yield components (YC). RSA features (shovelomics), FT, GY and YC were investigated in the field while BA and WUE were measured in plants grown in the high-throughput phenotyping platform PhenoArch (INRA, Montpellier). QTLs were identified for all investigated traits. Several QTLs for FT affected also RSA, BA, WUE, GY and/or GY, thus underlying the pivotal role of phenology in controlling morpho-physiological traits and yield. For several QTLs, a sizeable QTL x water regime interaction was evidenced in both experiments. A particularly interesting QTL for WUE in both WW and WD conditions was mapped on bin 9.01, with Gaspé Flint contributing the positive allele. Strong concurrent QTL effects on RSA and YC were identified on bin 1.03, 2.04 and 3.03. The most interesting QTLs are being considered for fine mapping and cloning.

Developmental aspects of barley drought tolerance


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2 Leibniz Institute of Plant Genetics and Crop Research, OT Gatersleben, Corrensstrasse 3, D-06466 Stadt Seeland, Germany
Contact: akuc@igr.poznan.pl

The POLAPGEN-BD (www.polapgen.pl) project was the largest coordinated plant genomics project in Poland which aimed at providing biotechnological tools for production of spring barley varieties with improved drought tolerance. Three RIL populations and a panel of varieties were subjected to various drought regimes and investigated by a systems biology approach with respect to morphological, anatomical, physical and physiological features, together with responses at gene expression, protein and metabolite levels. Phenotyping was done in experiments carried out in the greenhouse and in the field. However, there was no possibility to study the reaction of genotypes to drought during development, when the treatment was applied, in the way that is possible in modern phenotyping facilities. The EPPN transnational access program allowed us to perform the experiment on 100 RIL barley lines from the cross combination Maresi x Cam/B1/CI, and also four varieties: Maresi, Cam/B1/CI, Sebastian and Stratus, on the high-throughput automated plant phenotyping platform at IPK Gatersleben (Germany). The studied varieties are of European origin, with the exception of Cam/B1/CI which is a Syriac line and is characterized by a higher resistance to water deficit. Two watering regimes were applied. The first treatment was stress at the stage of flag leaf (phase 13 in the BBCH scale; soil moisture 3.2 pF) which is generally considered as the most important period for the establishment of yield-related traits. The second treatment was control with optimal humidity of the soil (2.2 pF). In addition to color-related and other traits derived from image phenotyping, yield-forming traits were observed after harvest, with a special interest in leaf area, biomass development, plant height, tiller number, and plant architecture. Preliminary results on the variability of most important traits will be presented. The population was also genotyped with microsatellite (SSR) and single nucleotide polymorphism (SNP) markers. On this basis a genetic map was constructed and QTLs determining the developmental traits and yield potential will be identified.

Project supported by the European Regional Development Fund under Innovative Economy Programme 2007–2013, Subaction 1.3.1 within the subject „Biological progress in agriculture and environment protection” and by the Transnational Access Program, European Plant Phenotyping Network (EPPN).
Whole plant vs. functional water saving: Different drought response of modern cultivars and landraces of durum wheat

GERNOT BODNER1, ALIREZA NAHKFOROOSH1
1 Department of Crop Sciences, Division of Agronomy, BOKU University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz Strasse 24, A-3430 Tulln, Austria
Contact: gernot.bodner@boku.ac.at

Identification of main drought stress response strategies in crop germplasm is of high importance to find ways towards improved adaptation to distinct drought environments.

In a greenhouse experiment we investigated the diversity within a set of 12 durum wheat landraces and three modern cultivars of different origin in terms of (1) whole plant morphology and (2) selected physiological traits. The sample was tested in a phenotyping experiment in the SCREEN House facility at FZ Jülich. Genotypes were planted in pots under two water regimes (75 % vs. 25 % plant available water) in five replicates. Canopy growth and architecture as well as water uptake via gravimetric water content were measured every second day by the SCREEN House phenotyping system. After five weeks when leaves were fully expanded, gas exchange and chlorophyll fluorescence measurements were done.

Landraces contained genotypes showing high water use under both well-watered and stress conditions (water spenders) as well as genotypes with a constituively low water use (water savers). The distinct water use strategies of landraces were mainly related to differences in canopy, particularly leaf area and leaf expansion. Modern cultivars had a low cumulative water use. They were characterized by a low leaf area and therefore were among water savers in terms of their canopy architecture. However modern cultivars showed a high water use per unit leaf area. This is related to their high stomata conductance and indicated their optimization in terms of leaf functionality, providing them a high assimilation potential particularly under well-watered conditions.

The experiment showed, that combining plant phenotyping with physiological measurements can reveal distinct drought response strategies of crop germplasm. While landrace showed a wide range of adaptation strategies via distinct canopy structure, modern cultivars have optimized water consumption in terms of their physiological assimilation potential providing them with water saving behavior compromising growth potential.

Leaf rolling in maize crops: contrasting behavior among cultivars evidenced from ground and UAV observations

FRED BARET2, KAMRAN IRFAN1, SIMON MADEC2, JEREMY LOPEZ2, ALEXIS COMAR3, MATTHIEU HEMMELE2, DAN DUTARTRE1, SÉBASTIEN PRAUD2
1 CAPTE, INRA-EMMAH, France
2 Biogemma, Chappes, France
3 HYPHEN SAS, Avignon, France
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Water is one of the main limiting factors of maize production. Resistance to water stress is therefore a highly desired trait for breeders. Water stress may show up under several ways including reduction of leaf expansion, increase senescence rate, stomatal closure inducing an increase of temperature and change in fluorescence yield. Leaf rolling may also be observed. It can be interpreted as a mitigation mechanism that will reduce the intercepted radiation, limit the excess of light as well as the evapotranspiration which will ultimately impact the vegetative growth and grain yield. Leaf rolling is also observed concurrently with other changes in the canopy structure corresponding mainly to leaves becoming more erect for maize canopies.

A dedicated field experiment was conducted near Nérac, France (44.17° latitude, 0.30° longitude) in 2015, where a large panel of cultivars was subjected to strong water depletion during the post-flowering stage in early August. A subset of 30 micro-plots corresponding to cultivars expressing large differences in leaf rolling was selected. Gap fraction measurements were completed over these 30 cultivars during the whole day using hemispherical photography. Leaf rolling was also visually scored for the same cultivars several times during the day. Concurrently, the Airphen multispectral camera with 6 bands was flown at an altitude of 70m ensuring a spatial resolution around 3 cm. The camera was each time carefully calibrated over a reference carpet to compute canopy reflectance. The camera was maintained in the nadir direction to be more sensitive to canopy structure possible variations. The illumination conditions were optimal during the whole day, with always clear sky.

The results of 30 genotypes were compared to determine the degree of resistance to heat and water stress. Results show important changes in the canopy structure after about 11:00 local time: leaves were starting to roll and to become more erect. Important differences in the onset time and magnitude of canopy structure variation were observed among the cultivars. This was quantified in terms of radiation interception efficiency that was compared to the visual estimation of leaf rolling. Finally, the UAV observations were tentatively transformed into the green fraction. The changes in the illumination geometry during the day combined with intrinsic canopy structure differences between cultivars make the interpretation more complex. All these results are discussed with emphasis on the capacity of UAV observations to phenotype this important trait with a high-throughput and a high accuracy.
ABIOtic Stress Part 2

Destructive and non-destructive quantification of drought resistance in isogenic tall and dwarf wheat

Joshua D. Klein1, Christian Jeudy2, Mathieu Chanis2, Mickael Lamboeuf1, Joshua Herskovitz1, Yonit Raz Shalev1, Christophe Salon2

1 ARO-Volcani Center, Bet Dagan, Israel 50250
2 UMR 1347 Agroécologie/AgroSup/INRA/uB, 17 rue Sully, BP 86510, 21065 Dijon cedex, France

Contact: vcjosh2000@yahoo.com

Dwarfing genes in wheat not only reduce lodging and increase grain yield but also enhance plant resistance to water stress. Investigating the physical attributes and the physiological mechanisms associated with drought resistance is often a time-consuming and destructive procedure, especially when studying roots. If the study takes place over time, a large number of plants must be prepared for harvesting and measurement at timed intervals. We investigated automated phenotyping of roots and shoots as a non-destructive method for quantifying physical and physiological aspects of drought resistance in isogenic tall and dwarf (Rht; Rht-B1c) Bérseewheat seedlings. During a month of growth, plants were exposed to no, medium, or severe water stress for 10 consecutive days, corresponding to 100, 60, or 40% of field capacity at the end of the stress period, followed by a return to full irrigation for a week of recovery. All experiments were carried out at the highly-automated Plant Phenotyping Platform facility at INRA, Dijon.

A series of 4 experiments were conducted during 2014 and 2015 to 1) select the most appropriate growth substrate, 2) adjust the watering regimes, and 3) monitor the extent of drought progression in both pots and rhizotubes. Non-destructive image acquisition and analysis allowed characterization of shoots and roots, when calibrated with results from parallel destructive measurements of biomass, leaf area and plant height, length of largest and smallest leaves, leaf and root specific weight, and membrane permeability. Calibration protocols were developed for use with the raw data acquired directly from images, which in turn allowed investigations of the dynamics of plant growth of both genotypes in a variety of treatments, including seed treatments with plant hormones.

The proportional effects of moderate or severe water stress in decreasing plant height and leaf area were comparable, whether measured destructively or not. Growth could therefore be measured continuously, rather than at static intervals. Even after a week of recovery, overall leaf area was reduced 25% in moderately-stressed and 60% in severely-stressed plants, although dwarf plants fared better than tall. Plant height recovered more rapidly than leaf area, with no residual effect found in moderately-stressed plants, and a 30% reduction in severely-stressed plants. The proportional effect of stress on both leaf area and height could be quantified automatically using three lateral views of shoots, which limited under-estimation resulting from leaves masking each other. Leaves that exceeded 25 cm often bent over, while some never grew taller than 5 cm. Despite calibration, in these extreme cases leaves could be miss-measured by as much as two-fold. Technical fixes for these problems are being developed in both hardware and software.

The number of main roots per seedling decreased 15–25% under moderate or severe stress, while the number of secondary roots increased 33% under severe stress. Main roots of dwarf wheat were 30% longer under moderate or severe stress, but secondary roots were up to 37% shorter, especially at the growing end of the root. Images from the rhizotron were analyzed manually using the programs ImageJ or Dart, since wheat roots were often too translucent and too intertwined for quantification by automated machine vision.

Chlorophyll, carotenoid, and anthocyanin concentrations in leaves changed with degree of water stress, and also in response to seed treatment with trinexapac-ethyl, which induces resistance to stress. We were unable to correlate leaf pigment values with R, G, B and hue values extracted from RGB color images. The automated methods that have been developed allowed efficient acquisition and analysis of data from individual wheat plants in 300 pots (shoots) and 100 rhizotubes (roots). Continued improvement of these methods will greatly increase possibilities and precision in large-scale experiments with agronomic and horticultural crops.

Early growth of barley cultivars and landraces in rhizotrons

Ridha Boudiar1, Kerstin Nagel1, Tania Gioia2, Fabio Fiorani2, Ana M Casas2, M Pilar Gracia2, Ernesto Igartua1

1 Aula Dei Experimental Station, CSIC, Avda Montañana 1005, 50059-Zaragoza, Spain
2 Jülich Plant Phenotyping Centre, Institute of Bio- and Geosciences (IBG-2), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Contact: igartua@eed.csic.es

The objective of this study was to explore the early growth features of three promising barley landraces, compared to three modern cultivars.

Three lines derived from Spanish barley landraces, part of the Spanish Barley Core Collection, presented good agronomic features at field trials: SBCC042 and SBCC073 out-yielded 26 cultivars in field trials at low production sites across Spain, and SBCC146 had an outstanding early vigour. This study was proposed to find out whether early growth, particularly under drought, was related to this good agronomic performance. Three modern cultivars, Orria, Cierzo and Scarlett were used as checks.
The six genotypes were grown under two water treatments in rhizoboxes-rhizotrons (12 plants per genotype and treatment, in balanced incomplete blocks), for four weeks, at the GROWSCREEN-Rhizo phenotyping platform (Nagel et al. 2012), at the Forschungszentrum of Julich (FZJ-Jülich), Germany. Each rhizobox was considered an incomplete block, holding 2 plants, with genotypes assorted to have a balanced number of occurrences with each other at each treatment.

Growth of roots was monitored by automatic image analysis. Images were taken 2–3 times per week. Growth of the aerial part was monitored by visual recording of morphological traits (number and size of leaves, number of tillers). At the termination of the experiment, the plants were extracted from the rhizoboxes, and shoot and root fresh and dry weights were determined. Several physiological measurements were taken at specific moments.

Landrace lines exhibited more vigorous growth than checks under no stress, although patterns differed among genotypes, with enhanced root growth for SBCC073 and larger shoot growth for SBCC146. Two of these lines will be further explored as parents of BC populations.


**High-throughput phenotyping of hybrid wheat under drought and heat stress**

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Drought and heat represent one of the major limiting factors of plant growth and production worldwide and hence play a crucial role for global food security (Turner et al. 2011). With a rising population, the pressure of food production on arable land available per capita is increasing. Due to the progress of climate change, this problem becomes increasingly serious.

In most countries cereals, especially wheat, are the basis of daily carbohydrate intake (Shiferaw et al. 2011). Therefore, it is important that breeding is focusing on the development of tolerant wheat cultivars regarding drought and heat stress. Wheat hybrids seem to be promising and occasional reports exist indicating an enhanced drought stress tolerance. Heterosis is leading to an increased grain yield and to an advantage regarding yield stability to abiotic stress (Hallauer et al. 1988). It is to be expected, that the yield increasing effects under drought conditions of heterosis in hybrids are not only depending on a single mechanism but also on a combination of various properties. However, detailed data about responsible mechanisms have not yet been elucidated. At the present, breeding efforts are high and potential varieties need to be tested in multi-annual field experiments. Spectral measurements offer new approaches to make non-destructive measurements of e.g. the plant water status.

In 2014 and 2015, 16 high performance wheat varieties and 4 wheat hybrids were grown under a rain-out shelter at the research station Dürnast in Bavaria. Drought stress was conducted during anthesis and grain filling by withholding rainfall. Heat stress was generated under the shelter during warm and sunny days. To compare the impact of drought and heat stress, control plants were grown outside the shelter.

During the stress period morphological and physiological parameters such as plant ground cover, relative leaf water content, N-translocation of the stem, the leaf and the spike, carbon-isotope discrimination as an indirect indicator for photosynthesis, stomatal conductance and plant height were measured. Furthermore, plant reflection was assessed with 5 passive and active sensors in a high-throughput mode with the mobile phenotyping platform PhenoTrac 4, developed by the Chair of Plant Nutrition from the Technische Universität München. Additionally, plant temperature was measured with a thermal camera. Results, illustrating the drought and heat tolerance of wheat hybrids and lines, will be presented.

References:


Identification of drought tolerance markers in potato

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Climate models predict an increased likelihood of seasonal droughts for many areas of the world. Potato (Solanum tuberosum L.) produces high calories per unit water invested, but is drought-sensitive. Drought tolerance breeding could be accelerated by marker-assisted selection. For marker identification, we used metabolite and transcript profiling by GC-MS and RNA sequencing. To validate markers, we determined genetic variance and potential costs of tolerance. In 14 independent pot or field trials, starch yield under optimal and reduced water supply was determined in 34 European potato cultivars. Quantifying drought tolerance as deviation of relative starch yield from its experimental median (DRYM), we found significant tolerance variation in the cultivar panel. Tolerance estimates from pot trials correlated with those from field trials. A negative correlation between drought tolerance and yield under optimal conditions in the field suggests that drought tolerance is linked to a yield penalty. The distribution of yield data versus DRYM indicated that tolerance can be combined with average yield potentials by adjusted breeding strategies. From the metabolite and transcript concentrations measured in leaves, we identified potential markers with significantly higher levels in tolerant than in sensitive varieties. With these markers, models were developed to predict drought tolerance using Random Forest method and cross-validation. The model for tolerance prediction based on samples from field experiments was validated on samples from breeders’ sites. In spite of very variable growth conditions, tolerance prediction was correct for about 90% of the samples within the same population panel. We thus developed and validated a predictive model for potato drought tolerance in Central European agro-environments.

Comparative performance of δ13C, δ18O and δ2H for phenotyping durum wheat adaptation to different water and nitrogen conditions

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Drought and low fertility can substantially affect plant growth and grain yield. Novel phenotyping tools may help breeding for yield potential and adaptation to drought and low nitrogen stresses simultaneously. This study evaluated the hydrogen isotope composition (δ2H) as a phenotyping trait for wheat breeding under different water and nitrogen conditions. δ2H performance was compared with those of oxygen (δ18O) and carbon (δ13C) isotope composition. The study was performed under field conditions in a Mediterranean-type dryland environment. A set of genotypes (cultivars and landraces), were evaluated during two consecutive years. Isotope composition was analyzed in dry matter, water soluble fraction and water from different tissues. δ2H performed better than the other two isotopes predicting grain yield (GY) and total N content under water stress but contrasting N regimes. δ2H similarly than δ13C correlated negatively with stomatal conductance. When analyzed in kernels δ18O performed much poorer than the other two isotopes predicting GY. This study illustrates the usefulness of δ2H independently or combined with δ13C and marginally with δ18O as a potential tool for genotype selection for yield potential and under a wide range of growing conditions providing time-integrated records of the photosynthetic and evaporative performance of the plant during crop development.

Integrating phenotyping for drought tolerance into biotechnological potato improvement for multiple resistance traits

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Potato is one of the most important crops in the world, ranking fourth in productivity. Classical breeding of potato has contributed to its production quality improvement at the cost of reduction in resistance to diseases and pests. Climate change adds new challenges for potato improvement, drought being one of the most important abiotic stress to face potato crop around the world.

The goal of our research was to integrate the new opportunities of plant phenotyping with biotechnological improvement of potato. In order to transfer multiple diseases and pest resistance traits from wild relatives Solanum
bulbocastanum (blb) and S. chacoense ( chc) into potato commercial cultivars, somatic hybridization through protoplast electrofusion was applied. Complex analysis of the somatic hybrids resulted in stalking two resistance genes to late blight from blb into potato cv. Delikat and its expression in the laboratory and field assessments. On the other hand, somatic hybrids Delikat + chc were characterized for resistance to Colorado potato beetle (CPB) in laboratory choice and non-choice assays. Selected somatic hybrids resistant two either late blight or CPB were pretested in in vitro culture for tolerance to drought and transferred to HAS-RSDS platform for phenotyping. By combining genomic analysis and phenotyping new genotypes with multiple resistances were identified. The new possibilities of integrating genetic and biochemical assays with phenotyping and their benefits for potato improvement are going to be discussed.
High-throughput shoot and root phenotyping to study drought and salt stress responses in wheat

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Every continent is affected by salinized soil and water deficiency. Soil salinization is one of the serious forms of soil degradations, which can arise from natural causes and human-mediated activity, such as irrigation in arid and semi-arid regions. Salt tolerance may be defined as a sustained growth of plants in a highly saline environment. Crops vary significantly in their threshold limits of drought/salt tolerance. Screening of crops for tolerance can strengthen the breeding programs by identifying genotypes with high drought/salt tolerance and yield potential.

The main objective of the study was to use high-throughput shoot and root phenotyping platforms to analyse salt tolerance of wheat genotypes under well-watered and drought conditions. The experiment was conducted with 14 wheat (Triticum aestivum L.) cultivars, from Serbia (5), Austria (4) and Azerbaijan (5), which were chosen on the basis of data available for their salt and drought tolerance. Plants were grown in a greenhouse under 4 watering/salt conditions:

1. well-watered (60% field capacity) and no salt (NaCl) added (control 1);
2. water limited (20% field capacity) and no salt (NaCl) added (control 2);
3. well-watered (60% field capacity) and saline conditions (0.2% NaCl);
4. water limited (20% field capacity) and saline conditions (0.2% NaCl).

Comparative investigations of various morphological, physiological, biochemical, enzymatic and ionic responses concerned with salt and drought tolerance were performed at different developmental stages by using semi-automated Complex Stress Diagnostic System, in greenhouse of CR Ltd., Szeged, constructed by HAS-BRC and CR Ltd.

The results have shown that salt stress (0.2 NaCl/kg soil) and drought stress when applied separately caused a retardation of water uptake and green biomass, but the negative effects of these stresses were not very strong. However, when both stresses were applied together these parameters were dramatically decreased. Regarding the grain yield the cultivars had different reactions to applied treatments. The most salt sensitive cultivar was Azerbaijan cv. Giymatly-2/17, while the most salt tolerant was Austrian cv. Galio. All other cultivars had very similar reactions to salt stress. Drought stress had stronger negative effect to the grain yield, but most of the cultivars had similar level of drought tolerance. In case when both stresses were applied together the best grain yield had cvs. Capo and Tale 38, while the cv. Azamatli-95 had the lowest yield. The root development was also affected by different combinations of water and salt stresses. However, the root density of different cultivars was not in significant and stable correlation with production of green biomass or yield. The results showed large difference among the studied cultivars, and revealed tolerant and sensitive genotypes. The tolerant genotypes can be recommended for cultivation in marginal salt-affected areas or for use in breeding programs for development of new high-yielding wheat cultivars with good level of drought/salt tolerance.

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Impact of domestication on the phenotypic architecture of durum wheat under contrasting nitrogen fertilization

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The process of domestication has led to dramatic morphological and physiological changes in crop species due to adaptation to cultivation and to the needs of farmers. To investigate the phenotypic architecture of shoot- and root related traits and quantify the impact of primary and secondary domestication, we examined a collection of 36 wheat genotypes...
under optimal and nitrogen-starvation conditions. These represented three taxa that correspond to key steps in the recent evolution of tetraploid wheat (i.e. wild emmer, emmer, and durum wheat). Overall, nitrogen starvation reduced the shoot growth of all genotypes, while it induced the opposite effect on root traits, quantified using the automated phenotyping platform GROWSCREEN-Rhizo. We observed an overall increase in all of the shoot and root growth traits from wild emmer to durum wheat, while emmer was generally very similar to wild emmer but intermediate between these two subspecies. While the differences in phenotypic diversity due to the effects of primary domestication were not significant, the secondary domestication transition from emmer to durum wheat was marked by a large and significant decrease in the coefficient of additive genetic variation. In particular, this reduction was very strong under the optimal condition and less intense under nitrogen starvation. Moreover, although under the optimal condition both root and shoot traits showed significantly reduced diversity due to secondary domestication, under nitrogen starvation the reduced diversity was significant only for shoot traits. Overall, a considerable amount of phenotypic variation was observed in wild emmer and emmer, which could be exploited for the development of pre-breeding strategies.

Physiological model greatly enhances GWAS when used to dissect multi-environment field phenomics for climate adaptation traits

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The GRiSP Global Rice Phenotyping Network mines genetic diversity for traits and genes conveying yield potential and adaptation traits, using sub-species diversity panels. An overview is presented. We then focus on the use of RIDEV model to extract physiological trait information from multi-environment field phenomics data describing time to flowering (phenology) and spikelet sterility (as affected by thermal stresses). Field experiments were conducted in 10 climatically diverse environments in Senegal and Madagascar, and time to flowering and incidence of sterility were observed on the ORYTAGE-diversity subpanel (200 accessions). The new RIDEV model simulating crop microclimate, photoperiodic and thermal control of phenology and thermal control of sterility (cold and heat) was fitted to each accession across all environments. Physiological model parameters, henceforth considered as traits, included cardinal temperatures (Tbase, Topt), basic vegetative phase (BVP) duration, photoperiod sensitivity, and critical temperatures for cold-sterility (at microspore stage) and heat-sterility (at anthesis). Fitted, genotypic model parameter values were used as trait values for GWAS. Genotyping and structure analyses were based on GBS. Large genetic diversity was found for all traits. Model-derived traits gave much stronger genomic association signals than did direct observations. GWAS results were further improved with the introduction into the model of a cold acclimation term, because many genotypes were more cold tolerant when exposed to cold during developmental stage. A genetic subgroup indigenous to Madagascar was identified that had strong acclimation ability. Among the candidate genes associated with the QTLs were (1) HD3a/RFT1 (cluster of two florigen genes) that was associated with the QTLs were (1) HD3a/RFT1 (cluster of two florigen genes) that was associated with cold acclimation capacity, indicating the involvement of epigenetic memory. Validation of QTLs and candidate genes is in progress.

Field-based phenotyping for abiotic stresses in maize – Focus on breeder’s wish-list

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Irrespective of breeding approach, whether conventional or molecular breeding, high-quality phenotyping data is the key of success for genetic improvement for targeted traits. Breeders generate different types of breeding populations, screen for the targeted traits and select among large number of segregating progenies, and the top-ranking best progenies for use in the their breeding program. In molecular breeding approach, progenies are genotyped and phenotyped, and selections are done the basis of genotype-phenotype association, and favourable alleles are taken forward for use in breeding program. However, key of success remain dependent on high quality phenotyping data support. In CIMMYT-Asia maize program we focus on precision in field-based phenotyping, including selection of appropriate phenotyping site/field, suitable cropping season in order to achieve targeted stress, managed stress phenotyping under field (or field-type) conditions to simulate stresses in target potential environment (TPE) and tools to keep track with stress intensity with uniformity within
phenotyping site. In terms of plant traits for phenotyping and selection criteria more emphasis is given on breeder’s wish-list in selecting genotypes, which could eventually be used in developing final product with agronomically desirable traits in TPE. In case of molecular breeding projects traits are further dissected into its components, which helps identifying favourable gene/alleles associated with desirable components traits, and that could be taken forward on introgression into elite genetic background but lack tolerance to a targeted abiotic stress.

QTL mapping for traits associated with terminal heat tolerance in spring wheat
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Due to global climate change, high temperature stress at the time of grain filling in wheat has become one of the major environmental threats to food security in different regions of the world. Hence, there is an acute need to identify new sources of resistance to terminal heat stress and develop cultivars which can produce in abundance even under high temperature. Limited number of heat tolerant cultivars in wheat and non-availability of reliable markers for terminal heat stress tolerance gene(s) are still hampering sustained breeding efforts. It is therefore necessary to appraise the work on QTL mapping for agronomic traits linked to terminal heat tolerance. An inter varietal mapping population in the form of recombinant inbred lines (RILs) developed from a cross between ‘Dharwad Dry’ (a terminal heat tolerant cultivar) and ‘HUW-468’ (a terminal heat susceptible cultivar) was used for the identification of QTLs controlling agronomic and physiological traits under heat stress regime. The 168 single seed descent (SSD) derived F$_6$ F$_7$–8 lines of ‘Dharwad Dry × ‘HUW-468’ were evaluated for terminal heat tolerance in randomized block design with three replications for three successive years i.e., 2012–13, 2013–14 and 2014–15 under timely sown (non-heat stress environment) and late sown (heat stress environment) conditions in NBPGR, New Delhi (India). The RILs showed a high level of variation for agronomic traits (spike length, no of tillers/plant, yield per plant and thousand kernel weight), physiological traits (chlorophyll content, membrane stability index and canopy temperature) and quality traits (Iron, Zinc and protein content) under heat stress regime. Membrane stability index and chlorophyll content showed high Pearson correlation coefficient (0.9 at 0.01%) to each other, were also found positively correlated with yield (0.55 and 0.6 at 0.01% respectively). To identify genomic regions for the traits associated with terminal heat stress tolerance, 540 genomic micro-satellite markers were screened for polymorphism between parents. Out of these, 94 (17.4%) simple sequence repeat (SSR) markers found polymorphic were used to identify genomic region contributing to terminal heat stress tolerance. The QTLs identified in the present work will be used in marker assisted selection (MAS) for terminal heat stress tolerant genotypes.
Deeper root systems during grain development for future farming systems: Selection by combining field and laboratory phenotyping

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Future farming systems require higher yields with less water. A potentially valuable target is to deepen the root systems to increase the productivity of water-limited systems. Deep water is protected from evaporation and may be predicted by the farmer at planting. Importantly deep roots arrive in deeper water around flowering and grain development, contributing to higher harvest indices and water use efficiencies. Deep roots at grain development in wheat provide the phenotyping challenges that they are (1) nearly impossible to select directly in pots, and (2) expressed in mature root systems. Working with wheat in Australia and India we had to: first, identify genotypes with deep roots at grain filling in the field, because laboratory seedling screens did not reliably predict field depth, and second, to develop a fast selection method to use in breeding populations for deep roots. A tractor-mounted hydraulic corer was used to make root counts to 2 meters within 4 minutes per genotype, of 100s of Australian, Indian and international wheat genotypes over five field seasons. Despite large spatial and seasonal variability, lines were identified with the potential to increase yields by 0.5 tonnes per hectare and increase water use efficiency, if root phenotypes are incorporated into varieties for farmers. To develop a breeding screen, a retrospective dissection of deep and shallow field sources was undertaken using agar and paper rhizotrons, columns of soil, and baskets in soil. Rapid, early axile root growth, and root angles in baskets of soil were related to deep roots, depending on season and soil type. Further development of these methods is underway to establish reliable relationships with mature root systems in the field. This work highlights lack of knowledge of mature root system traits in crops, which may become increasingly valuable in future including for the capture of deep N for growth and grain quality.

Random forest models and Support Vector Machine analysis enhance trait selection and cultivar differentiation of mature pea root systems

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Grain legumes represent a significant source of protein for food and feed but are currently grown on 1.8% of arable land in the EU only. However, while distinct leguminous crops are used locally for food and feed in many European countries, and local cultivars are kept in numerous collections in gene banks, research institutions and also in farms/families, this genetic pool cannot be used at its full potential for large scale agriculture and breeding programs until important traits have been determined. While the increasing number of studies addressing crop root systems indicates the growing awareness regarding their importance for crop productivity, especially root traits of mature plant are still underexplored. This is surprising because root traits can differ tremendously between seedlings and more mature plants. Thus, a standardized greenhouse experiment in large lysimeters was performed to phenotype 16 Pisum sativum cultivars originating from Southern and Northern Europe. After manually excavating 5–6 root system of 70 days old, mature plants per cultivar, we measured 16 root traits. We identified the most discriminative root traits with random forest models and differentiated cultivars with support vector machine analysis based on root traits. Previously, these methods have been used occasionally on larger numbers of replicates but we can show for the first time that they can be successfully used on smaller, manually derived trait data sets as well. This project received funding from the European Union’s Seventh Framework Program for research, technological development and demonstration under grant agreement no 613781 (EUROLEGUME).
Exploring root development and architecture in one of the most heat and drought tolerant cereals, pearl millet

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Higher global temperatures due to climate change place our agriculture at risk. Pearl millet is a subsistence cereal crop adapted to heat, drought and poor soils, and has potential to produce high grain and fodder yields at high temperatures where other crops fail. Root architecture contributes to this adaptation and thus provides promising traits that could be targeted by selection, but little is known about the genetic regulation of root traits in pearl millet.

To better understand pearl millet root growth pattern in soil and the impact of drought stress, we used the Hounsfield CT Facility (University of Nottingham). This allowed us to non-invasively analyze over time the soil volume explored by the pearl millet root system under different moisture conditions.

Using the RootTrace facility (University of Nottingham), we performed high-throughput phenotyping of a set of 108 genetically diverse pearl millet inbred lines, monitoring their early seminal root growth. These phenotypic data will be combined with genotyping-by-sequencing (GBS) data in order to identify genomic regions controlling this trait via genome-wide association studies (GWAS).

**X-ray CT for growth tracking of belowground organs and root systems**

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**Introduction**

During the last years, the formerly in medical applications established technique of X-ray computed tomography (CT) is used for non-destructive material analysis as well. Adapting this technique for the visualization and analysis of growth processes of plants above and underneath the soil enables new phenotyping possibilities. Using State-of-the-art CT systems the computed 3D volume datasets allows the visualization and virtual analysis of hidden structures like roots (Metzner et al., 2015) or tubers (Ferreira et al., 2010) in the substrate, or a detailed structural analysis of sprout features like caulis, leaves and branches. This is a completely renewed approach to phenotyping without the need of a huge amount of plants. Due to the non-destructive technique time resolved CT measurements can be conducted at the same plant.

**Materials and Method**

The geometry mainly used is the axial 3D-CT, where a conical x-ray beam projects the object onto a flat 2D image detector. The pot size limits the resolution due to the available standard detector size. i.e. a pot size to about 20 cm in diameter leads to a spatial resolution of about 85 µm. Using smaller pots, as done for beans, increases the resulting spatial resolution.

We used pot sizes of 20 cm, 15 cm, 10 cm and 8 cm in diameter for cassava, potatoes, maize and bean, respectively. The potato pots were scanned regularly 4 weeks after tuber initialization. In different batches the results for drought stressed and unstressed tubers are evaluated.

The crucial task of X-ray CT plant phenotyping is the segmentation of plant material and the surrounding soil. Thus, we developed powerful algorithms to analyze automatically reconstructed CT data.

**Results and Discussion**

The developed algorithms are used for the segmentation of potato tubers, bean, maize and cassava storage roots in pot diameters up to 20 cm. Thus, it is possible to study growth rates of individual organs in much more detail compared to destructive methods. For large belowground organs like potato tubers we show exemplarily the tuber development with and without external stress factors, whereas for maize, bean and cassava we will demonstrate the powerful capabilities of segmenting root structures with our algorithms.
Conclusions

The main task in the analysis of X-ray CT data is the post processing itself. To bridge the gap towards medium throughput phenotyping algorithms are needed to automatically extract the root system as good as possible from the surrounding soil. In contrast to common algorithms based on thresholds or region growing we developed 3D segmentation algorithms based on homogeneity and the variance of the local surrounding. We think that this versatile algorithm is the next step for automatic volume processing of X-ray CT datasets to enable the live tracking of underground growth processes in a greenhouse phenotyping environment.

Fine phenotyping of root and shoot dynamics on drought conditions in rice

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Cirad set up a high-throughput system for roots and shoot phenotyping, named “Rhizoscope” (Courtois et al., 2013). The system is based on the use of 2-D "Nail board rhizoboxes" filled up with glass beads and connected with a nutrient solution irrigated system. The transparent glass bead substrate allows to study root growth and development under uniform mechanical impedance with mineral nutrient constraint. However as substrate, glass beads have no water retention ability and don’t give the opportunity to apply water constraint. The objective of this study was to test the feasibility to improve substrate by additioning to initial glass beads a proportion of water beads, containing a water-absorbing polymer gel.

The experiment using Azucena cultivar was conducted in growth chamber in April–Mai 2015 in Montpellier. The substrate was a mix of glass beads (3/4) and superabsorbent beads (1/4). Until the 5th leaf on main culm starts emerging stage (20 days), plants were managed with optimal condition. Then 2 treatments were applied: 1) control plants (C) with maintenance of irrigation; 2) water stressed plants (S) without any irrigation. Plant shoot and root development were monitored up to 3 weeks after beginning of treatments application. In the stressed treatment, rhizoboxes were weighed daily in to follow the transpiration. Plants progressively deplete the average 800ml solution reserve of Stressed rhizoboxes during the dry-down period, and reserve was 30% after 3 weeks. On stress treatment, plant development rate progressively slowed down to reach a Haun Index value of 7.1 at the end of experiment while Control plants were at 8.6 stage. Moreover, Stressed plants showed a drastic decrease in the 6th leaf elongation rate, which was expanding during stress, affecting maximum rate and final size. This first experiment is promising and prove that rhizoscope system can be used for fine phenotyping under water deficit conditions.
Combining information from multiple experiments in platforms and field

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Phenotyping experiments most often aim at evaluating traits or performances of large number of genotypes for genetic analyses. When experiments are repeated, they provide different values between experiments for each trait, and different rankings of genotypes. If all experiments are performed in strictly controlled environmental conditions, this can be interpreted via biological variability, plus the effect of differences in experimental procedures or measurement protocols. In semi-controlled phenotyping platforms and in the field, environmental effects are added to the latter sources of variability. In most cases, this results in several-fold differences between sites for studied traits, and in a genotype x environment interaction about equal to the genetic effect. A way to analyze these multi-site experiments is to relate traits observed in each site to environmental conditions as sensed by plants, precisely measured during experiments. I will report the results of (i) a multi-site series of 10 experiments in controlled or semi controlled conditions performed in the EU project EPPN, (ii) a multi-site multi-scale experiments performed in the UE project DROPS, with 3 experiments in a platform and 30 experiments in the field. Briefly, the plant developmental program as estimated by the progression of leaf number or flowering time is common to all experiments, provided that time is corrected for the temperature effects. Growth is largely dependent on conditions such as light, evaporative demand or water deficit, with consistent relationships between environmental conditions and studied traits. This allows one to identify genomic regions conditionally associated with traits or performances, with clear relationships between allelic effects and measured environmental conditions.

Caring about standards? Does phenotyping need this?

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Phenotyping is becoming a mature technology and has started producing significant amounts of data. Most of these data sets are however still generated in a particular consortium. However if there is anything “omics” sciences have taught us, it is that making data comparable not only paves the way for machine learning approaches but that it will help individual researchers as well.

This is because once federated and curated, data sets can be used to unravel novel associations, and the data allow finding new candidate genes or loci. Whilst these large scale data mining approach comes at the price of high computational costs, here the ongoing cost reduction in IT will come to help to properly analyse these data. Some examples will be presented how unexpected associations can be mined to unravel novel biological hypotheses and/or to find candidate genes based on already standardized biological data sets to provide a direct incentive to properly standardize phenotyping data.

Exploring the phenome

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Within the biomedical community, one of the most successful strategies for achieving standardization and integration of biomedical knowledge, data and associated experiments was proposed more than a decade ago with the advent the advent of the Gene Ontology. Since then, the biomedical community has invested a considerable amount of effort, research and resources in the development of ontologies that are now becoming and increasingly successful as information management and integration tools. This presentation will focus on how phenotype data ontology-based standardisation can enable a variety of applications. It will employ examples stemmed from the biomedical domain and attempt to provide directions of how these could be applied in the plant domain.
Plant phenotyping results at your fingertips: Design, manage, analyse, and visualize with PIPPA

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Nowadays, phenotyping has a central role in many plant research projects. At the VIB research institute, several phenotyping systems have been developed for both Arabidopsis and maize, all including imaging acquisition and the analysis of the associated data through image analysis analysis, and where appropriate automated weighing and irrigation. All phenotyping systems are supported by PIPPA, a central database and web interface with image and data visualization and analysis. The interface allows scientists to setup and analyse their own experiments, while keeping all data together in a structured database. The database takes care of the data management and integration, linking images, metadata, environmental data, and image analysis and measurement results. As the software package is developed as a web interface, the tool is available on every computer within the department. PIPPA communicates with our suite of WIWAM phenotyping systems in two ways: information to be stored in the database is automatically retrieved from the different platforms, and platform-specific experiments, designed in PIPPA, can be transfer to the automated systems. Preprocessing of images, such as cropping, can be automated and image analysis is performed by starting a task on the server or computer cluster, for fast processing. The analysis framework is designed to support the integration of external image analysis scripts. Furthermore, environmental measurements, weighing and irrigation output, the experimental design, and image analysis results can all be graphically visualized within PIPPA, bringing the plant phenotyping results to your fingertips.
Large-scale genetic screens with O3 to reveal components involved in ROS signaling

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We have conducted two large scale genetic screens with the model plant Arabidopsis thaliana to discover components that are involved in the sensing and immediate downstream signaling of apoplastic reactive oxygen species (ROS). The harmful air pollutant O3 can be used as a convenient tool to generate apoplastic ROS and screens for O3 sensitivity have revealed components involved in ROS-related signaling cascades and antioxidant biosynthetic pathways. However, the components involved in the early ROS sensing and immediate downstream signaling are mostly unknown. Cysteine-rich receptor-like kinases (CRKs) are transmembrane proteins characterized by the presence of two domains of unknown function 26 (DUF26) in their ectodomain. The CRKs form one of the largest groups of receptor-like protein kinases in plants but their biological functions have so far remained largely uncharacterized. We conducted a large-scale phenotyping approach of a nearly complete crk T-DNA insertion line collection showing that CRKs control important aspects of plant development and stress adaptation in response to biotic and abiotic stimuli in a non-redundant fashion. In particular, the analysis of ROS-related stress responses, such as regulation of the stomatal aperture, suggests that CRKs participate in ROS/redox signalling and sensing. Since O3 primarily enters plants through stomata, stomatal conductance determines the effective O3-dose and critical flux rate and therefore impaired stomatal regulation can result in increased O3 damage. In a second large-scale screen of nearly 400,000 EMS-mutagenised individuals, we have isolated mutants for O3 sensitivity caused by stomatal dysfunction. This large-scale effort identifies components involved in ROS sensing and early signaling. Results from both approaches are presented and discussed.

Phenomics analysis of Arabidopsis thaliana chromatin remodeling mutants during drought stress

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Plant growth and crop production can be greatly affected by common environmental stresses including drought. Chromatin modification and remodelling factors impinge on different mechanisms of abiotic stress responses in plants. Understanding the function of epigenetic mechanisms in the plant responses to drought stress will lead to major advances in the area of crop productivity and tolerance to abiotic stresses. Herein, drought responses of several Arabidopsis chromatin related mutants were characterized using phenomics. Particularly, data from mutants of SWR1 complex in plants will be presented. Young seedlings were grown under short days until the first emergence of their fifth leaf and then three watering regimes were applied, 80% (optimum watering), 60% and 40% of field capacity, for two weeks. The vegetative development was captured, by a series of photos taken from top view every other day and the rosette growth was estimated. After that period, the effect of drought stress on fresh and dry weight, endoreduplication rate, chlorophyll fluorescence and gene expression profile was examined. We found that some components of SWR1 complex could affect drought responses in Arabidopsis without developmental problems. These results could be the basis for further exploitation to engineer enhanced drought tolerance in plants.

Identifying novel regulators of the auxin dependent gravitropic response in Arabidopsis thaliana roots using RootTrace and a modified vertical confocal

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Root gravitropism plays a key role in the evolution of root system architecture. Recent studies have shown that a gravity stimulus induces a lateral auxin gradient that promotes a differential growth response in the elongation zone, causing root bending. Detailed analysis has revealed that the auxin asymmetry builds up within just a couple of minutes. Auxin generally
exerts its function through binding with auxin receptors and the following derepression of ARF transcription factors, regulating downstream genes. Auxin response factors ARF7 and ARF19 play a key role in this differential growth response since the double mutant exhibits an agravitropic root phenotype.

To study the genes involved in the bending response, a detailed transcript profiling experiment was performed on dissected meristematic and elongation zones of gravistimulated roots of wild-type (ecotype Col-0) and the double mutant, arf7arf19. Over 500 differentially expressed genes were identified. Homozygous T-DNA insertion lines were screened and tested for agravitropic behaviours. Mutants were selected on the basis of altered dynamics of the gravity response by performing time-lapse root imaging using a robotised imaging setup coupled to digital image analysis with RootTrace software. Several promising candidates were identified that encoded novel gravitropic signalling components. I will present a summary of our recent results.

The Microphenotron: A new high-throughput platform for chemical biology applications
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A novel 96-well microphenotyping system has been developed to meet the challenge of growing seedlings in the small volumes needed for chemical screens, while still enabling detailed analysis of both root and shoot development [1]. In this system, Arabidopsis seed sown on the surface of agar-filled microtubes is allowed to germinate in the absence of any chemical treatment and the small molecules being tested are applied 2–3 d later by diffusion from below through the cut ends of the tubes. The plasticity of roots, together with their phenotypic complexity and their sensitivity to chemical treatments, provides a highly information-rich read-out for the phytoactivity of small molecules. Phenotypic traits that can be visually monitored include: root elongation; root branching; root hairs; root morphology; gravitropism; hypocotyl elongation; shoot colour. The method has been validated by demonstrating its ability to distinguish the phenotypic effects of a range of plant hormones and other phytoactive compounds, and by using it to identify, from a collection of >1500 yeast bioactive molecules (http://cutlerlab.blogspot.com/2008/05/latca.html), two classes of molecule that act as glutamate antagonists [1]. We are now developing an automated version of the system (The Microphenotron) that will use robotics to capture images of roots and shoots on a daily basis and novel image analysis software to automatically track changes in seedling growth and development. This robotic platform will be capable of up to 8,600 individual assays per month in a growth room footprint of just 1.2 m².


INTERNATIONAL INITIATIVES

Wheat Initiative Expert Working Group – Wheat Phenotyping to Support Wheat Improvement
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The Wheat Initiative was established in 2011 as part of the Action Plant of the G20 Ministries of Agriculture to meet the expected 60% rise in demand for wheat by 2050. It currently brings together 16 countries, 2 international CGIAR centres, and 9 private breeding companies. The Wheat Initiative aims to foster the collaboration and communication between the research community, funders and policy makers. An important aspect of the Wheat Initiative organisational structure are numerous Expert Working Groups that bring together experts in a specific area of wheat research to provide advice and consultation on wheat research in general, the specific area of expertise more specifically.

The Expert Working Group – Wheat Phenotyping to Support Wheat Improvement was established in 2014 and brings together over 50 experts from over 20 countries. It contributed to the preparation of the Strategic Research Agenda of the Wheat Initiative and will continue to consult on priority areas for investment in wheat phenotyping.

More information can be found at http://wheatinitiative.org/
Increasing yield potential and improving crop adaptation to climate change: Strategies and genetic gains
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Crossing programs that have implemented the use of phenotyping tools have resulted in a new generation of wheat lines with an increased yield potential and improved drought adaptation based on strategic crossing of complementary physiological traits (PT). 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. The first aerial remote sensing platforms for large scale genetic resource screening was developed at CIMMYT in NW-Mexico and more than half of the accessions of the World Wheat Collection have been screened. High-throughput and precision phenotyping has been applied to evaluate specific traits in elite lines, or genetic resources such as landraces and products of interspecific hybridization- and the best sources of their expression are identified as potential parents. Strategic crosses are designed to combine as many potentially complementary traits as feasible in selected progeny. Crossing and the subsequent selection of progeny serve a number of purposes. One is to achieve cumulative gene action for yield in the respective target environments based on expansion of the genepool for alleles associated with superior physiology. Another is to permit the testing of alternative hypotheses associated with assembling different constellations of traits and alleles by evaluating phenotypic expression and genetic gains at target sites. A third is to study the genetic basis of PT expression in association panels constituting diverse sources, or in populations derived from bi-parental crosses. Outputs can assist breeding decisions by revealing traits with common genetic bases, and identifying QTL networks as potential building blocks for gene-based modeling. Precision phenotyping has application in all of these steps, and the use of airborne platforms for thermal imaging and multispectral measurement are increasing throughput and predictive power of remotely sensed traits. The application of physiological approaches has clear benefits at a number of stages in the breeding pipeline. For instance, genetic gains associated with selection for canopy temperature and spectral water indices have shown that such remotely sensed traits can serve as proxies that reliably estimate water relations characteristics impacting on yield. Proof of concept comes from international trials of new PT lines showing significant genetic gains over conventional checks in a range of environments.

Diversity Seek (DivSeek): An international partnership to harness the genetic potential of crop diversity

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More than 1,700 genebanks globally conserve approximately seven million accessions of crop species and their wild relatives. In addition, farmers conserve and adapt germplasm to social, economic and ecological conditions across diverse farming systems. The genetic diversity encapsulated in all these materials underpins and drives crop improvement. Yet efforts to identify and mobilize beneficial genetic variation into breeding programs have been limited, compared to the size of this global resource. Game-changing ‘omics’ and ‘big data’ technologies now enable a more effective and broad-based approach to harnessing crop diversity. The recently launched Diversity Seek initiative (DivSeek; http://www.divseek.org) aims to capture this opportunity to accelerate the development of climate-ready, high-yielding and nutritious varieties for a growing global population. DivSeek provides a platform to generate synergies and add value to like-minded, but otherwise autonomous efforts by germplasm holders, breeders, geneticists, and database and computational experts to make crop diversity more readily accessible and usable. It is a community-driven initiative based on voluntary membership, which focuses on common challenges encountered by individual projects. Priority areas of work will include (i) the establishment of a ‘cross-crop meeting platform’ to share experiences and rapidly spread innovative research approaches and techniques, (ii) the development and advocacy of common data standards and interoperable data sets/repositories, (iii) a broadly accepted framework for ‘rights management’ that helps individual projects comply with data-sharing principles, and (iv) capacity-building efforts in these areas. We welcome colleagues and organizations interested in mining crop diversity for food security to join the growing group of the 60-some institutions that have come together to establish DivSeek.
EMPHASIS – European Multi-environment Plant phenotyping And Simulation InfraStructure

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The EMPHASIS proposal aim to establish a European Infrastructure within the ESFRI framework, based on national phenotyping infrastructure (Belgium, France, Germany and UK). The partnership will be extended to other European member states.

EMPHASIS will develop and provide access to infrastructures addressing multi-scale phenotyping for analyzing genotype performance under diverse environmental conditions and quantify the diversity of traits contributing to performance in diverse environmental scenario (i.e., plant architecture, major physiological functions and output, yield components and quality).

EMPHASIS will address the technological and organizational limits. EMPHASIS will:
• develop an integrated pan-European network of instrumented phenotyping platforms using current and future agro-climatic scenarios.
• link data acquisition to a European-level data management system and to state-of-the-art crop models to simulate plants and crops in current and future climates.
• develop, evaluate and disseminate novel technologies, thereby providing new opportunities for research involved in phenotyping and precision agriculture.
• make these infrastructures and concepts accessible to European plant science community in academia and industry.

EMPHASIS infrastructures will include
• platforms in (semi-)controlled conditions for high resolution and throughput phenotyping
• Intensive field experimental sites
• a coordinated network of field experiments with phenotyping infrastructure
• Modelling platforms for testing existing or virtual combinations of alleles.

The Finnish Plant Phenotyping Infrastructure – The road to high throughput and high precision

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Current strategies on development of the Bio economy strongly depend on sustainable plant production of food, feed, fiber and biofuel. In this process fundamental and applied plant research approaches should form a single continuum aiming at meeting the demands of the growing world population. To facilitate fundamental plant research in Finland and the translation of the vast amount knowledge accumulated from model plants including extensive molecular and genomic data to crop plant performance, we have recently established a Finnish Research Infrastructure (FIRI) platform, the National Plant Phenotyping Infrastructure (NaPPI) at the University of Helsinki Viikki campus. This high throughput facility accommodates analysis tools for both small model plants such as Arabidopsis, large crops (legumes, cereals, potatoes, berries) and trees (spruce, pine, birch, poplar). The aim of NaPPI to provide services for the whole plant research community in Finland and in the Baltic region as well as to supplement existing research infrastructures on the different omics - (transcriptomics, genomics, proteomics, and metabolomics) with multi-analysis of plant phenotypes on campus. This system is based on automated plant transportation, imaging and data processing provided by Photon Systems Instruments (psi.cz). The imaging tools allow automated detection of morphological and fitness parameters, analysis of the photosynthetic capacity and physiological responses. These high throughput facilities are part of a distributed infrastructure together with the University of Eastern Finland high precision unit (www.spectromics.org) that focuses on hyperspectral imaging and multimodal approaches, including chemical imaging. Automation of these research aspects enhances data collection and analysis while it also makes the data more accurate since the imaging tools exceed human capacity of morphological and fluorescence detection. At University of Helsinki our flag ship research projects focus on high throughput screens on e.g. Arabidopsis mutant collections and breeding populations of crops. Examples of initial screens of will be presented.
**POSTER ABSTRACTS**

**ABIOTIC STRESS**

**Effect of isoprene on drought tolerance of *Arundo* ecotypes**


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Isoprene is the most abundant biogenic VOC emitted from vegetation to the atmosphere. It has been suggested that isoprene could prevent the oxidation of the photosynthetic apparatus under water deficit. The biosynthesis of isoprene appears to be remarkably resistant to drought stress and it may trigger the production of other secondary metabolites in plants with antioxidant effects such as phenols and carotenoids. In order to expand our understanding about the isoprene function in plants, we investigated the interaction between isoprene and non-volatile isoprenoid compounds during drought stress in different *Arundo* ecotypes differing in their isoprene emission capacities.

*A. donax* is an important biofuel crop in Mediterranean regions known to emit high amount of isoprene. During this study we used three ecotypes of *Arundo*, namely: Srebarna (Sr) and Plovdiv (Pl) from Bulgaria and Sesto Fiorentino (SF) from Italy. Bulgarian ecotypes were characterized by higher isoprene emission than the Italian ecotype at control conditions, however the difference between Pl and SF was not significant. The results of our experiments under control conditions showed that the net photosynthetic rate and efficiency of PS2 was higher in the Bulgarian ecotypes compared to the Italian ecotype. Under severe drought all three ecotypes had the same photosynthetic capacity and isoprene emission, however, Sr and Pl ecotypes recovered better than SF. Isoprene biosynthesis was more resistant to drought than photosynthesis in all the ecotypes. On the other hand, Sr and Pl ecotypes had higher chlorophyll A and B and carotenoid contents than SF. The production of phenolic compounds was also induced under drought, especially in the higher isoprene emitting ecotypes while in SF with lower isoprene emission the zeaxanthine content was higher than in the other two ecotypes during drought stress. After re-watering, the biochemical damage to the Calvin cycle was recovered largely in the high isoprene emitting ecotypes.

The results of this study demonstrate that isoprene enhances the stability of the chloroplastic membranes resulting in better photosynthetic efficiency of high isoprene emitting ecotypes especially under drought conditions. Our study also suggests that isoprene could induce the biosynthesis of other compound with protective role for plants under stress. The investigated ecotypes seem to have different biochemical approaches to deal with stress.
Plant hormones are small molecules that regulate plant growth and development, as well as responses to changing environmental conditions. By modifying the production, distribution or signal transduction of these hormones, plants are able to regulate and coordinate both growth and/or stress tolerance to promote survival or escape from environmental stress. A central role for the plant growth regulators in the response to abiotic stress is becoming increasingly evident. The main objective of my work was to monitor the impact of plant growth regulators on physiological parameters under drought stress. The experiment was conducted on a field experimental station in Žabčice on variety Matylda. Within this experiment following growth regulators and fungicide with growth regulation effect were used: Retacel extra R68 (chlormequat chloride 720 g.l\(^{-1}\)), Moddus (trinexapac-ethyl 250 g.l\(^{-1}\)), Cerone (ethephon 480 g.l\(^{-1}\)), Amistar (strobilirin 250 g.l\(^{-1}\)). From our preliminary results it can be concluded that almost all growth regulators increase the water use efficiency and definitely positive effect was found in growth regulator – ethephon. The results show that all regulators increased yield, which demonstrate a positive effect under dry conditions because the vegetation season was very dry and lodging did not occur. Furthermore, the most pronounced mitigation of drought stress was found for CCC and partly also ethephon. Our results confirm that the effects of different growth stimulators are very different and their selection can greatly affect not only the growth of aboveground part, but also the growth of the root system which can be very essential for example in dry years. Our results exclude the possibility of using the mentioned plant growth regulators as a partial alternative to the elimination of the drought.

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Water stress does not modify phenotype of two varieties of lentil (Lens esculenta Moench) but triggers the formation of stress proteins and some enzyme activities

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The present work proposes to study the effect of drought stress by arrest of watering on the phenotype and physiological behavior of two varieties of Lens culinaris: L Balkan 755 Amir and xChampagne.

Our results show that under the drought stress, growth and physiology of seedlings are not significantly affected. At the cellular level, stress causes a decrease of proteins content of leaves and some parameters associated with photosynthesis which are the total chlorophyll and the carotenoids. Drought also disrupts the water supply of seedlings but induces an accumulation of proline which could play a main role in osmotic adjustment. Electrolyte leakage and the malondialdehyde a reactive substance thiobarbituric acid does not change in stressed Balkan seedlings while these two parameters increase significantly in Champagne variety subjected to drought stress. Ascorbate peroxidase and Catalase enzyme activities are more important in the Champagne variety than in the Balkan range. At the protein level, we record a decrease in total protein content, result of reduced photosynthesis and mineral uptake. At the qualitative level, we have demonstrated by SDS-PAGE the appearance of two new stress proteins of approximately 30 kDa and 60 kDa. The first would be a 30 kDa protein which appears in stressed seedlings for both varieties. This protein would correspond to a CDSP (chloroplastic drought-induced stress protein). The role assumed to this chloroplast protein would be in relation to the tolerance of photosynthetic apparatus to dehydration. The 60 kDa protein is detected in seedlings stressed of the Balkan variety. We assume that this protein would be a dehydrin (Cap 60). These proteins prevent water loss and help to stabilize cellular proteins.

Keywords: drought stress, lentil, stress proteins
Evaluation of growth, proline gene expression and catalase activity in an autochthonous ecotype of cowpea (Vigna unguiculata L.) grown under salt stress

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Soil salinity is a major abiotic stress that limits plant growth and agriculture productivity. The ability of plants to tolerate salts is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions and maintain ion homeostasis. Essential pathways include those that lead to synthesis of osmotically active metabolites such as proline and induction of activities of certain antioxidative enzymes such as catalase which scavenge reactive oxygen species (ROS).

This work proposes to study the effects of salt stress induced by increasing concentrations of sodium chloride (NaCl): 50 mM, 100 mM and 150 mM on Vigna unguiculata L.

The results show that under the effect of 50 mM of NaCl, phenotype is not changed: growth and leaf areas of seedlings are not significantly affected. However, at higher levels of salinity, seedlings manifested a modified phenotype: significant reduction in shoot length of seedlings and leaf areas was observed.

At the cellular level, the salt induced an accumulation of proline which could play a main role in osmotic adjustment. This accumulation is shown to be related to an over expression of P5CS gene (pyrroline 5-carboxylate synthetase) which is involved in proline synthesis. However, ProDH expression in salt-stressed seedlings did not vary significantly compared to the control.

The effect of oxidative stress induced by salt stress on cowpea seedlings is determined by evaluating the membrane integrity of cells, the results showed that salinity increase the relative ion leakage in stressed seedlings compared to the control. However the oxidative damage seems to be counterpoised by increasing the catalase activity in stressed seedlings.

These results suggest that cowpea seedlings accumulate proline and increase catalase activity to mitigate detrimental effects of salt stress by decreasing level of ROS accumulation.

Keywords: salt stress, growth, Vigna unguiculata, osmotic adjustment, ROS, P5CS, ProDH.

Image-based phenotyping to elucidate salinity stress responses in rice

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Salinity is one of the major environmental limitations for irrigated agriculture with yield losses estimated to exceed $12 billion annually. Only 17% of agriculture is under irrigation, yet it provides a third of the global food supply. Rice is arguably the most important crop for global food security, but is also the most salt-sensitive among staple cereals. It is therefore important to discover the genetic and physiological basis of salinity tolerance in rice by better characterizing the existing germplasm.

To this end, we are combining the genotypic resources for a rice diversity panel with high-throughput image-based phenotyping for elucidating the physiological and genetic basis of salinity tolerance in rice. The diversity panel includes ~400 rice accessions collected from more than 80 countries and has been genotyped with high density SNP arrays. Using non-destructive phenotyping, we were able to capture the dynamic responses at both early and late stages of salinity stress. Results from quantitative genetic association analysis with this dataset will be presented. Loci identified from this study have enabled us to identify novel genes and allelic variants involved in salinity tolerance.
PHENOPLANT, a feature of PHENOTIC: Phenotyping horticultural products and the interactions between host plants and pathogens

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Within the PHENOPLANT offer, the platform PHENOTIC developed phenotyping activities on whole plants. Mainly two objectives are developed:

Objective 1: Quantification of the impact on plants of various biotic and abiotic stresses:

Non-conventional imaging approaches allow imaging of processes that are not visible by human eyes, especially through the use of various wavelength in the UV or in the infrared. Thermal, hyperspectral and chlorophyll fluorescence imaging started to be used for studying plant biology only very recently. These techniques are powerful tools to visualise transpiration on leaves as well as photosynthetic activity of plant tissues. Yet biotic and abiotic stresses impact these two parameters. On PHENOPLANT, we use contrasts obtained through these non-conventional imaging techniques to measure the foliar surface impacted by these stresses using image analysis.

Using the PHENOPLANT tools, pathogens may be localized within plant tissues in a non-destructive manner, even though no symptom may be visible by human eye. In particular, we use chlorophyll fluorescence imaging to quantify plant resistance towards pathogens using image analysis. We developed an automated service for image analysis and shaping the obtained results that is available on PHENOTIC web page.

Objective 2: Characterization of the quality of horticultural products:

The architectural analysis of bushes such as rosebush aims at, first, evaluating the effect of environmental factors on the shape and the aesthetics of plants, and secondly, study the genetic determinism of plant architecture. Such an analysis necessitates the acquisition of morphological, topological and geometrical data. On PHENOPLANT, such data can be acquired at various scales, with a precision depending on the targeted organ, using sensors adapted to the acquisition of architectural data.

Frustration & elation – Six years using UAVs in breeding trials

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In 2009, an encounter between a crop physiologist and a roboticist led to a collaboration in which we have used a CSIRO-designed autonomous ‘PhenoCopter’ to characterise breeding and physiology trials (Merz and Chapman 2010). These trials have been on wheat, sorghum, cotton and sugarcane and included abiotic stresses associated with water and nitrogen deficits, typically comparing 10 to 100 genotypes with flights over 1 to 5 ha areas.

When our research began, only large machines (> several $100 000 in price) were available and this platform has since been used in multiple applications including remote rainforest surveys. In the last six years in more than 200 flights, we have developed a workflow to make best use of the image data in both native form and after post-processing through image mosaic and digital surface model software. Rapid development of aerial platforms, specialty cameras and processing software have greatly reduced the cost of these systems and provide convenient and quickly deployed phenotyping tools (e.g. we now use $1000 multicopters). This paper traces our experiences with developing these systems and our expectations of where they are heading, in particular in the combining of such systems with wireless stress monitoring sensors in field trials.
Monitoring canopy temperature patterns in two grapevines varieties: a tool to compare genotypes and optimize deficit irrigation?

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Modern Mediterranean viticulture faces increasing risks due to more stressful climate conditions and scarcer water resources. In addition, the sector must also address increasing concerns of stakeholders on sustainable production and environmental impact of wine production. Irrigation water must be judiciously used in the vineyard in dry areas such as the Mediterranean. A better understanding of grapevine physiological responses to drought and heat stress will help to optimize crop/soil management, increase water savings and berry quality. Canopy temperature (Tc) can be used as a non-destructive parameter to characterize stomatal physiology and to monitor water stress in grapevine. Thermography permits the remote determination of Tc in field and to assess the range and spatial distribution of temperature from different zones of the canopy (and soil). Our hypothesis is that diurnal Tc patterns can differ between genotypes due to different stomatal control and heat loss strategies. To that extent, we have monitored the diurnal and seasonal course of Tc in two V. vinifera cvs Aragonez (syn. Tempranillo) and Touriga Nacional subjected to deficit irrigation under typical Mediterranean climate conditions in South Portugal. The diurnal course of Tc was complemented by measurements of leaf water potential (ψwad), and leaf gas exchange. Measurements were done in 2013 and 2014, along different phenological stages: i) mid-June (green berry stage), ii) mid-July (veraison), iii) early August (early ripening) and iv) end August (before harvest, late ripening). The relationship between Tc(14–17h), gs and ψ was more robust in 2013 (drier and hotter conditions). Correlations between Tc and physiological parameters were similar in the two genotypes. Strategies to optimize the use of thermography in field conditions are suggested.

Keywords: thermography, evaporative cooling, diurnal and seasonal water stress, grapevine phenotyping, water use.

Development and use of a fully automated PHENOMOBILE for field phenotyping

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The capacity for detailed high throughput phenotyping in field conditions is mandatory to better describe structural and functional traits over large collections of micro-plots. An Unmanned Ground Vehicle called PHENOMOBILE-V1 was developed for this purpose within the framework of the PHENOBLE and PHENOME projects. The vehicle moves automatically over the micro-plots following predefined trajectories within a centimeter accuracy using GPS-RTK positioning. It is equipped with four drive and directional wheels to be able to operate in most conditions of ground. The PHENOMOBILE-V1 spans over the micro-plots with an adjustable width. All the systems are powered by electricity produced by a generator. A platform with automatically adjustable height is equipped with several sensors including RGB cameras for organ counting and green fraction measurement, spectroradiometer working in the visible and near infrared domain for canopy biochemical composition estimation, and LIDARs for 3D description of the canopy architecture. All these measurements are performed from nadir and inclined directions to acquire complementary information on canopy structure. The sensors are running in active mode using synchronized flash for measurements fully independent from the natural illumination conditions. They are automatically triggered as defined in acquisition scenarios.

The system is currently providing a throughput around 150 micro-plots per hour, allowing sampling around 1000 micro-plots within a day. The system may also run during the night. A dedicated processing chain was developed to transform the raw data into biophysical variables while significantly reducing the data volume.

After describing the main functionalities of the PHENOMOBILE-V1 we will present its first operational application over a large wheat experiment conducted in 2015 studying crop response to water stress. Results consists of dynamics of canopy height, green fraction, and spectral indices on both well-watered and water stressed micro-plots. Perspectives on new data processing modules and sensors will finally be addressed.
Plant phenotyping is the assessment of morphometric traits such as growth, development and yield, and the functional traits like photosynthesis, respiration, transpiration, quantum yield, and chlorophyll fluorescence. Plant phenotyping has become a widely used tool in the characterization of plant growth and stress responses. Tolerance to drought is a complex phenomenon and controlled by multiple genes imparting a number of physiological and biochemical changes and no single trait explains the mechanism of drought tolerance. Many physiological characters are known to be involved in drought tolerance. It’s the case of photosynthesis and respiration. In this study, the response to water deprivation was compared between *Nicotiana sylvestris* wild type (WT) plants and the CMSII respiratory complex I mutant, which has low efficient respiration and photosynthesis and drought tolerance. This mutant presents higher ratio respiration: net photosynthesis. Thus, this ratio could represent a good index of screening plant genotypes for drought tolerance.

**Keywords:** mitochondria, drought, respiration, photosynthesis, *Nicotiana sylvestris*.

**Impact of domestication and post-domestication on phenotypic diversity of tetraploid wheat genotypes under contrasting nitrogen fertilisation**

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**Introduction**

The process of domestication has led to dramatic morphological and physiological changes in crop species due to adaptation to cultivation and to the needs of farmers. To investigate the phenotypic architecture of shoot- and root-related traits and quantify the impact of domestication and post-domestication, we examined a collection of 36 wheat genotypes under optimal and nitrogen-starved conditions. These represented three taxa that correspond to key steps in the recent evolution of tetraploid wheat (i.e., wild emmer, emmer, and durum wheat).

**Materials and Method**

Time series images of root growth at the seedling stage were collected using the automated phenotyping platform GROWSCREEN-Rhizo (Nagel et al. 2012). Additional measurements were performed to quantify shoot growth, chlorophyll content, photosynthetic performance, and biomass.

**Results and Discussion**

Overall, nitrogen starvation resulted in reductions in the growth parameters of shoots for all genotypes, while it induced the opposite effect on root traits. An overall increase in all of the shoot and root growth traits from wild to durum wheat were observed, while emmer was in general very similar to the wild, but intermediate between these two subspecies. Indeed, while the differences in phenotypic diversity due to the effects of domestication were small and never reached significance, the post-domestication transition from emmer to durum wheat was marked by a large and significant decrease in the coefficient of variation. In particular, this reduction was very strong under normal nitrogen, and less intense under nitrogen starvation. Moreover, while under normal nitrogen both root and shoot traits showed significantly reduced diversity due to post-domestication, under nitrogen starvation the reduced diversity was significant only for shoot traits.

**Conclusions**

Overall, our data indicate that post-domestication episodes of breeding have greatly affected the plant architecture, both in terms of shoots and roots, which has promoted the ability of the plants to increase their growth under high nitrogen fertilisation, and to a lesser extent, to increase the above-ground growth also under low nitrogen availability. In contrast, tetraploid wheat domestication did not substantially influence the plant growth, at least in the very early stages, as seen here for both optimal nitrogen and nitrogen starvation regimes. Finally, our results indicate that there is considerable genetic variation for the studied phenotypic traits in wild and landrace germplasm which could be exploited for the development of
pre-breeding programmes. Similar to other studies, our findings support the view that there is the need for the conservation evaluation and use of wild progenitors and landrace populations for further crop improvements.

References

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**LeasyScan – A novel semi-field platform to phenotype traits controlling plant water budget**

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We describe the concept and realisation of a high-throughput phenotyping platform (LeasyScan) combined with lysimetric capacity, to assess canopy traits affecting water use. The platform is based on a novel 3D scanning technique to capture canopy development, a scanner-to-plant concept to increase throughput (2500 plants/h), and analytical scales to combine gravimetric transpiration measurements. We present how the technology functions, how data are visualised via a web-based interface, and how data extraction and analysis is interfaced through ‘R’ libraries. Close agreement between scanned and observed leaf area data of individual plants in different crops was found (R² between 0.86 and 0.94). Similar agreement was found when comparing scanned and observed area of plants cultivated at densities reflecting the field conditions. Example of the monitoring, the plant transpiration by the analytical scales is presented. Moreover we present some on-going applications of the platform to target key phenotypes: (i) the comparison of the leaf area development pattern of fine mapping recombinants of pearl millet; (ii) the leaf area development pattern of pearl millet breeding material targeted to different agro-ecological zones; (iii) the assessment of the transpiration response to high VPD in sorghum and pearl millet.

**Field Scanalyzer delivers phenotypic data for plant improvement**


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Grand challenges of the future, such as feeding increasing human population, dealing with climatic uncertainties, environmental stress, as well as conserving biodiversity and natural resources, demand for substantial improvements in plant breeding and crop management. Multi-level detailed analysis of plant properties, reaching from genome to phenome, is essential for making plant breeding, cultivation, and management compatible to future scenarios. Substantial improvements in plant production and adapting plants to novel cases of use in bio-based materials, energy, and pharmaceuticals demand for rapid and reliable characterisation of plant traits, in particular phenotypes. At the same time plant production and management has to gain strongly in resource use efficiency in order to deal with increasing limitations in resource availability, and to minimise negative effects on natural ecosystems.

Phenotypic analysis takes place at laboratory-, greenhouse-, and field- scale and therefore demand for platforms that carry appropriate sensor technology enabling non-invasive measurements. Such technology delivers reliable, repeatable, and standardised datasets for plant science and breeding. Sensor fusion makes data acquisition and evaluation comprehensive, but complex at the same time. Complex data sets in turn enable mathematical models to describe and predict plant functionality and structure.

In particular field performance is of outstanding interest for researchers and breeders and therefore the novel Field Scanalyzer was developed to assess phenotypes of plot-grown plants. The first installation was recently inaugurated at Rothamsted Research in a joint project with technology provider LemnaTec. Preliminary data from the 2015 pilot study give a first impression of the performance of the novel setup. The machine allows high precision sensor positioning that enables repeated measurements of individual plant positions. Sensors, such as laser scanners, RGB cameras, thermal cameras, hyperspectral cameras, and chlorophyll fluorescence imaging units delivered data that show differential responses of tested cultivars towards the applied nitrogen fertilisation levels. Thereby, NDVI index derived from sensor data characterises
Genotype-specific plant growth responses to temperature as a case example for the capabilities of the field phenotyping platform FIP at ETH Zürich

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Crop phenotyping is gaining increasing importance in modern breeding. Investigations ideally should be performed in the target environment on large numbers of replicates for critical traits related to yield. We developed a platform enabling the monitoring of multiple plant traits using remote-sensing techniques. For this purpose a rigged sensor carrier system was installed at ETH Zürich research station for plant sciences in Eschikon Lindau, Switzerland. It can be freely positioned in 3D over a square field of 1 ha area. Its capabilities were recently tested in a wheat association panel comprising 300 genotypes, which were monitored with respect to their growth reaction to temperature at different times of the year.

The complete rigging system including the sensor head was designed and manufactured by Spidercam®. At each of the four field corners, a 24 m high pole was set up with a pulley mounted on top and a winch located in a hut at the base. The pulleys deflect the ropes from the sensor head to the winches. Coordinated adjustment of rope lengths by the winches allows free positioning of the sensor head in three dimensions above the field. Six sensors were mounted on the head i.e. a visible-, a near infrared, a thermal camera, two spectrometers and a terrestrial laser scanner. The sensor unit includes a pan-and tilt-drive which allows for arbitrary observation directions of the sensors. An integrated operational camera gives an image of the sensors’ perspective for positioning purpose. Considering the maximum payload of 12 kg the sensors can be exchanged keeping the system flexible for future applications and measurements.

A high spatial reproducibility is achieved by an integrated position calibration of the system.

The complete system of positioning and sensor release is freely programmable. Therefore, measurement scripts were set up for each experiment, enabling the automated acquisition of all plots of an experiment.

To date various test measurements were taken in order to get the system in automatic, supervised operation. This includes plot-wise monitoring of the above-mentioned winter wheat experiment of 700 plots with a near infrared camera which enables robust segmentation and determination of canopy coverage. Such data was used to calculate differences between genotypes with respect to their growth response to cold temperatures in winter (proof of concept with manually acquired data published in Grieder et al. 2015). Moreover, it was evaluated, whether terrestrial laser scanning can be used to measure genotype-specific responses to fluctuating meteorological conditions during the critical stage of stem elongation.

During execution of the measuring scripts the positions of the plots are successively approached by the sensor head and the sensors measure at each position. Data are automatically stored in the experiment file system and named appropriately.

The setup of the system offers an economic, robust and fast acquisition of large data sets for plant phenotyping in the field. It therefore represents a novel and promising phenotyping platform, complementing the spectrum of existing systems. Apart from the depicted analyses of genotype-specific plant growth responses to temperature, other abiotic and biotic stress experiments can be performed at high temporal and spatial resolution.

The homeodomain transcription factor TaHDZipl-2 from wheat regulates flowering time, frost tolerance and a grain size

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Homeodomain leucine zipper class I (HD-Zip I) transcription factors (TFs) are important players in regulation of plant growth and development under different environmental stimuli and stresses. The TaHDZipl-2 gene was isolated from endosperm of developing grain. It is expressed in most young wheat tissues and down-regulated by cold. Molecular characterisation of TaHDZipl-2 included analysis of its protein and DNA binding properties. In transient expression assays TaHDZipl-2 can activate artificial promoters, and this activation is boosted two-fold by elevated levels of ABA. For further functional characterisation the TaHDZipl-2 gene was constitutively expressed in transgenic barley using a maize ubiquitin promoter. Transgenic TaHDZipl-2 lines showed characteristic phenotypic features that included reduced growth rates, plant height and biomass, early flowering, light coloured leaves, narrow elongated spikes and small grain. Transgenic lines produced 25–40% more biomass accumulation during time and analyses on colour and texture allow plot classification. Via laser scanners, 3D surface of the growing canopy can be measured. In next steps of data processing, interpretation, and validation, sensor-derived parameters have to be translated into plant data that can be used by scientists and breeders.
seeds per spike than control plants. However, because of the 50–60% smaller grain size the yield of transgenic lines was lower than yield of control plants. A detailed analysis of grain composition revealed no significant differences between transgenic grain and control grain. In vivo lipid imaging exposed changes in the distribution of lipids between the embryo and endosperm of transgenic seeds. Transgenic barley seedlings demonstrated significantly higher frost tolerance than control seedlings. Comparison of expression levels of potential downstream-regulated genes in control and transgenic plants revealed significant changes in transcript levels of several known regulators of flowering time, vernalisation, cold adaptation and spike development. Our data suggest a role of TaHDZipl-2 in control of several key processes responsible for transition to flowering and spike development.

Root traits and stable isotopes composition of durum wheat under water stress

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The aim of our work was to investigate the effect of water regime and genotype on root traits and stable isotopes signatures ($\delta^{13}$C and $\delta^{15}$N) of durum wheat seedlings. Ten durum wheat genotypes were cultivated under two contrasting water regimes, well-watered (WW) and moderate water-stressed plants (WS) in a growth chamber. Plant growth, photosynthetic and gas exchange parameters and expression level of Aquaporins (AQP) and Glutamine Synthetase (GS2) were also assessed. Our result showed that water stress decreased the value of root volume (RV), specific root length (SRL), root length density (RLD) and shoot/root ratio, as well as photosynthetic rate ($A_{uw}$), stomatal conductance ($g_{s}$), transpiration rate (E) and leaf chlorophyll content (SPAD), whereas $\delta^{13}$C and $\delta^{15}$N in plant dry matter, together with the expression of AQP and GS2 were increased. Aerial dry weight (ADW) was positively correlated with root dry weight (RDW), RLD and RV and negatively correlated with $\delta^{13}$C regardless of the water regime. $\delta^{15}$C was correlated negatively with RDW, RLD and SRL and positively with AQP expression. GS2 showed a positive correlation with $\delta^{15}$N and RDW. Our study highlights the potential applicability of some root traits together with the isotope signatures and some physiological traits to assess in durum wheat adaptation to water stress during crop establishment.

Keywords: Water stress, durum wheat, root traits, $\delta^{13}$C, isotopes.

VPD Response variation in pearl millet and wheat

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The development of society and industry joined to global warming dwindle the water resources available for agriculture. In a near future, the crops must enhance their productivity with less available water. Under conditions of high evaporative demand (hot and dry air), restricting transpiration is an important avenue to gain in efficiency of water use. During the last years large genotypic variation in the restriction of water loss under high VPD has been found in different crop species. How much the VPD-response depends on the environment where the genotype/line has evolved or for which they have been bred, is unknown. In this presentation, we conducted two VPD response experiments in glasshouse, one in India (ICRISAT) with 8 F1-Hybrid and parental line of Pearl millet (adapted to zones varying in rainfall), and another in Spain (UB) with 20 wheat lines (post-green revolution since 1977 to 2006). In all cases, we found a genotypic variation on the response-slope to high VPD, with environment-specific variation. For Pearl millet, the genotypes adapted to lower rainfall had higher transpiration response curves than genotypes from higher rainfall zones. In wheat, the transpiration response-slope to high VPD was lower in 790 ppm CO$_2$ than in 400 ppm CO$_2$. In terms of biomass, we found genotypic, growing conditions and adaptation-environment variations. All this differences in the transpiration response clearly show the genotype/line strategies to save water depended on the species and breeding history.
Biomass removal in spring as a method to differentiate wheat genotypes for drought tolerance

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Field testing for drought tolerance depends on precipitation and sometimes fail if too much rainfall occurs. Removal of wheat above ground biomass prior to stem elongation makes crop invest again in building biomass and utilize moisture. The degree of recovery after biomass removal and associated yield losses may depend on the root system development and reflect drought tolerance of the genotypes tested. In 2012–2013 set of winter wheat genotypes was subjected to biomass removal under irrigated and rainfed conditions. There was genotype x treatment interaction demonstrating that yield losses from biomass removal weakly associated with the yield under drought. Segregating populations F2 and F3 were subjected to selection under biomass removal control and conditions and originating lines are being tested for drought tolerance.

Phenotyping of barley resistance to powdery mildew (Blumeria graminis f.sp. hordei) using thermal imaging, spectral reflectance and chlorophyll fluorescence imaging

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The aim of this study was to test the potential of optical diagnostic methods for phenotyping resistance to fungal diseases based on chlorophyll fluorescence, spectral reflectance and thermal imaging. Within this experiment 6 genotypes (Pallas lines) of spring barley (Hordeum vulgare) carrying different resistance genes to powdery mildew and exhibiting different reaction types were tested. The infection was performed using two isolates of powdery mildew with contrasting virulence against resistance genes used.

Promising results for phenotyping of disease resistance have been obtained using thermal imaging. Following infection the increase of leaf temperature was observed and this was more pronounced in virulent isolates. Significant temperature change was found on the third day after infection, but the strongest change in temperature occurs in the leaves on 4–6 days, i.e. shortly before the appearance of visual symptoms.

Chlorophyll fluorescence imaging showed significant changes after infection particularly in parameter NPQ, however, these changes were not consistent with virulence of isolates and reaction types. Therefore the discrimination of resistant and sensitive genotypes is not easily possible using this method. Integration (point) measurements of fast fluorescence kinetics parameter 1–Vj showed response to disease progress already one day after infection, particularly in sensitive genotype. However, the interactions with individual resistance genes and reaction types should be further studied and understood.

Measurement of spectral reflectance on leaf level showed no significant effect of powdery mildew infection before 6th day after infection. The analysis of vegetation indices from 6th day revealed a response particularly of indices based on reflectance in the red-edge (e.g. ZM = R750/R710) and also in the green bands.

Using automated high-throughput phenotyping to quantify and visualize early stress responses in plants

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Non-invasive capturing and interpreting of plant structural and functional phenotypes in controlled or dynamically changing environment is long-standing and necessary requirement for genetic and physiological research by crop breeders, agricultural industry, and academia. To sustain global food security the major challenge global agriculture and plant biology field has been facing is the identification of new high-yielding genotypes of agricultural crops that are adapted to our future climate. The use of automated high-throughput phenotyping approaches offers the tool for accelerating crop breeding progress by linking the selected gene function with phenotypic traits in relevant environment.

Here we describe the concept of a high-throughput phenotyping platform (PlantScreen™ System) for dynamic quantification and visualisation of phenotypical signatures, which provide a wealth of information about plant development, physiological status, and performance. The platform incorporates imaging technologies for RGB and morphometric analysis, thermal analysis, hyperspectral analysis and, critically, in-depth analysis of chlorophyll fluorescence kinetics. The latter
technique, as a rapid tool for monitoring photosynthetic processes, is key to the identification of early onset of stress, and recovery from stress after amelioration. The concept of the platform is based on using integrative approach when multiple non-destructive image-based technologies are combined in order to capture multi-dimensional phenotypes in spatial and temporal resolution.

We present the different imaging techniques used for monitoring plant growth, physiological performance and biochemical composition and discuss their application in screening for phenotypic traits associated with early response of plants to various abiotic factors. Two application examples are presented, which demonstrate the capacity of the integrative phenotyping approach in monitoring growth dynamics and physiological performance of plants subjected to adverse conditions. First quantification and visualization of early stress responses of plants to non-selective, broad-spectrum, postemergence herbicide glyphosate will be discussed. Second quantification of early plant responses to salinity, independent of shoot-ion accumulation, in Arabidopsis thaliana ecotypes will be shown.

The integrative concept of PlantScreen™ high-throughput phenotyping platform provides a powerful tool for acquisition and selection of morphological, physiological and biochemical parameters, which can be used for identification of various components underlying early plant responses to environmental conditions.

**Phenotypic and metabolic responses to ozone of two Fraxinus excelsior provenances**

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Environmentally-induced phenotypic variations in plants are considered to be functional responses that maximize fitness in variable environments. Provenances of a species may have evolved mechanisms to cope with stress and to complete their life cycles under hostile conditions. In particular, the magnitude of metabolic alterations in response to stress correlates with sensitivity/tolerance. Many phenylpropanoid compounds play a crucial role in plant defense against several environmental stressors such as tropospheric ozone (O₃). Leaf physiological and metabolic traits of two Italian provenances [Piedmont (P) and Tuscany (T)] of Fraxinus excelsior (two-year-old plants) exposed to O₃ (150 ppb, 5 h d⁻¹, 35 consecutive days) were investigated to verify if the geographical gradient affects O₃ sensitivity. Analysis of visible injuries, chlorophyll a fluorescence and phenylpropanoids were performed. In P plants exposed to O₃, the onset of visible injuries [widespread chlorosis which developed in minute (1–2 mm Ø) roundish dark-reddish necrosis scattered among the leaf veins of the adaxial surface of completely expanded leaves] occurred after 12 days of treatment; in treated T plants it occurred after 21 days from the beginning of the exposure. Throughout the whole period of exposure, chlorophyll a fluorescence analysis has been applied with success in a high-throughput phenotyping system. Differential responses to the pollutant between provenances were observed in terms of physiological responses. Negative effects of O₃ on PSII were evident in P plants [the variable and maximal fluorescence ratio and the maximum quantum yield of PSII (ΦPSII) decreased throughout the whole period]. By contrast in T, these effects were shown intermittently and plants recovered at the end of the treatment (ΦPSII remained unchanged). Differential responses to the pollutant between provenances were observed also in terms of metabolic responses. A non-targeted metabolomics approach was applied using HPLC, detecting 18 phenolic compounds in both provenances and other de novo peaks (potential phytoalexins) in O₃-elicited plants. The application of this approach provides a wider perspective of metabolic responses to O₃ and supports the discovery of stress tolerance-specific metabolic phenotypes. Specific metabolite indicators were identified. Among hydroxycoumarins (Fraxinus-specific compounds), only scopoletin showed differential responses, decreasing only in T plants (-53%). Still among species-specific compounds, verbascoside decreased in comparison to controls only in T plants (-70%); oleuropein increased up to more than 8-fold only in P. Also trans-chalcone (present in the middle of the flavonoids biosynthesis pathway) increased up to 4 times over the controls only in treated P plants. Flavonoids were affected by O₃ only in T plants: rutin (a quercetin glycoside) decreased by 56%; isoquercetrin and quercetrin showed higher values than controls (+152% and +120%). Among the hydroxibenzoic acids, O₃ exposure decreased the syringic only in P plants (-48%). Also the hydroxycinnamic acids, placed at the beginning of the coumarin biosynthetic pathways, showed differential responses to the pollutant: p-coumaric increased only in T plants (+126%). We can conclude that: (i) the phenotypic variability and the geographical gradient affect the O₃ response mechanisms, (ii) P plants activated different mechanisms (in terms of visible injury and PSII functionality) in comparison to T, (iii) P treated plants showed the highest biosynthesis within some specific phenolic metabolites (i.e. oleuropein, trans-chalcone), and (iv) metabolomics is a diagnostic tool that provides a powerful means to gain a better understanding of structural and physiological responses to O₃.
Benchmarking of an affordable thermal camera for plant phenotyping

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High-throughput and non-invasive plant phenotyping devices are expected to greatly improve the speed and quality of plant breeding and research, by relieving the so-called ‘phenotyping bottleneck’. In many cases these devices still have high initial costs and, therefore, access to this technology is usually restricted to a limited number of research institutes and private companies, mainly in developed countries. A way of widening the access to these tools is to develop affordable alternatives, which could be deployed with low initial and maintenance costs. Off-the-shelf consumer devices, such as pocket digital cameras or 3D sensors used in videogames, have usually been proposed as alternatives for plant phenotyping. However, their low cost might be associated with limited capabilities (i.e., precision, accuracy, automation), as compared to custom and/or sophisticated phenotyping equipment. Benchmarking of these devices is therefore necessary, in order to establish the range of conditions under which these affordable devices yield useful results.

In this work we evaluated the FLIR One (FLIR Systems, USA), which is the first low-cost (ca. EUR 300), consumer-oriented thermal camera that has been released on the market. This device is attached to a smartphone (an Apple i-Phone 5 in the evaluated version), and is based on a low resolution (80 x 60 px) sensor. A comparison was made to a high spatial resolution (320 x 240 px) industrial thermal camera (FLIR A320). The thermal sensitivity of both sensors is similar (0.05 °C), but while the accuracy of the FLIR A320 is reported to be ±2 °C, that of the FLIR One is not supplied by the manufacturer. Cameras were tested under controlled environmental conditions by analyzing the measured temperatures of two rapeseed plants, which were imaged with both cameras every 30 seconds during 2 hours. The temperature of the background was simultaneously registered with a temperature sensor. Plants were first kept in the dark, and after 30 minutes they were illuminated with LED lights (700 μmol m⁻² s⁻¹ PAR), which increased the temperature of the background by 2.4 °C and that of the leaves by about 1.9 °C. The FLIR One camera showed very high fluctuations in the measured temperatures, which resulted in a very low accuracy in the measurement of the image background temperature (RMSE of 6.1 °C vs. 1.0 °C for the FLIR A320). As a result of this, the measured temperatures of the plant leaves were not correlated between the two cameras (R² < 0.01). These fluctuation could, however, be corrected using the background temperature as a reference. After this correction, the measured temperatures of the leaves were highly correlated between both cameras (R² > 0.95).

These preliminary results show that it is possible to obtain non-invasive temperature data from plants using an affordable consumer device, with results that are similar to those obtained with a more expensive, scientific-grade camera. Further testing is required in order to evaluate the performance of this device.

Disease detection in seed potatoes using hyperspectral imaging

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Virus and bacterial diseases are one of the biggest problems in the cultivation of seed potatoes. Once found in the field, virus and bacteria diseased potatoes lead to rejections of the tubers resulting in a big financial loss. The estimated direct damage caused by diseases for seed potato growers in the Netherlands annually counts up to approximately € 12 million. Currently detection of diseased seed potatoes is done by human selectors, with an annual labor cost of approximately € 6.5 million. Much damage occurs when the disease is not detected in an early stage, which is often the case, when the bacterial infection is latent present, showing no symptoms in the plants. Therefore there is a need for fast and adequate disease detection, which also detects diseased plants without visible symptoms to human observers. Early detection of diseased plants with modern vision techniques can significantly reduce costs.

In 2011 a small laboratory experiment was carried out with potato plants grown in the greenhouse. Amongst other techniques, hyperspectral imaging was used to classify virus and bacteria infected plants from healthy plants. Hyperspectral images were taken from the top of the plants. Supervised classification using a Fisher linear classifier on a random selection of pixels of the top leaves was used to separate healthy from diseased plants, using the bacteria concentration from the inoculation as ground truth. For virus infected plants using images of all growing stages, 1 out of 8 diseased plants was classified wrongly as healthy, and 3 out of 22 diseased plants were wrongly classified as healthy. When measuring full-grown plants, all plants were properly classified. For bacteria diseased plants the results were worse, but our method found 50% of the diseased plants, where the crop experts only found 30%. Plants not detected by the crop expert didn’t have visual symptoms, but the hyperspectral camera obviously found enough differences in the leaf pixels to classify the plant as sick.
In 2015 a new experiment was set up, using potato plants grown in the field. Since bacteria accumulate in the vascular bundles especially in lower parts of the stem, images were taken from the side of the plants. This way the stems can be analyzed much better compared to the previous experiment. Using hyperspectral imaging the stems could be easily separated from the leaves, in order to analyze them separately. Currently the analysis to classify diseased from healthy plants is carried out.

Elucidation of the functions of the plant hormones cytokinins during the drought stress response in Arabidopsis
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The role of cytokinins (CK) during drought stress response was explored using Arabidopsis thaliana transformants over-expressing gene for CK deactivating enzyme cytokinin oxidase/dehydrogenase (CKX) and for CK biosynthetic enzyme isopentenyltransferase (IPT). Transgenes were under the control of constitutive (35S:CKX), dexamethasone induced (pOp/LhGR:CKX, pOp/LhGR:IPT) or senescent activated promoters (SAG12:IPT). CK levels were modulated also by application of stable aromatic CK. Drought stress caused inhibition of plant growth (detected after 4 days) and decrease of leaf water content. Re-watering led to growth renewal at elevated speed. CKX transformants (35S:CKX and pOp/LhGR:CKX) were smaller in control conditions and their drought induced growth suppression was relatively low. These transformants did not accelerate growth rate with age, and during recovery, stressed variants balanced growth rate on the same level as their same-old controls.

Drought response of all experimental variants (with exception of SAG12:IPT and pOp/LhGR:IPT) was associated with down-regulation of active CKs trans-zeatin (tZ) and its riboside (tZR), which was partially compensated by elevation of less active dihydrozeatin (DZ), isopentenyladenine (iP) and cis-zeatin (cZ). Levels of cZ, cZR and its storage form O-glucoside were elevated during drought in all variants except pOp/LhGR:CKX. Recovery was associated with up-regulation of active CKs (even in comparison with well-watered controls), which was not so high in 35S:CKX and pOp/LhGR:CKX. Content of active CK was lower after application of exogenous CK, and remained lower during drought.

Senescence-inducible promoter SAG12 strongly enhanced IPT expression in drought and all CK metabolites were strongly up-regulated (in order to prevent excessive elevation of active CKs, as very high CK levels may induce programmed cell death). pOp/LhGR:IPT had also higher level of IPT expression and content of active CKs and their metabolites was raised.

Transcription of endogenous CKX1 gene elevated after exogenous CK application and in IPT transformants, pOp/LhGR:CKX maintained low CKX1 transcription. IPT3 transcription was low during drought in variants with elevated content of CK, but its expression increased during recovery. By contrast, CKX mutants had higher level of IPT3 during stress.

Level of auxin (indole-3-acetic acid, IAA) decreased during drought stress in WT Col-0 and pOp/LhGR:IPT, although drought imposed strong increase of inactive auxin metabolites in all stressed variants. IAA was slightly increased after recovery in comparison with control plants (except CKX transformants), which correlated with faster growth rate.

Stress hormones abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) were strongly up-regulated during dehydration responses in all variants. Increase in ABA during drought was accompanied by elevation of its metabolites, which were much higher in pOp/LhGR:IPT and after exogenous CK application (and also the levels of stress marker genes were higher than in control plants). After re-watering, ABA decreased below the level in control plants.

CKX transformants exhibited lowered levels of active CKs in comparison with Col-0 under drought and control conditions, and their increase during recovery was not as high as in Col-0. Surprisingly, 35S:CKX plants had higher content of cZ, less active CK associated with stress response. Lower CK levels in CKX transformants coincided with lower level of IAA, but furthermore with reduction of ABA.

Lower level of CKs negatively affected growth rate, but were associated with higher drought tolerance. Even though the total content of CKs was elevated in IPT transformants and after application of exogenous CK, its impact on stress tolerance was not different from control Col-0.

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High-throughput field phenotyping of apple tree response to drought by UAV thermal imaging: Supervised image classification for unravelling intra-canopy temperature variation
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In the context of climate change, general temperature increase and summer drought periods are expected, notably in the Mediterranean region. Research of tolerant varieties to water stress is therefore relevant regarding future breeding scope. This research aimed at phenotyping the apple tree response to water stress in a field trial where a segregating population (123 hybrids) was submitted to contrasting water regimes. Our assumption was that differences in stomatal closure among progenies can be efficiently estimated through high-resolution airborne imaging, including thermal signature, since reduction in transpiration rate and subsequent loss of latent heat flux is traduced by an elevation of canopy surface temperature.

By using Unmanned Aerial Vehicle (UAV) as vector, and multispectral and thermal image acquisition, a series of flights were planned on sunny days with high evaporative demand, at 40m elevation, making it possible to characterize the tree response at individual scale, simultaneously across the trial. Zenithal images were pretreated, i.e. orthorectified and normalized, then georeferenced and mosaicked. Retrieval of spectral values from orthomosaics was performed on tree individuals within a central 60cm radius buffer zone. This allowed computing individual vegetation and water stress indices. Since indices computation relied on composite images, we tested the contribution of supervised classification of thermal images for unravelling well-illuminated leaves from shadowed vegetation or soil pixels. Comparison between different image analysis methods revealed usefulness of classification to improve the interpretation of thermal images, in relation to water stress and genotype effects. It opens the perspective of high-throughput pipelines for image analyses.

Protocol under field conditions with water gradient for plant phenotyping to drought and water use efficiency
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In the Brazilian savanna region it is important to have two different biotypes for sustainable agriculture. One genotype needs to be drought tolerant (DT) for the non-irrigated crop season and the second genotype needs to show a sufficient water use efficiency (WUE) as crops being irrigated during winter season. The long dry period in the winter month facilitates field phenotyping experiments with a water deficit question without the necessity of rain out shelters. A reel pulling one irrigating bar system where each sprinkler can simulate different irrigation levels and therefore create a water gradient has been used for phenotyping experiments at Embrapa Cerrados Station (Planaltina, Brazil). The obtained information help to develop new genotypes adapted to this region for both irrigated and non-irrigated system for several crops. Different drought stress experiments using the real pulling system were carried out between 2005 and 2015, during dry season, using several species such as wheat, soybean, sugar cane, sorghum, barley, coffee, millet, quinoa and amaranth. Several agronomic morphologic and physiological parameters have been evaluated in different water stress level. The results show that the methodology using bar and reel irrigation generating gradients has been efficient to select and characterize genotypes tolerant to drought, efficient water use and to assess responsiveness to irrigation.

Physiological phenotyping of salinity and drought cross-tolerance in tomato conferred through ectopic overexpression of a cell wall invertase
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Abiotic stress conditions such as salinity and drought modify source-sink relations, thereby influencing plant growth, adaptive responses and consequently crop yield. Invertases are key metabolic enzymes regulating sink activity through the hydrolytic cleavage of sucrose into hexose monomers, thus playing a crucial role in plant growth and development. However,
the physiological role of invertases during adaptation to abiotic stress conditions is not yet fully understood. Here we show that salinity tolerance and water use efficiency (WUE) can markedly be improved in tomato by ectopic overexpression of the cell wall invertase gene CIN1 from Chenopodium rubrum. Under moderate salinity, CIN1 overexpression conferred higher fruit sink activity and reduced flower abortion, thereby increasing fruit yield up to 4-fold compared to wild type. Further, CIN1 overexpression caused a strong increase in WUE, markedly improving drought stress tolerance. Physiological phenotyping revealed that the dual abiotic stress cross-tolerance correlated with various metabolic changes. The determination of enzyme activity signatures revealed that these transgenic plants maintained a high extracellular invertase activity resulting in an outstanding drought tolerance. They showed an increased activity of enzymes involved in primary carbohydrate metabolism, a high photosynthetic activity and, notably, an increased water use efficiency. Antioxidant metabolism and the determination of phytohormone profiles revealed also an effect on hormonal balances. Ectopic CIN1 expression also induced cross-tolerance against salinity and resulted in increased tomato fruit sink activity and yield. Furthermore, metabolic changes induced in the CIN1 plants correlated with changes in the fruit hormonal balance. The determination of metabolic fingerprints showed that cell wall invertase critically functions at the integration point of metabolic, hormonal and stress signals, providing a novel strategy to overcome drought- and salt-induced limitations to crop yield, without negatively affecting plant fitness under optimal growth conditions.

References

Natural variation in Arabidopsis thaliana response to water deficit
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Plant response to soil water deficit involves complex survival strategies. Among others, they include efforts to maintain constant water potential by limitation of water loss e.g. decrease of transpiration rate and arrest of growth. Plants have also evolved dehydration tolerance mechanisms, leading to reorganization of global transcription patterns and accumulation of osmotically active solutes inside cells. We utilized the phenotyping platform PHENOPSIS to conduct an experiment on 18 Arabidopsis accessions in order to study natural variation in response to water deficit. Our set of accessions was chosen on the basis of annexin 1 (AtAnn1) mRNA level, that was previously related to drought tolerance. Plants were grown in control conditions or were subjected to moderate and severe soil water deficit. Measured traits were mainly associated with growth and transpiration, allowing us to assess responses of different accessions to applied environmental stresses. Additionally, we harvested leaf samples to determine changes in gene expression patterns induced by dehydration. Used set of accessions displayed high variation in response to water deficit, however unrelated to AtAnn1 transcript level. Principal component analysis performed on physiological data revealed condition-dependent clustering, as well as presence of individuals behaving in a significantly distinct fashion, suggesting their extreme low or high response. We are joining phenotypic and molecular analyses together to acquire more complete description of plant response to water deficit and we are also looking for links between measured variables of both types of data.

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The effect of some bioregulators in determining of relationship between leaf rolling response and dehydrins

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Dehydrins (DHNs) are a group of plant proteins that accumulate in response to abiotic stresses and have specific protective functions in plant cells. This study aimed to identify the protein profiles and dehydrin accumulation in osmotic stressed maize (Zea mays L) seedlings after treatment with salicylic acid (SA) and abscisic acid (ABA) and to determine relationship between dehydrin and leaf rolling. The leaves were pretreatment with SA (in low and high concentrations) and ABA for 6 h and then kept in polyethylene glycol (PEG) for 6 h to promote osmotic stress. The level of dehydrin proteins was increased by low SA concentration (25 μM), but decreased by high SA (200 μM) concentration. Similarly, ABA pretreatment (100 μM) also increased in dehydrin proteins. Furthermore, it was seen that remarkable changes in leaf rolling response at different dehydrin levels. Low SA and ABA pretreatments decreased lipid peroxidation and H₂O₂ contents in the osmotic stressed seedlings while lipid peroxidation and H₂O₂ content enhanced by high SA pretreatment. There was also a similar trend in prolin contents by SA and ABA pretreatments. The results showed that low SA and ABA pretreatments might regulate dehydrin protein accumulation. But high SA pretreatment could suppress the accumulation of dehydrin proteins. It is concluded that it may be a relationship between leaf rolling and SA and ABA-mediated accumulation of dehydrin proteins.

Keywords: Dehydrin, Leaf rolling, Salicylic acid, Abscisic acid, Drought stress

High-throughput phenotyping of a maize introgression library under water deficit conditions

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A maize Introgression Library (IL) of 75 lines was derived from the cross between Gaspé Flint (an extremely early Canadian landrace, used as donor parent) and B73, used as recurrent parent (Salvi et al., 2011). The population was formerly shown to segregate for several flowering time QTLs as well as for Seminal Root Architecture (SRA) traits. In this experiment, the IL was characterized using the high-throughput phenotyping platform PhenoArch (INRA, Montpellier), a greenhouse platform equipped for large-scale automated plant imaging and transpiration measurements. Objective of this investigation was to a) to screen the IL for the presence of growth and water use efficiency (WUE) QTLs under different water regimes and b) to test the effect of previously identified QTLs for flowering time and SRA on the growth and WUE.

The maize IL lines were tested under well-watered and water-deficit conditions (soil water potential > -0.05 and ~ -0.4 MPa, respectively). Each pot was weighted at least once per day allowing for the accurate estimation of daily transpiration. By means of image analysis and suitable models (multiple regression and sigmoidal fitting), it was possible to estimate biomass accumulation and thus WUE. Preliminary results showed statistically significant genetic effect for several traits, including biomass accumulation, leaf emission rate and WUE (P < 0.05, Dunnet test).

Based on the collected phenotypic data and highly dense SNP genotyping, several QTLs for biomass accumulation and WUE were mapped on Chr. 1, 2, 3, 5, 8, 9, 10, both in well-watered and water-deficit conditions. QTLs for phenology and RSA traits seems to affect differently plant development, biomass accumulation and WUE. A particularly interesting QTL conferring increased WUE in both WW and WD conditions was mapped on chr. 9.

The data collected using the platform PhenoArch will contribute to the comprehensive multi-trait phenotypic characterization of the IL. The most interesting IL lines and corresponding QTLs will be considered for fine mapping and QTL cloning.

Breeding bio-fortified aerobic rice: Phenotyping challenges

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Direct seeded, drought resistant rice that is grown without flooding the field with water at anytime during the crop growth is referred to as Aerobic rice. It has immense potential to solve several challenges faced by the farmers. While water saving is the key aspect, of this method of cultivation, other equally significant benefits include labour saving, reduced
methane emission and complete absence of eutrification of lakes that is caused due to excess nitrogen leaching out from irrigated rice fields. The varieties developed for this habitat can tolerate periods of water stress as the have been selected for deep rooting habit and faster growth. Highest grain yields have been recorded under low-moisture stress and numerically lower (statistically not significant) yields under well-watered conditions. Breeding for drought resistance has been done by adopting selection methods for roots among segregants.

Biofortification of aerobic rice has been accomplished by increasing the grain Iron and Zinc content. Conventional strategies were adopted to select for aerobic adaptability, productivity and biofortification. When compared to irrigated condition (standing/impounded water) the aerobic grown rice had higher mineral content in the grain. All parts of the plant had higher contents at all stages of growth. Plant endophytes, soil rhizosphere microflora significantly different between aerobically grown rice and rice that was grown under flooded/water impounded conditions.

The challenges that were encountered and addressed, to the best possible extent, were related to methods of estimation of nutrients. Atomic absorption Spectroscopy and X-Ray fluorescence techniques were used for estimation of grain mineral concentrations. Estimations were done in brown rice and polished rice too. Grain Zinc content in brown rice, among the best lines ranged from 32.2–33.25 PPM as against 20–22.8 PPM in the popularly cultivated/consumed varieties. Iron content was 12.08 PPM in the improved line as against 7.5 PPM in the commonly consumed varieties. The intricacies of phenotypic of drought resistance and mineral nutrition are discussed in the paper.

Phenotypic evaluation of a tomato mutant line with increased carotenoid and ABA levels

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A mutant collection of Red Setter tomato plants was established via the TILLING technology. One mutant line, having an allelic variant of the LCY-E gene (lcy-e1) involved in carotenoid metabolic biosynthesis, was identified and selected for further study. In plants grown in greenhouse the specific mutant allele caused both a decrease in the levels of lutein and an increase of violaxanthin and abscisic acid (ABA) in tomato leaves. The mutant line was further investigated by imaging phenotyping. Wild type Red Setter variety and lcy-e1 mutant plants were grown until sixth green leaf stage in a greenhouse, when regimes of normal irrigation and drought stress were initiated. Plants were treated to their respective regime for one week while they were imaged under fluorescent inducing, visible and NIR light. In addition, the stomatal conductance of the plants was measured immediately prior to imaging.

Analysis of images from visible light revealed colour differences in the mutant genotype which are likely due to the change in carotenoid profile. Stomatal conductance measurements with a porometer showed differences between wild type and mutant plants, revealing lower conductance levels in the mutant line. This result is consistent with the expected closure of the stomata in the mutant plants associated with the increased production of ABA. This behaviour was also associated with a lower biomass volume both under normal watering and drought stress regimes.

Stress phenotyping of *Arabidopsis* mutants disrupting mitochondrial electron transport genes

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High salinity or drought produces the accumulation of reactive oxygen species (ROS), leading to oxidative stress. Although chloroplasts are main source of ROS, mitochondria are also important in ROS production, where Complex I and III of mitochondrial electron transport chain are major sites for ROS. Thereby the mitochondria are important in the maintenance of the cellular redox homeostasis. To reveal the importance of genes encoding the mitochondrial proteins in stress responses, Arabidopsis thaliana mutants have been characterized, in which the T-DNA inserts are localized in genes encoding the subunits of Complex I and III of the mitochondrial electron transport. When compared to wild type, several mutants showed morphological and physiological changes under stress conditions. Phenotypic alterations and differences in tolerance to drought and salinity were revealed through in vitro germination and growth tests, as well as by complex phenotyping in collaboration with European Plant Phenotyping Network. In the EPPN facility of FZJ Jülich, Germany, multiple parameters (eg. rosette growth, chlorophyll fluorescence) were monitored in standard, water-limiting and high salt conditions. Several mutants were identified, which showed altered tolerance to drought and/or salt stress. Results of leaf area measurements suggested, that ndusf8, cyc1-1, mppa-2 and ppr40-1 mutants were hypersensitive to salt, while the mppa-2 mutant was more sensitive to drought than wild type plants. Changes in chlorophyll fluorescence under stress treatments suggested that these Complex I mutants influence photosynthesis as well. Our data shows that several nuclear genes which encode mitochondrial proteins is important in plants stress responses. This research was supported by OTKA Grant NN-110962 and the European Plant Phenotyping Network.
Exogenous proline alleviates hazardous effects of osmotic stress on photosynthetic performance of maize seedlings

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Osmotic stress limits crop productivity and to counteract osmotic stress, many plants accumulate osmoprotectant compounds such as proline, glycine betaine and sugar. On the other hand, exogenous osmoprotectant application is an important shotgun approach to mitigate detrimental effects of environmental stresses on plants. However, knowledge about the role of exogenously applied proline in counteracting the negative effects of abiotic stresses on photosynthesis is still insufficient. Here, we conducted a hydroponic experiment to determine the role of exogenous application of proline in alleviating the adverse effects of osmotic stress on water status, photosynthetic pigments, gas exchange and chlorophyll fluorescence parameters in maize seedlings. Osmotic stress (-0.6MPa) was induced by dissolving 15% Polyethylene glycol (PEG6000) in the Hoagland nutrient solution. Different concentrations of proline from 0.1 to 50 mM were applied through rooting medium, as a foliar spray and seed soaking treatment. The stress caused a significant decrease in leaf water potential, photosynthetic pigment content, net photosynthesis (Pn), transpiration (E), stomatal conductance (gs) and sub-stomatal CO2 concentration (C). Membrane damage and proline content increased under the stress condition. Proline treated seedlings maintained higher leaf water content, pigment contents and net photosynthesis compared to the non-treated plants. Rooting medium was the most effective mode of proline application compared to those of foliar spray and seed soaking treatment. Moreover, 20 mM proline concentration at rooting medium was more suitable than that of other concentrations. On the other hand, proline application increased Photosystem II activity. In conclusion, exogenous proline ameliorated the detrimental effects of osmotic stress induced by PEG6000 on photosynthetic performance of maize seedlings.

Keywords: Chlorophyll fluorescence, maize, osmotic stress, photosynthesis, exogenous proline

Evaluating hyperspectral reflection and transmission measurement for phenotyping of barley disease resistance

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Powdery mildew (Blumeria graminis f. sp hordei) is an important disease in barley production and causes major yield loss. Cultivation of resistant barley cultivars is one main measure to prevent powdery mildew infection. Thus the identification of resistant barley genotypes is an important task in plant breeding. It has been shown, that hyperspectral imaging is a reliable method to recognize powdery mildew infection in early stages for plant phenotyping. Furthermore hyperspectral imaging proved to be sensitive enough to distinguish different plant diseases through changes in the hyperspectral data. In this work a hyperspectral imaging setup for simultaneous measurement of reflection and transmission of powdery mildew on different genotypes of Hordeum vulgare near isogenic line cv. Ingrid is introduced. Hyperspectral transmission imaging of plant diseases is a novel technique that offers additional information to common reflection measurement. Results of time-series measurements from healthy and inoculated barley leaves are presented. The acquired imaging data of a nine day time series measurement was analyzed with principal component analysis (PCA) in order to evaluate changes of the leaves over time for every measured waveband. PCA analysis has shown to be highly sensitive towards changes of symptom development in early stages of powdery mildew infection.

The results show, that not only the susceptible and resistant lines can be identified via hyperspectral imaging. Moreover reflectance and transmittance measurement datasets exhibit distinct differences for the powdery mildew infected leaves in different wavebands. Besides identification of powdery mildew infection, the long term aim of this approach is to use the information gathered by hyperspectral imaging, to further identify specific resistance reactions involved in plants defense against the pathogen in a non-invasive manner.
Mapping QTLs for root architecture and yield in a maize introgression library grown in well-watered and water-stressed conditions

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The genetic control of root architecture in maize was studied using a collection of 75 introgression-lines (IL) developed from the maize cross B73 x Gaspé Flint. The aim of our study was to optimize a protocol for maize root phenotyping in the field, to map QTLs for root trait architecture in the field in the B73 x Gaspé Flint introgression library and to investigate the relationship between root traits and grain yield under drought.

For this purpose, the IL collection was grown in a replicated field trial at two water regimes (well-watered, WW, and water-stressed, WS). Forty-four traits covering phenology, plant architecture, yield, and root architecture were analyzed. Root architecture was investigated by means of coupled with software-assisted digital image analysis for a total of c. 1,400 analyzed root images. The stress imposed in the WS experiment resulted in significantly lower yield (-42%) and longer anthesis-silking interval (+9%) when compared with WW. In WS, statistically significant lower root dry weight and higher number of brace roots were also observed. Relatively high correlation values (up to r = 0.66) were observed between visually or manually scored root traits and corresponding traits based on software-based analysis. QTL analysis showed a complex genetic control for most root traits, and only limited overlap between root and yield or yield components QTLs. Together with root and drought-related QTL information based on hydroponics and pot experiments for the same population, these results contribute to unveil the role of root architecture variation on maize yield.

A fast-forward genetics approach for cloning genes for root architecture in barley

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Until recently, searching for mutations responsible for phenotypes-of-interest in a forward-genetics fashion was typically considered a multistep and challenging process. The advent of highly efficient mapping and next-generation-sequencing approaches considerably facilitates this procedure. For this purpose, we screened a mutagenized population (TILLmore; see http://www.distagenomics.unibo.it/TILLMore/) in barley (cv. Morex background, 3,071 M5 mutant families) to identify root morphology alterations. Four randomly chosen mutants were out-crossed to generate recombinant populations and to confirm their Mendelian inheritance. To speed-up the mapping procedure an SNP array (Illumina9K)-based analysis was applied on bulked recombinants allowing for a fast and cheap identification of the target interval. An exome-capture, mapping-by-sequencing approach of the mutant lines has been deployed to link the observed mutant phenotype with the underlying gene. A candidate gene for a short-root mutant has been identified by analysing mutant allele frequency of called variants in the target region. The missense mutation, located in a conserved domain, is predicted to severely affect the protein function (SIFT score 0.01). Currently, we are carrying out a TILLING experiment in order to find alternative alleles affecting the candidate function. These preliminary results indicate our population as a useful resource for functional genomics and gene cloning in barley.

Use of the full-range spectral signature for the high-throughput phenotyping in durum wheat

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In the last decades the increased on temperature associated with climate change as well as the increased of occurrence of abnormal weather events has led to an increase on the abiotic stress conditions threatening food security. Wheat is the most widely cultivated worldwide and durum wheat is specifically the most cultivated cereal in the south and east margins of the Mediterranean Basin that are especially sensitive to climate change. Therefore it is of strategic importance for Mediterranean agriculture to develop new varieties of durum wheat with greater production potential and better

38
adaptation to increasingly adverse environmental conditions. At present field phenotyping remains the major bottleneck for breeding process, but hyperspectral High-Throughput Phenotyping systems have shown promise for the characterization of weather-resilient crops. In the following study the spectral signature of plant material is tested with a full-range (400–2500 nm) spectroradiometer at different development stages in a panel of 24 durum wheat genotypes cultivated under different growing conditions. When considering different growing conditions together at the canopy level, the spectral signature in the red and in the short-wave infrared were highly correlated with grain yield \(r > 0.8\), being better than that in the near-infrared \(r = 0.4\). Correlations were always stronger at the grain-filling stage. The combination of some wavelengths as indices for their use as phenotyping criteria is also discussed. Regarding the spectral signature of leaves and spikes within growing conditions, significant correlations with grain yield were also found but in a minor extent than that at the canopy level. Future research will focus on the allocation of relationships between spectral signatures of the plant material with their respective metabolic composition.

**Growth and physiological responses to drought need to be combined in phenotyping of drought tolerance**

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The aim of this study was to evaluate the potential of non-invasive optical methods for drought tolerance phenotyping on the model of natural accessions of *Arabidopsis thaliana*. A projected leaf area of rosette, light-saturated CO\(_2\) assimilation rate \((A_{\text{max}})\) and relative water content (RWC) of leaves were estimated together with thermal imaging (leaf temperature) and spectral reflectance (in the range of 350–2500 nm) measurements. Within the six accessions tested, we were able to identify three major groups of genotypes according growth and physiological responses to drought: i) genotypes their growth is not affected by drought stress but there is a significant reduction in the \(A_{\text{max}}\); ii) genotypes which significantly reduce both the growth rate and \(A_{\text{max}}\); iii) genotypes with significant reduction of growth rate due to drought stress but only small decrease of \(A_{\text{max}}\). These results document that a substantial reduction of leaf area growth reduces total transpiration of the plants resulting in the maintenance of both plant water status and basic physiological processes.

Non-invasive thermal imaging and spectral reflectance methods provided a reliable tool for evaluating drought-induced changes in \(A_{\text{max}}\) and RWC across all accessions tested and represent thus useful tools for phenotyping stress tolerance. Given the fact that the final response to drought stress is result of cross-interaction of growth and physiological responses, it is desirable to complement the thermal imaging and spectral reflectance methods e.g. by image analysis of leaf area.

**Automated detection of *Mycosphaerella melonis* infected cucumber fruits**

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The fungus *Mycosphaerella melonis* causes internal fruit rot to cucumbers which is not visible on the outside during the first stage. The infected cucumber are not saleable and could infect the healthy fruits. During sorting, the contaminated cucumbers are difficult to recognize, even by experienced personnel who separates these as good as possible. Due to sorting on *Mycosphaerella* the processing speed must be reduced and automation of this process would be highly desirable.

255 fresh cucumbers were collected in two time points: 131 cucumbers out of which 44 healthy and 87 infected at one time point; and another 62 cucumbers out of which 46 healthy and 16 infected one month later. The collected fruits were measured within 2 days after the collection. Measuring included: 1) measuring shape features using cucumber images that were made by a Nikon D70 colour camera with 3008 x 2000 pixels resolution and 2) transmission spectra point measurements of each cucumber using S2000 fibre optic near-infrared (NIR) spectrometer with the wavelength range 350–1000 nm. The spectral transmission was measured in 3 points at the upper tip of the cucumber where the infection occurs.

We present and compare two fully automated methods to classify between healthy and unhealthy cucumbers using the results of the measurements.

In the first method, the cucumbers were segmented from the excessive green images (2G–R–B) using thresholding. The upper tip of each cucumber, with length equal 10% of the full cucumber length is further processed. All cucumbers are scaled to unity length and widths of the cucumbers extracted orthogonally along the central cucumber were used as features. The features are utilized by Fisher classifier in order to separate healthy from infected fruits.

In the second method the transmission spectra measured in three points of the healthy and infected cucumbers were classified using linear classifier with 1286 features that were approximated with a lower dimensional feature space using principal component analysis (PCA). Features represented an averaged transmission signal over three points at each discrete wavelength.

Both methods were trained on 50% of the data and tested on the remaining 50%.
The shape based method reached accuracy of 86%, while the NIR spectroscopy based method in combination with the shape analysis had 95% accuracy. The accuracy of manual classification was 94%.

Collecting more representative set of training samples, having more controlled measuring conditions and combining the two methods presented are possible directions for the improvements of the method.

We presented a method that can automatically sort *Mycosphaerella* infected cucumber fruits more accurately than the manual sorting can.

**PHENOARCH, a multiscale phenotyping platform for plant architecture, growth rate, water use efficiency and radiation use efficiency**


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PHENOARCH (https://www6.montpellier.inra.fr/lepse/M3P/plateforme-PHENOARCH) measures traits associated to the plant adaptation to climate change with a throughput of 1650 plants. Typical measured variables are the timing of the plant cycle (phylochron, duration of phenological phases), plant growth rate in terms of area and biovolume, plant architecture in terms of ramification and angles, light interception, radiation use efficiency and water use efficiency. Soil water potential is controlled individually for each plant via scales; changes in weight are attributed to changes in soil water content after correction for the increase in plant biomass. Microclimatic data fluctuate naturally within a range fixed by users. A set of sensors measures meristem temperature, incident light, air temperature and VPD every minute. A 3D model of the greenhouse allows inferring the environmental conditions sensed by each individual plant by calculating spatial gradients in the greenhouse each day of the year. Plants are imaged every day with 12 side views and one top view, which allow reconstructing a digital ‘avatar’ of each plant of the platform. Calculations related to plant architecture and to individual organ size are carried out on these virtual plants. Light interception by each plant in the platform is calculated via a virtual scene consisting of the 1650 3D virtual plants in their real positions. The measured time courses of biomass and of intercepted light, combined with local measurements of light intensity, allow estimation of radiation use efficiency. Responses to water deficit and evaporative demand can be analysed by combining time courses of leaf area or biovolume with environmental data. PhenoArch is associated to an information system for real time monitoring of experiments, post-analysis of large datasets and identification of genotypic parameters such rates, architectural parameters or sensitivities. It has been used for (i) association genetics in panels of genotypes (diversity, MAGIC or biparental crosses), (ii) in-depth analyses of smaller panels such as panels of genetic progress or introgression lines, for morphological or physiological traits, (iii) estimation of parameters of crop models that are then tested and used in the field. PhenoArch has been used with maize, rice, sorghum, wheat, apple tree and vine. It is part of the M3P facility (https://www6.montpellier.inra.fr/lepse/M3P) and is accessible to public or private scientists via the website of the national project Phenome-FPPN (https://www.phenome-fppn.fr/). Accesses have also been provided via the infrastructure EU project EPPN.

**PHENODYN: A high throughput platform for measurement of organ elongation rate and plant transpiration with high temporal resolution**

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Leaf elongation rate (LER) is the first trait affected by water deficit or high evaporative demand, with typical time constants of 30 min for change in LER upon rapid changes in soil water content or air vapour pressure deficit (VPD). The same applies to other organs such as maize silks. Phenodyn (https://www6.montpellier.inra.fr/lepse/M3P/plateforme-PHENODYN) measures organ elongation rate and transpiration rate of hundreds of plants with a temporal resolution of 3 min (or more if required) in order to follow the changes in LER and transpiration in fluctuating conditions of soil water content, evaporative demand and temperature. Phenodyn imposes known soil water potentials to each plant via independent automatic irrigation. Climatic conditions are either imposed in the growth chamber or left to naturally fluctuate in the greenhouse. Elongation rate is measured with 500 rotational displacement transducers with a precision of 0.2 mm. Transpiration and soil water content are measured with scales; changes in weight are attributed to changes in soil water content after correction for the increase in plant biomass. A set of sensors measures meristem temperature, incident light, air temperature and VPD every minute. Phenodyn is associated to an information system for real time monitoring of experiments, post-analysis of large datasets (around 700,000 data points are generated in each experiment) and
phenotypic parameters such as rates or time constants. It has been used (i) for analyzing the response of LER to soil water potential and to VPD in mapping populations, diversity panel for association genetics or insertion lines, (ii) for establishing response curves to temperature in different species and genotypes, (iii) for following jointly changes in transpiration and in elongation rates of leaves or silks together with hydraulic variables. It has been used in maize, rice, wheat, sorghum, millet, apple tree and vine. Phenodyn is part of the M3P facility (https://www6.montpellier.inra.fr/lepse/M3P). It is accessible to public or private scientists via the website of the national project Phenome-FPPN (https://www.phenome-fppn.fr/).

PHENOPSIS: From a phenotyping platform to a whole pipeline of analyses
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A major goal of the life sciences is to understand and model how molecular processes control phenotypes and their alteration in response to biotic or abiotic stresses. The study of Arabidopsis thaliana genomics is providing new insights into the understanding of these processes. The functional analysis of genes associated with these responses is made possible by the phenotypic analyses of mutants or natural genetic variants, high-throughput genetic mapping and large-scale analyses of gene expression. Ten years ago, an important bottleneck was the phenotypic analysis of the genetic variability, which requires simultaneous analysis of hundreds to thousands of plants. The PHENOPSIS platform provide since 2002 large quantities of micro-meteorological data, images and phenotypical data for the study of genotype x environment interaction effects on different plant processes in A. thaliana. Protocols have been standardised to allow reproducibility between experiments and facilitate meta-analyses. Phenopsis is part of the M3P facility (https://www6.montpellier.inra.fr/lepse/M3P). It is accessible to public or private scientists via the website of the national project Phenome-FPPN (https://www.phenome-fppn.fr/). Database, ontologies, image analyses and statistical tools ensure that the data produced by specific groups can benefit other groups in analyses of which the purposes extend beyond the ones that have been published.
**ROOT PHENOTYPING**

Can electromagnetic induction accurately detect differences in water use between field grown cotton genotypes?

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Measuring soil water extraction of cotton can be used as an in-situ surrogate for tracking root growth in the field. Currently, we use neutron moisture meters to regularly measure soil water in the field to calculate differences in water extraction between different genotypes. Whilst effective, these devices are time consuming, spatially limited to an access tube and contain radioactive sources. As an alternative, electromagnetic induction, specifically the EM38-Mk2, is a non-intrusive, portable device that may allow soil moisture information to be gathered quickly and efficiently. This two month study was conducted on a furrow irrigated cotton field employing an approximately 10 day irrigation cycle. This study determined that electromagnetic induction could accurately detect differences in water use in cotton field experiments, including differentiating water use between genotypes and planting strategies. The technique was able to resolve significant differences in soil water use over 24 hrs between both genotypes and planting. These results indicate that the EM38-Mk2 can accurately detect soil moisture extraction non-intrusively and quickly and could be an important research tool for both cotton phenotyping and agronomy studies.

Drought induced changes in rooting patterns, water extraction and shoot biomass of three commercial *Brachiaria* hybrids

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*Brachiaria* forage grasses are widely planted in the tropics and their demand is increasing in East Africa. Water limiting conditions greatly affects the productivity of *Brachiaria* grasses. Deep root systems, with greater root length densities with increasing soil depth, have been generally linked with uptake of stored water in lower layers of soil. However, variation in patterns of root length density down a soil profile and water uptake of commercial *Brachiaria* hybrids is largely unknown. For that reason, a greenhouse study was conducted to determine patterns of root length density and water uptake of three commercial *Brachiaria* hybrids using soil cylinders. Our results showed that larger root system at depth of one hybrid contributed to greater water uptake, in turn reflected in greater shoot biomass production under progressive soil drying conditions.

The development of two-dimensional root phenotyping method on vertical black filter paper

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Plant root system architecture (RSA) has an important role in crop production, particularly for water and nutrient uptake under limiting conditions. Therefore, the understanding of the mechanisms regulating RSA is important for future crop improvement for drought conditions and nutrient deficiency. In the last few years, several root phenotyping method have been developed. Here we present a new technique which has been developed for non-destructive root growth studies and RSA measurement. To illustrate the potential applications, this method was tested in experiment with nutrients deficiencies (Knop's hydroponic solutions deficient in N, P, K), affecting different parameters of RSA of winter wheat and spring barley. This technique was developed as hydroponic system which enables control of root environment and supply of plant nutrients. The root were grown on the surface of vertically fixed black filter paper sheets (30×60cm), covered from both sides by black plastic sheets (PVC-P). This system allows easy access to roots for their measuring and sampling. Moreover, the system was setup with a micro-irrigation channel in the top of sheets to ensure circulation of hydroponic medium and evenly wetting of paper sheets. After 18 days after transplanting, we took the root images by the standard RGB digital camera. To evaluate the root architecture parameters the “SmartRoot” software was used. The results showed that this technique has potential for non-destructive root growth studies, RSA measurement and root sampling. The software SmartRoot allowed precise evaluation of root development but needed substantial user inference. Moreover, there were no effect of nutrient...
deficiency on seminal root length. P deficiency promoted lateral root length, mean root diameter and total root area for both of winter wheat and spring barley however slightly decreased lateral density. Root/shoot ratio of winter wheat and spring barley was enhanced by the absence of N. There were Positive correlation between root and shoot dry weight.

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Exploring the rhizosphere: Imaging root-soil interactions using X-ray Computed Tomography

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Although roots play a crucial role in plant growth and development through their acquisition and delivery of water and nutrients to the above-ground organs, our understanding of how they interact with their immediate soil environment largely remains a mystery as the opaque nature of soil has prevented undisturbed in situ root visualisation. The spatial arrangement of roots and the soil structure are extrinsically linked to the overall productivity of a plant, as they control the ability of a plant root to extract essential resources for growth. In a world with a rapidly increasing population and the threat of climate change maximising plant productivity is vital. Therefore the visualisation and quantification of root growth in soil is needed to understand plant root growth dynamics. The use of non-invasive techniques such as X-ray Computed Tomography (CT) means that it is now possible to visualise a growing root within an undisturbed soil core. X-ray CT is a technique that enables non-destructive 3-D investigations into root:soil interactions at the micro-scale. By imaging the actual 3D geometries of the soil structure and visualising the interfaces between roots, soil, water and air filled pores an accurate representation of water movement and root growth in soil is achieved. Previous commonly employed destructive methods for root studies, such as root washing could not provide detailed information on root architecture, including branching characteristics and extension rate, which are inherently linked to conditions within the soil matrix. This information is crucial if laboratory research is to be translated to an understanding of responses under field conditions. It is envisaged that this research will inform interdisciplinary scientists and farmers to improve current mathematical models on root water uptake and soil management techniques.
STANDARDS IN PHENOTYPING

Differenced flag leaf and ear photosynthetic performance under elevated CO₂ conditions during grain filling period

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Even if during the past decades increases in wheat cereal productivity have matched population growth, the average increases in wheat yield have stagnated after the green revolution. This is a matter of major concern as long as, wheat represents a target crop in worldwide food security programmes. Grain filling, and consequently crop yield is sustained, in part, by photoassimilates from the flag leaf and by the C fixed by the ear itself. Although classically, flag leaf has been considered as the main organ providing photoassimilates to the grains, it is now generally accepted that ears make an important contribution to final grain yield. Ear and flag leaf photosynthetic performance of durum wheat (Triticum durum, cv. Amilcar) exposed to ambient and elevated [CO₂] (i.e. 400 and 700 µmol mol⁻¹ respectively) were assessed. CO₂ enrichment increased photosynthetic rates in flag leaves but, the larger [CO₂] availability did not significantly affect ear photosynthesis. Moreover, compared with flag leaf, ears showed lower photosynthetic rates. Expression analysis of carbon and nitrogen metabolism related genes showed that the strong repression of genes involved in Calvin-Benson cycle (Rubisco small and large subunits, Rubisco activase and Sedoheptulose-1,7-bisphosphatase) and photosynthetic electron transport chain (ferredoxin, ferredoxin-NADP reductase and ATP synthase β subunit) were involved in ear photosynthetic down-regulation. Elevated CO₂ also decreased the transcripts of genes involved in nitrogen assimilation, including the glutamine synthetase cytosolic and plastidial, the asparagine synthetase and others, in the ears, whereas these were induced or unaltered in flag leaves. Finally, our study also remarked the fact that the strong increase in ear respiration rates, together with the depletion of organic acids in ears exposed to elevated [CO₂], suggest that ear contribution to grain filling was lower than that of the flag leaf during post-anthesis period. Such findings underscore the significance of characterizing spike physiology and the importance it may have for research supporting wheat breeding programmes.

phenoVein – A software tool for leaf vein segmentation and analysis

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We introduce a powerful and user-friendly image analysis tool, named phenoVein. It is dedicated to automated segmenting and analyzing leaf veins of images acquired with different imaging modalities (microscope, macro photography, etc.) including options for comfortable manual correction. Advanced image filtering automatically emphasizes veins from background and compensates for local brightness inhomogeneities. The most important phenotypical traits being calculated are total vein length, vein density, piecwise vein lengths and widths, areole area and skeleton graph statistics like the number of branching or ending points. For the determination of vein widths, a model based vein edge estimation approach has been implemented. We demonstrate the capabilities of phenoVein on a set of vein structure mutants of Arabidopsis thaliana.

Diallel cross analysis in melon (Cucumis melo L.): Identification of best parents for yield and related traits

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A complete diallel cross study of 6 Iranian melon cultivar (Eyvanaki, Abasali, Tashkandi, Hose-sorkh, mashhadi, and Nahavand) and one foreign cultivar (Ananasi) was carried out for number of fruits, average weight of fruits per plant, flesh thickness, yield and acceptable yield. The analysis of variance revealed that differences among the genotypes were significant for all characters. Additive gene effects were most important in governing fruit number per plant, average weight per fruit, flesh thickness and yield while dominance genetic effects mainly controlled acceptable yield. The reciprocal effect was only significant for average weight of fruits per plant. Nahavand was the best general combiner for average weight, flesh thickness and yield. Heterosis over the mid parent was found for all traits except number of fruits per plant while heterosis over the better parent was not found.

**phenoSeeder – A robot system for phenotyping and handling of individual seeds**

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The phenoSeeder system is based on a pick-and-place robot to analyze and handle individual seeds of very different sizes from *Arabidopsis* to maize. By default, morphometric traits are monitored at 3 different sensor stations. (1) At a 2D-imaging station seeds are disseminated on a glass plate where they are selected according to user-defined selection criteria. A selected seed gets an ID and its traits (projected area, length, width, color distribution) are measured. (2) It is then picked up and conveyed to a 3D-imaging station where a series of images are taken from different angles and a surface reconstruction procedure delivers 3D information (volume, length, width, height, etc.). (3) The seed is then carried to a balance to get the actual weight which, divided by the measured volume, provides seed density. Finally, the seed is directly planted into substrate, sorted into storage containments according to user-defined classification criteria, or individually stored in a multi-well plate. This cycle is repeated for each single seed. All obtained seed data including the current position of a seed are stored in a database which also delivers the parameters needed for the workflow of the system.

We want to systematically study how the different seed traits correlate and which seed traits are important for a developing plant of various species or genotypes under optimal or adverse environmental conditions. The main objective of the phenoSeeder approach is thus seed-to-plant tracking which includes monitoring seed germination and measurement of plant growth at early developmental stages and beyond. The modular implementation of the phenoSeeder system allows for easy integration of additional sensors enabling measurement of additional parameters of individual seeds, beside the morphometric traits described above. Modalities we plan to integrate are near infra-red (NIR) providing information about seed content, nuclear magnetic resonance (NMR) delivering water content or biomass, or X-ray CT for imaging internal seed structures allowing to extract e.g. embryo or endosperm size. Due to the versatile design of the phenoSeeder it can contribute to different applications including better seed characterization and improvement of seed quality for breeding.

**The German (DPPN) and European (EPPN) Plant Phenotyping Networks**

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Progress in plant and agricultural research is crucial for solving some of the future grand challenges: in order to guarantee sufficient food supply for the world’s fast growing population as well as for non-food needs. There is an increasing need for higher crop yields and plants which are optimized for higher resistance to environmental stresses. DPPN, the German Plant Phenotyping Network (http://www.dppn.de), intends to collect quantitative information on the structural and physiological properties of plants and to apply this for basic plant research and plant breeding. For this purpose, new and better methods to determine the phenotype of plants, their resistance to stress or the concentration of important metabolites or precursors will be developed. The DPPN is a national infrastructure developed and run by the Research Center Jülich (coordination), the Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben and Helmholtz Center Munich. The main target of the network is to foster the development of basically non-invasive new technologies to be applied for plant research and breeding. The implementation phase 2012–2017 is funded by the German Federal Ministry of Education and Research with a total amount of € 34.6 million. Within period these institutions will intensify and optimize their cooperation and will give access to potential users in industry and to academia. The DPPN also accounts for the German core of the European Plant Phenotyping Network (EPPN, http://www.plant-phenotyping-network.eu/), a corresponding initiative sponsored by the EU. The EPPN is coordinated by the Research Center Jülich and aims at creating synergies between the leading plant phenotyping institutions in Europe as a nucleus for the development of a strong European plant phenotyping community. In particular, EPPN provides opportunities for user groups to access plant phenotyping facilities available within the network which enable a growing number of collaborative projects.
A tool for phenotyping based on mobile terminals
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Plant breeding and genetics require fast and exact phenotyping that is reproducible independent of scientist and location. Efficient statistical evaluation furthermore requires standardized data storage. Safe data storage must ensure long-term availability of data, ideally over organizational barriers while maintaining intellectual property rights. All this is already state of the art at large phenomenics centers. However, these resources are unavailable for most scientists. For these, we develop a simple and cost-efficient system that employs mobile data recording systems for onsite data entry. The system uses personal digital assistants (PDA) to enter phenotyping data on-site into user defined forms, so called ‘phenotyping schemes’. These schemes can be defined by the user on a web-based graphical user interface ‘Test Program Composer’ from controlled vocabulary compatible with published ontologies (e.g. the plant ontology consortium). XML-based downloads of the phenotyping scheme from the webpage allow easy transfer to the recording system and exchange between users. On the terminal, the interface ‘Mobile Phenotyper’ displays the schemes and stores recorded data in XML and CSV format. For long-term data storage, data can be uploaded to data management pipeline based on the systems developed for the TROST project (Billiau et al. 2012 http://dx.doi.org/10.1071/FP12009). Web tools store the original data files, upload data from the mobile device into the standardized storage format of a database and provide download tools.

Aiming for an open source, low cost solution, we used free software components for database (MySQL) and website (Apache Tomcat, Apache httpd2 webserver, CakePHP). After field testing, by academic groups and breeding companies, we built a web-based distribution and user support system to make the system available to a wider community.

Variation of yield-related traits in wheat hybrids inoculated with Fusarium sp.
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Resistance to Fusarium head blight (FHB) caused by fungi of the genus Fusarium is an important from agronomic and economic point of view. Infection of cereals by these pathogens causes decrease in quantity of yield and its quality by contamination of the harvested grain with toxic fungal secondary metabolites. Resistance to FHB is a quantitative trait modulated by genetic factors and environmental conditions. The level of resistance can be assessed after artificial inoculation by observation of disease symptoms on spikes (resistance type I and II) and/or on grain, including an assessment of the reduction in yield and yield-forming traits (resistance type III). In plant breeding it is important to distinguish at early stages of breeding process such cross combinations, which give the possibility to select homozygous genotypes with desirable traits, including resistance to diseases. For evaluation of plant resistance to FHB type III several characteristics should be considered simultaneously, such as the rate of kernels damaged by pathogen (FDK), number of developed kernels in spikes, kernels weight per spike, thousand-kernel weight in control and artificially infected plants. In the present study susceptibility of F2 hybrids to FHB of sixteen winter wheat cross combinations was observed after inoculation with F. culmorum, F. graminearum and F.avenaceum.

Hybrids of each cross combination were grown under field conditions. Inoculation was performed at the full anthesis (Zadoks scale 65). In the harvested samples, kernels were visually scored and divided into two categories: Fusarium-damaged kernels and healthy-looking kernels. Furthermore, the number and weight of kernels per spike of infected and control plants were recorded and 1000-kernel weight was calculated. Results of two-way analysis of variance showed significant effects of hybrids (H), treatments (T) and interaction H x T for all analysed traits except number of kernels per spike. Coefficients of variation in all cross combinations were higher for inoculated than for control plants. Among studied traits thousand kernel weight appeared to be the most stable—in control and infected hybrids coefficients of variation for that trait were relatively small. The division of the tested hybrids onto homogeneous groups for individual characteristics permitted us to distinguish two crosses, which were characterized by high mean values of thousand kernel weight and kernel weight per spike both in control conditions and after artificial inoculation, which may suggests that these hybrids are more resistant to FHB. Future study on doubled haploid lines derived from these hybrids will allow us to verify these findings.

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Thorvald, a versatile robotic platform for agricultural applications, including phenotyping in field trials
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Much effort has been put into developing robotic systems for the agricultural domain that are able to perform specific tasks such as yield estimation, phenotyping, sampling, precise application of pesticides, and so on. Some robots have also been developed for more energy-demanding tasks such as seeding, but little work has been done to make more versatile systems that are able to perform tasks with variety in energy demand, precision, operation speed, etc. Thorvald is a novel robotic platform designed and built at the Norwegian University of Life Sciences that is powerful enough to perform energy-demanding operation in the soil, and at the same time has the beneficial properties of lightweight, autonomous robots. The robot is designed to be capable of crop scouting and gathering information in the field, as well as performing energy demanding, physical work. The robot is equipped with all sensors required for reliable, autonomous navigation, and different tools, or agricultural implements, are attached within the robot frame according to the task that is to be performed.

Some preliminary results from field-testing of the robot during the 2015 crop season is presented, from experiments with precision seeding and phenotyping in field trials with cereals. The feedback from these tests were mainly good, and an improved version is being developed for further testing in 2016.

Agronomic traits affected by dwarfing gene Rht-B1, Rht-D1 and Rht-8 in DH wheat populations (\textit{Triticum aestivum} L.)
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Plant height is a typical quantitative trait, which is influenced by environmental and genetic factors. The genetic control of the plant height depends on the large complex of genes located on multiple chromosomes which could lead to a reduction or increase in plant height (Worland et al. 1990). The dwarfing genes have been associated with large increases in the yield potential of cereals. The most current wheat varieties contain \textit{Rht-B1b} and \textit{Rht-D1b} genes, which are located on homeologous chromosomes 4BS and 4DS and encode proteins involved in the gibberellin (GA) signal transduction. These genes were transferred from the Japanese variety Norin 10 and exhibit pleiotropic effects on plant growth. \textit{Rht-B1b} and \textit{Rht-D1b} reduce plant height and increase the index yield which could be associated with higher photosynthesis productivity and higher alpha-amylase activity. Other height-reducing \textit{Rht8} gene was introduced into Italian wheats from the Japanese landrace Akakomugi, and has been widely used in wheats adapted to southern and eastern European conditions. Xgwm261 microsatellite marker, closely linked to \textit{Rht8} gene, has been used to its alleles identification; a 192 bp allele at this locus (\textit{Rht8c} allele) has been taken as diagnostic and it was associated with about 7–8 cm height reduction. The aim of the study was (1) the \textit{Rht-B1}, \textit{Rht-D1} and \textit{Rht8} alleles of two winter wheat homozygous populations using molecular markers characterization; the effects of alleles on the flowering time and yield parameters were examined (2) lines possessing different \textit{Rht} alleles were phenotyped with respect to plant height, heading date, full maturity date, no. fertile tillers, spike length, spikelets no. spike\textsuperscript{3}, grain no. spike\textsuperscript{3}, plant yield, thousand grain weight.

Plant architecture measurement using a 3D reconstruction method suitable for high throughput plant phenotyping
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In plant phenotyping, there is a demand for high-throughput, non-destructive systems that can accurately analyse various plant traits by measuring features such as plant volume, leaf area, and stem length. Existing vision-based systems either focus on speed using 2D imaging, which is consequently inaccurate, or on accuracy using time consuming 3D methods. On this poster, we present a computer-vision system for seedling phenotyping that combines best of both approaches by utilizing a fast three-dimensional (3D) reconstruction method. We developed image processing methods for the identification and segmentation of plant organs (stem and leaf) from the 3D plant model. Various measurements of plant features like plant volume, leaf area, and stem length, are estimated based on these plant segments. We evaluate the accuracy of our system by comparing the measurements of our methods with ground-truth measurements obtained destructively.
EPPN phenotyping facilities at the Jülich Plant Phenotyping Centre (JPPC)

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Plant phenotyping is technically a very challenging task in many aspects, mainly because of the nature of the plant’s organs. Recent technological developments at the Jülich Plant Phenotyping Centre (JPPC) allow us to measure and quantify important features of plants in an automated way.

In the poster, the available platforms for root and shoot phenotyping at the JPPC, provided for EPPN, will be highlighted. SCREEN Root LP (Nagel et al., 2012) as well as SCREEN Root SP (Nagel et al., 2009) were developed for quantifying the architecture of entire root systems automatically in an image-based approach, either in a soil-based or agar-based system. SCREEN House and SCREEN Chamber (Jansen et al., 2009) were developed to measure image based shoot morphology, growth and biomass for smaller plants in growth chambers and for larger plants in the greenhouse. During the lifetime of the EPPN project we have offered access to these facilities to several user groups realizing a total of 13 experiments. In several cases these experiments have already brought to publication of the results, for example, regarding studies of root and shoot architecture responses to low nitrogen in durum wheat (Gioia et al., 2015). Overall, we were successful in providing access and training users groups in the use of automated phenotyping platforms.

References


Phenotyping of Brazilian wheat genotypes under field conditions

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At the Cerrado region (Brazil), wheat is planted in two different environmental conditions. The first one is in the cold and dry winter, which requires irrigation of the wheat plants. The second one is in hot and rainy summer, when no irrigation is applied but wheat plants grow under water deficit. These two situations require different strategies for wheat genotype selection. The selection based only on productivity is not sufficient to understand the interaction between genotype and environment. Non-invasive phenotyping methods can help to understand this interaction, and give added information to physiologic studies and plant-soil management approaches. The aim of this work was to test selected non-invasive phenotyping methods on a selection of Brazilian wheat genotypes. The experiment was carried out under field conditions from April to August 2015 at Campus Klein-Altendorf (University of Bonn, Germany). Ten different Brazilian wheat genotypes were selected for the study. Three of them are developed for winter (BRS 254, BRS 264 and CPAC 544) and seven for summer season (Brilhante, Aliança, PF 020037, PF 020062, PF 100660, BR 18 and PF 080492). The experimental design was a randomized block with 12 replications and each plot had a size of 12 m². Non-invasive plant traits which were evaluated in the beginning of the grain filling stage are: plant height (canopy and flag leaf levels), leaf chlorophyll content (SPAD) and leaf reflectance. Destructive plant traits such as plant biomass (separated in leaves, stem and spikes) and leaf area of the flag leaves and the remaining green leaves, was evaluated subsequently. Starch and protein content of grain was determined post-harvest. The genotypes developed for rainfed and irrigated season showed differences in terms of plant height, leaf morphology other parameters, with exception of the PF492. Despite the fact that PF492 was developed for rainfed season, the phenotype developed similar to the irrigation varieties. As a preliminary outcome of this study PF492 could be recommended to be planted in both seasons. For total plant biomass, there were no differences among materials, but there were significant differences considering single plant parts. The materials developed for irrigation produced more spikes than
the ones for rainfed, which in turn produced more leaf and stem. The Normalized Different Vegetation Index (NDVI), which was derived from leaf reflectance spectra, had a significant positive correlation with leaf chlorophyll content, productivity, and weight of spikes. Furthermore there was a negative correlation with plant height. Finally, protein and starch have the opposite tendencies between genotypes due to the dilution effect since high starch meant greater productivity.

Cost-effective imaging in plant phenotyping from information-based approaches

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A conventional metrological approach in plant imaging consists in producing, a representation of the observed object that is the most faithful possible to the underlying biology (fluorescence chlorophyll efficiency, watercontent, leaf area index, ...) of the plant. Yet, in this way, only one faithful reproduction stage is targeted, and little or nothing is actually started at this level in terms of information processing. The faithful image has to be further processed by higher-level operators, in order to achieve an information processing task, such as plant classification, pathogene detection, organ counting, ... If such high-level task to be achieved are targeted from the very beginning, it appears that the perfect faithful representation of the biological measurement in the processing chain is only an intermediary step, which may not be necessary to achieve the final information-based phenotyping task. Such an information-based approach is specifically relevant in the context of high-throughput phenotyping to produce imaging systems less computational demanding or working with lower spatial resolution or lower bit dynamic than with the conventional metrological approach while preserving the quality of the extracted information. This is of high value if imaging systems have to be multiplied to monitor large population of plants or if one seeks to control the rate of data production of his/her phenotyping center. We present a review of such a cost-effective informational approach that integrates, as soon as the low-level stage of image acquisition, design principles dictated by the final high-level information task of the imaging system. The approach is illustrated with various case studies published during the period of the EPPN project including an optimal image compression scheme for the characterization of seed [1], a 3D characterization of shoots [2], a leaf counting task from low-cost depth images [3] and the detection of pathogens from low resolution spectral imaging [4].

References


Field based high-throughput phenotyping using proximal sensing and UAVs

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Proximal hyperspectral/multispectral sensing or aerial phenotyping using UAVs represent high-throughput and non-invasive phenotyping methods which can be applied in large breeding field trials. Both methods have their pros and cons in terms of high-throughput and accuracy, but finally the method’s selection depends on the specific plant traits to be observed.
Phenotyping offers the horticultural sector a powerful tool for optimising plant performance and productivity. For effective use phenotyping platforms need to be fast, accurate and objective. Several phenotyping systems are already intensively being used world-wide and one of the objectives within the EPPN project (WP4 – Good Phenotyping Practice) is to test the reproducibility of these existing phenotyping platforms from partners within the EPPN project. So, how comparable are the results of the different phenotyping platforms as a same set of plants is measured by every platform. Measuring the reproducibility is done with a set of fake plants, on all available phenotyping platforms. Of course differences are found between the resulting measurements of the phenotyping platforms. The interesting challenge is to explain and explore the differences in outcome. After the determination of the cause of the differences, guidelines have been setup to improve the results and open up the possibility to run multi-site experiments.
MODEL PLANT PHENOTYPING

High throughput estimation of incident light, light interception and radiation-use efficiency of thousands of plants in a phenotyping platform

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We developed a non-invasive method to measure light interception and radiation-use efficiency (RUE) in thousands of maize (Zea mays) plants at the PHENOARCH phenotyping platform.

Different models were interfaced to estimate (i) the amount of light reaching each plant from hemispherical images, (ii) light intercepted by each plant via a functional-structural plant model, (iii) RUE, as the ratio of plant biomass to intercepted light. The inputs of these models were leaf area, biomass and architecture estimated from plant images and environmental data collected with a precise spatial and temporal resolution. We have tested this method by comparing two experiments performed in autumn and winter/spring.

Biomass and leaf area differed between experiments showing a high GxE interaction. Difference in biomass between experiments was entirely accounted for by the difference in intercepted light. Hence, the mean RUE was common to both experiments and genotypes ranked similarly.

The methods presented here allowed dissecting the differences between experiments into (i) genotypic traits that did not differ between experiments but had a high genetic variability, namely plant architecture and RUE (ii) environmental differences, essentially incident light, that affected both biomass and leaf area, (iii) plant traits that differed between experiments due to environmental variables, in particular leaf growth.

Post Green Revolution genetic advance in durum wheat: the case of Spain

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In recent decades, the genetic progress in grain yield of wheat has reduced due mostly to a narrow genetic base and lack of new breeding techniques. Understanding of changes in yield and linked traits related to breeding advances is critical for improving our knowledge of factors limiting yield and for designing future breeding strategies. This is particularly important for wheat in the Mediterranean basin, where the negative effects of ongoing climate change are already evident.

This study evaluates the breeding advances of durum wheat (Triticum turgidum L. var. durum) after the Green Revolution, with Spain as a case study. A set of twenty cultivars, covering the last 45 years of breeding, were evaluated during two consecutive crop seasons (2013/14 and 2014/15) under different locations and water regimes, totaling nine trials and a wide range of yield conditions (3–8 Mg ha\(^{-1}\)). Besides grain yield, carbon isotope composition of mature kernels was also analyzed, as an indicator of intrinsic water use efficiency. Genetic advances, as calculated for the whole set of trials, was lower (10.21Kg ha\(^{-1}\) yr\(^{-1}\)) than values reported in scientific literature for post Green Revolution durum and bread wheat under Mediterranean conditions. A different pattern was observed when the trend was studied separately within the three subsets of trials with high (>6.6 Mg ha\(^{-1}\)) average (5.5–6.5 Mg ha\(^{-1}\)) and low (<5.5 Mg ha\(^{-1}\)) yields. Except for few genotypes, no clear genotype by environment interaction was observed. Moreover, no clear genotypic trend was observed for carbon isotope composition across time.
Monitoring micro-plots for high throughput phenotyping using a UAS
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Breeders are eager to identify QTLs to drive the genetic improvements. Traits should be as independent as possible from the environment subjected by the plant, and rather a measure of the potentials and sensitivity to several biotic and abiotic factors. The traits should therefore be closely related to the functioning of the crop. The plant functioning may be accessed through the dynamics of few state biophysical variables including the Green Fraction (GF), the fraction of intercepted photosynthetically active radiation (fiPAR), the green area index (GAI), Leaf Chlorophyll content (Cab) or crop height. Further these noninvasive variables must be characterized in field to better represent realistic agricultural conditions. Non-invasive measurements made with a multispectral camera may provide good estimates of these variables in experimental fields that are representative of actual agriculture conditions. Unmanned Aerial Systems (UAS) are well suited to monitor with a high throughput these canopy state variables. Their time course will provide proxies of the plant vigor, senescence rate, allows to compute the radiation use efficiency, evaluate the impact of stresses on the leaf development and detect the main phenological stages.

Several field experiments were conducted on wheat, maize and sugar beet in France in 2015 with a large panel of cultivars grown under several modalities. The Airphen multispectral camera with 6 bands was flown aboard a hexacopter at an altitude of 70 m ensuring a spatial resolution around 2.5 cm at the ground level. The camera was carefully calibrated over a reference gray carpet to compute canopy reflectance. It was maintained in the nadir direction to maximize the sensitivity to canopy structure, i.e. leaf area and orientation. The monitoring was achieved under contrasted meteorological conditions in terms of cloud cover, sun position and wind during the flights. A dedicated and processing pipeline was developed to, extract the estimates of all these variables other each microplot.

Results show very consistent time profile for the considered state variables despite the range of illumination and wind conditions during the flights. Heritability was investigated for the characteristics describing the main temporal patterns. Results are discussed with due attention to application for plant breeding.

High-throughput plant phenotyping at the Vienna Biocenter: CSF 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 (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: UV375, blue405, blue460, white and red650), and different gas conditions (e.g. CO₂) 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, sensor-to-plant screening system linked to LemaTec image analysis software. The phenotyping system is fully integrated into one of the chambers allowing highly precise control of environmental conditions throughout the experiment.

Besides high-throughput screening of Arabidopsis we are currently evaluating LemaTec’s software for various other applications e.g. low-throughput (side-view) phenotyping of crop plants, screening of seedlings (agar-plates), root phenotyping (agar-plates) and confocal microscopy image analysis.
UAV HTPPs for wheat and maize climate change risk mitigation

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Current breeding methodologies have been successful in breeding drought tolerance; however, the acceleration of breeding advances is urgently needed to confront increases in the frequency and intensity of climate change-induced extreme weather events. Extreme and abnormal weather events, as well as the more gradual meteorological trends associated with climate change often coincide with not only increased abiotic stress, but also increased pressure from biotic stressors due to conditions that are often favorable to the rapid spread of crop pests and diseases. Durum wheat is by extension the most cultivated cereal in the south and east margins of the Mediterranean Basin. It is of strategic importance for Mediterranean agriculture to develop new varieties of durum wheat with greater production potential, better adaptation to increasingly adverse environmental conditions (drought) and better grain quality. Similarly, maize is the top staple crop for low-income populations in Sub-Saharan Africa and is currently suffering from the appearance of new diseases, which, together with increased abiotic stresses from climate change, are challenging the very sustainability of African societies. Current constraints in field phenotyping remain a major bottleneck for future breeding advances, but High-Throughput Phenotyping Platforms (HTPPs) using multispectral and conventional RGB cameras have shown promise for rapidly developing both disease-resistant and weather-resilient crops. RGB cameras have proven cost-effective in studies assessing the effect of abiotic stresses, but have yet to be fully exploited to phenotype disease resistance. Recent analyses of aerial and field images of durum wheat in Spain and maize in Kenya and Zimbabwe have shown RGB vegetation indexes to consistently outperform NDVI in disease and yield prediction. Results using UAVs (Unmanned Aerial Vehicles, or drones) with multispectral and conventional RGB cameras demonstrate the strengths and limitations of lower resolution multispectral sensors and the more cost-effective, high spatial resolution conventional RGB digital cameras.

The system for acquisition and reconstruction of 3D model of plant chlorophyll fluorescence

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3D modeling of plant photosynthetic capacity is one of the basic prerequisites for further progress in plant breeding to improve plant architecture, nutrient allocation, activity of photosynthetic processes and assimilate storage in the vertical profile of plant, thereby improving the light use efficiency of the entire canopy.

For the purpose of creating a 3D model of chlorophyll fluorescence a new system was developed, combining spatial arc manipulator, 3D scanner based on structured light technology and adapted fluorescence camera. The process of data acquisition consists of three separate phases. First phase is the 3D model acquisition, where object is scanned, the data are transferred to the PC and basic model processing is done. In the second phase the chlorophyll fluorescence transient is measured for each pixel and images of basic chlorophyll fluorescence parameters are derived. Within the last step the chlorophyll fluorescence image is co-registered to the 3D model of plant and 3D model of chlorophyll fluorescence is produced together with calculated basic morphological parameters. Processes in individual steps are performed automatically and are controlled by separate software modules. Model analysis is running under Matlab environment in two sub-phases. In the first one, plant morphological parameters (leaf area, leaf angle) are computed and output is written to the xml file. In the second sub-phase, the fluorescence image is mapped to the 3D model and the result is exported. Later the algorithm for correction the effect of changing excitation energy and pixel size with leaf distance and angle was developed and this will be programmed into model analysis software.

Characterization of wheat sowing pattern and plant density from RGB images taken at the ground level

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Plant density is an important variable in farming practice. With the development of high-throughput phenotyping, increasing non-invasive techniques have been introduced into the decision making for agriculture by characterizing the plant architecture and canopy functioning. The scaling from the plant to the canopy is generally achieved simply through the plant density: the error on plant density will be directly propagated to other stand scale parameters. Further, the uniformity of...
plant distribution starts to attract attention: recent research showed that the in-uniformity of plant distribution pattern could intensify the competition between adjacent plants and reduce that with the weeds, while increasing the differences between plants.

With the development of high-resolution commercial camera, RGB images have been used to extract LAI, plant structure, leaf shape and other information, which proves its applicability and advantages compared with other techniques in terms of cost and efficiency. However, only little attention has been paid to the plant density and sowing pattern from non-invasive techniques.

In this work, we developed the processing pipeline to extract plant distribution pattern and automatically estimate the density from RGB images taken in the field. The experiments over wheat crops were conducted in Toulouse with 5 densities, 100, 200, 300, 400, 600 plants m⁻², and in Avignon with 4 densities, 100, 200, 300, 400 plants m⁻². This paper presents this method and the main results obtained:

1) The sowing pattern could be characterized by:
   a. Gamma distribution of plant location along the row and normal distribution across the row.
   b. Along the row direction, the plants are distributed independently. Conversely, significant spatial correlations were found across the row for the highest densities.
   c. The distribution law along the row direction allowed using a data-count model to estimate the required number of plants to be sampled to get a given precision level. Results show that for counting 90 successive plants and measuring the corresponding row length provides an accuracy of 10% independently from the plant density.

2) After image classification, the features of each object in the image can be applied to train neural network to identify the number of plants per object. Such supervised approach provides reliable estimates of the plant density with rRMSE 10%.

Affordable, robust and standardized plant phenotyping with Phenotiki

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In the last decade several systems and software solutions have been proposed to obtain reliable phenotyping information from plant experiments. However, currently, most high-throughput solutions are too expensive and many labs instead develop highly customized (hardware and image analysis) solutions tailored to their experimental setting. Even when they are affordable this variability in methods and set-ups creates standardization problems.

To overcome such limitations, we have developed Phenotiki: an affordable solution for phenotype acquisition and analysis based on distributed sensing and analysis. Affordable and easy-to-install sensors based on the Raspberry Pi device can be deployed in a greenhouse or growth-chamber setting. Sensors collect time-lapse images of growing plants and transmit them over the network to the cloud or to a local workstation, where sophisticated computer vision algorithms extract visual phenotypes. Our cloud-based approach offers several key advantages: a cloud application is always up to date, Web-based interfaces offer transparent access to algorithmic and computational resources, and the user is relieved from maintaining locally a computing infrastructure. Our sensor is truly affordable (equipment investment is less than €100), thus multiple sensors can be deployed to cover wide areas. Standardizing equipment and analysis permits consistency in experiments among different labs, while an open and expandable architecture still offers the possibility to change or add new camera sensors (e.g., near infrared) to meet different phenotyping needs.

We validated the Phenotiki system in an experiment aimed at studying diurnal and nocturnal growth of five different genotypes of Arabidopsis thaliana. We automatically extract plant size, and leaf count driven by machine learning based algorithms. Based on size, colour related traits, and leaf count the system highlighted statistically significant phenotypic differences among the genotypes. To favour adoption of our system, software and sensor set-up instructions will be released in the public domain, at http://www.phenotiki.com.

References


**Chlorophyll fluorescence imaging for screening cold tolerance in natural accessions of Arabidopsis thaliana**

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Cold is one of the major factors affecting growth and geographical distribution of plants and adversely affects crop yields. Traditional methods for screening cold tolerance, i.e. survival rate and quantification of the lethal temperature (LT₅₀) at which 50 % of electrolytes released from the plasma membranes, are labor intensive, time-consuming and thereby of limited value for large scale screening. Therefore, we have tested the capacity of chlorophyll a fluorescence (ChlF) imaging based methods for the first time on intact whole plants in nine natural accessions of *Arabidopsis thaliana* for (1) non-acclimated (NAC, six week old plants grown at room temperature), (2) cold acclimated (AC, NAC plants acclimated at 4 °C for two weeks), and (3) sub-zero temperature (ST) treated (STT, AC plants treated at −4 °C for 8 h in dark) state. We report that ChlF transients are more informative as cold acclimation broadened the slow phase of ChlF transients in cold sensitive accessions. Similar broadening in the slow phase of ChlF transients was observed in cold tolerant plants following ST treatments. The classical ChlF parameters well categorized the cold sensitive and tolerant plants when measured in STT state. Further, we trained several statistical classifiers with the sequence of captured ChlF images and selected a high performing quadratic discriminant classifier (QDC) in combination with sequential forward floating selection (SFFS) feature selection methods and found that combinatorial imaging showed a reasonable contrast between cold sensitive and tolerant *A. thaliana* accessions for AC as well as for STT states. We shall present our newly developed methods for selection of cold tolerant and sensitive accessions as well as demonstrate the applicability of chlorophyll fluorescence based methods for investigating cold tolerance induced modulations in photosynthetic mechanisms.

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**References**


**Phenotyping of a sequenced tomato core collection for plant growth and fruit quality traits**

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Recently, more than 500 different tomato accessions have been (re)sequenced, providing an excellent and untapped resource of promising genetic variation. However, there is a lack of phenotypic information about the accessions used and this hampers the selection of promising accessions for genetic studies. In this project we aim to phenotype a core collection of 104 (re)sequenced tomato accessions consisting of wild relatives, land races and old cultivars to unravel the genetic and physiological basis underlying processes affecting plant growth and fruit quality traits. For this phenotyping analysis the core collection was grown in triplicate plots in a controlled greenhouse and a variety of (semi-) automated and manual techniques were used to obtain information of several attributes:1) Crop plant phenotyping platform including: flowering time, inflorescence architecture, fruit number, sympodial index, leaf area, photosynthesis efficiency, fruit yield; 2) Fruit quality traits such as: weight, firmness, fruit color, locale number and 3) Metabolomics platforms for the detection of aroma volatiles, primary metabolites and semi-polar secondary metabolites (flavonoids, alkaloids, polyamines). The most promising genotypes of the first screen were re-grown and analysed to validate initial results. For all traits analyzed, a lot of variation was present in the core collection. Promising genotypes for several fruit quality traits were selected for follow-up genetic studies. Current efforts are geared towards associating allelic variation in selected candidate genes with several fruit quality traits.

**Keywords:** Phenotyping, Tomato, Trait, Genotype, Resequenced
Field phenotyping requires characterizing the biophysical properties of the vegetation through non-destructive measurements to monitor the whole vegetation cycle. Remote sensing thus appears as a powerful tool to achieve this goal over large experiments. The green fraction (GF) is one of the most easily accessible characteristic of the vegetation structure from remote sensing observations is. GF is closely linked to the efficiency of light interception, the plant density, the plant development, the vigor and the green biomass. High resolution RGB cameras aboard UAVs or on ground based systems allow deriving the green fraction by classifying the images. Because of the large volumes of images that have to be processed, automatization of the classification process is mandatory. However, the illumination conditions, setup of the camera and variability of soil optical properties may pose problems in the classification process.

We present a new algorithm able to perform automated classification of green vegetation and background pixels within digital RGB images to estimate the green fraction. This algorithm is based on support vector machines and is able to process images provided by different sources including UAVs and UGVs, in different light environments and over 3 crop types (wheat, sugar beet, potatoes) at several growth stages. Further, aside from providing a green/non green status, it allows to assess the probability that a pixel belongs or not to green vegetation. However, the SVM technique is a supervised classification method that requires a crucial step consisting in building a learning dataset.

Results show that SVM provide better results in terms of accuracy as compared to classical thresholding techniques. While these latter require some tuning when applied in different conditions (light, crop, development), we show that SVM is very robust by comparing its performances when using different learning datasets to process the whole set of images. Finally, we also evaluate the dependence of the SVM estimated green fraction to the probability threshold applied on the processed image.

Combining high-throughput phenotyping and GWAS to accelerate the dissection of rice genetic architecture
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As plant genomics rapidly develops with the use of high-throughput sequencing techniques, traditional plant phenotyping lags far behind. To relieve this bottleneck, our work describes a combination of high-throughput phenotyping and GWAS to unlock genetic information coded in the rice genome for controlling the complex traits and demonstrates the feasibility of replacing laborious manual phenotyping with objective, efficient, and non-destructive phenotyping tools such as HRPF. Based on a performance evaluation of the HRPF and GWAS results, we demonstrated that high-throughput phenotyping has the potential to replace traditional phenotyping techniques and can provide novel gene identification information. With appropriate modifications on the image analyses, we anticipate that the combination of HRPF and GWAS can be used in a wide spectrum of other plant species to determine their genetic architecture and provide insights into basic biological processes. And we also developed some complementary phenotyping tools to HRPF, such as high-throughput leaf scorer for rice leaf traits, micro-CT for rice tiller traits, and so on. In conclusion, the combination of the multifunctional phenotyping tools HRPF and GWAS provides novel insights into the genetic architecture of important traits.
INTERNATIONAL ACTIVITIES

PhénoField®, a high-throughput phenotyping platform to screen genotype response to drought in field conditions

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Phenotyping is now a major limitation for breeding genotypes that increase crop performance under climate change and reduced inputs in a context where agriculture needs to be both highly competitive and environmentally friendly. The French Plant Phenotyping Network (FPPN-PHENOME) provides academic and private communities with a network of nine instrumented platforms able to deal with most crop species, biological questions and environmental stresses.

PhénoField®, the platform managed by ARVALIS, is a highly instrumented field research facility allowing detail analysis of yield build-up of maize, cereals and other crops genotypes under managed water deficit. Located in the Beauce Region in France, it is composed of 8 mobile rain shelters equipped with controlled irrigation. The available water capacity is monitored by an ensemble of sensors distributed in many places selected to represent the variability of soil properties that are scaled up using a sub-metric spatial resolution map. Phenotypic traits are measured by a suite of novel sensors including RGB cameras, LIDARs and spectrometers which periodically acquire from a fully automated gantry, along with consistent monitoring of plot micro-meteorology. A team of specialists on instrumentation, signal processing, plant modeling, plant physiology and applied agronomy is dedicated to run the experiment and transform the raw measurements into pertinent traits to be directly used by breeders. As an applied institute for farmers and breeders, the experience of ARVALIS on technological transfer and its active role in developing innovative methods in the framework of the FPPN, make it a strong candidate to design, conduct or host tailored protocols and research programs for genetics, breeding, and crop physiology of genotypes harboring promising traits.

For its first year of operation in 2015, PhénoField screened a historic panel of maize hybrids in the framework of the AMAIZING project so as to evaluate the genetic progress in drought response and identify physiological traits underpinning it. Along with methodological studies and a first commercial service, the 768 plots that occupied 10 000 m² of experimental area were conducted under 3 irrigation regimes.

In 2016, PhénoField will principally welcome a comprehensive panel representing the last 25 years of wheat breeding in the framework of BREEDWHEAT project. This trial should provide insight into the genetic and physiological architecture of the drought response of wheat. Following exploitation years will be distributed between the screening of genetic diversity panels for the BREEDWHEAT and AMAIZING projects and new coming academicals or industrials partnerships or commercial services.

French and German elevated CO2 set ups in agricultural fields: The start of a European Consortium of FACE rings for breeding and agriculture

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It is predicted that worldwide CO2 concentration will continue to increase (IPCC 2014) and that regional weather patterns will alter substantially. Elevated atmospheric CO2 concentrations can stimulate photosynthesis and induce stomatal closure affecting carbon uptake and water use. However little is known about the interaction between CO2 and abiotic/biotic constraints and about the genetic variability of the response to its atmospheric concentration. Thus, the development of strategies for adaptation and acclimation of crop productions to changing environmental conditions requires large scale studies in all fields of plant research and production.

Systems to study elevated CO2 on plants grown under open field conditions, so called Free Air CO2 Enrichment (FACE) systems, have proven their relevance in providing information in conditions closer to agronomic situations than plants grown in greenhouses and open top chambers. The urgent need for new FACE facilities was already expressed in 2007 at a European Science Foundation meeting. The focus was on analysing long-term soil impact and plant response in the context of breeding.

Almost 10 years after, two national plant phenotyping platforms have incorporated a FACE system in their infrastructure. These are the Phenome: French Plant Phenomic Center (www.phenome-fppn.fr) and the German plant phenotyping network (www.DPPN.de). Both platforms equipped with the latest phenotyping techniques will be completed in the coming
year. The similar technology and design used in both FACE systems allow for the unique opportunity of testing breeding populations or specific crop cultivars at elevated CO\textsubscript{2} in two different regional climates. The synergy between the two FACE installations could be the first step towards a European consortium of FACE rings to answer important questions on increasing yield for food security under future climate conditions.

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Climate change causes an increased frequency of unfavourable environmental scenarios with abiotic and biotic stresses, requiring the development of novel adapted varieties. Phenotyping is the major limitation for selecting genotypes in this context. Phenome (www.phenome-fppn.fr) develops a versatile, high-throughput infrastructure and a suite of methods allowing characterisation of hundreds of genotypes of different species under environmental scenarios of climate changes (e.g. drought, high CO\textsubscript{2} and high temperatures).

The infrastructure consists of (1) two platforms in controlled conditions (capacity of 1700 plants each) for in-depth analysis of leaf or root system architectures and growths under ranges of water deficits, CO\textsubscript{2} concentration and temperature; (2) two field platforms with semi-controlled environments, in particular large rainout-shelters and one free-air carbon enrichment (FACE) system (capacity 800 individual plots each); (3) three field platforms with higher throughputs (capacity 2000 individual plots each) equipped with soil and climate sensors. All platforms can cope with throughputs of 200-300 genotypes with the necessary number of repetitions and manipulate and/or control environmental conditions in order to impose well-characterised scenarios. Platforms are equipped with a consistent set of 3D functional imaging techniques, namely detailed imaging of roots and shoots in controlled conditions, canopy imaging with an autonomous 'phenomobile' that captures functional and 3D images of each plot, and drones that image hundreds of plots jointly. Two supporting platforms centralise metabolomic and structural measurements associated with phenotyping experiments. Platforms are accessible to public and private partners via the project website.

Applications with technological jumps are developed at infrastructure level, with partnerships with French SMEs. They (1) improve our capacity to measure plant traits at different resolutions in field and platforms (eg root and shoot architectures, light interception, transpiration rate) and environmental conditions (novel sensors); (2) organise phenotypic data originating from different platforms, so that they can be saved and analysed for a long period by a wide scientific community; (3) handle very large datasets with applications on data cleaning via artificial intelligence and analyses of time-related data; an interface with plant and crop models is developed. These methods and techniques are widely transferred towards the phenotyping community, academic and industrial. Phenome has already resulted in the development of SMEs aimed at phenotyping and/or precision agriculture (including one spin off, several patents and new activities of already existing SMEs. Networking and training activities are developed towards seed companies, SMEs and the extension system. Phenome is part of an Infrastructure (I3) European project (EPPN) and is participating to an initiative for a preparatory phase for a European ESFRI infrastructure. It participates to the French national roadmap of Infrastructures with a widened partnership (CEA, CNRS, INRA).
M3P: The "Montpellier Plant Phenotyping Platforms"
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The research group LEPSE, ranked among the top 5% research group in ecophysiology worldwide in 2014 by a panel of experts is developing high throughput phenotyping platforms for more than 15 years. Three complementary platforms, embarking 500 to 1700 plants simultaneously, aim to analyze and model genetic variability of plant responses to environmental stresses and climate change (mainly drought and elevated temperature). These platforms host large collections of genotypes of the same species, evaluate their tolerance and obtain relevant parameters that will be injected into predicting models allowing the selection and the breeding of future, tolerant and more efficient varieties.

These platforms are gathered into "Montpellier Plant Phenotyping Platforms" (M3P), that is a full member of the "Investment for the future" initiative PHENOME. The platforms host ~ 50% of external access in the frame of national and international projects on a variety of species (maize, wheat, grapevine, apple tree, sorghum, millet, rice, A. Thaliana).
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