

4th International Plant PHENOTYPING SYMPOSIUM

December 13-15, 2016
El Batán, Texcoco, Mexico



ABSTRACTS

CIMMYT

Headquartered in Mexico, the International Maize and Wheat Improvement Center (CIMMYT) is the global leader in publicly funded research for development for wheat and maize and for wheat- and maize-based farming systems. CIMMYT works throughout the developing world with hundreds of partners, belongs to CGIAR and leads the CGIAR Research Programs on Wheat and Maize. CIMMYT receives support from CGIAR Fund Donors, national governments, foundations, development banks and other public and private agencies. For more information, visit: www.cimmyt.org.

IPPN

International Plant Phenotyping Network is an association representing the major plant phenotyping centers. IPPN aims to provide all relevant information about plant phenotyping. The goal is to increase the visibility and impact of plant phenotyping and enable cooperation by fostering communication between stakeholders in academia, industry, government, and the general public. Through workshops and symposia, IPPN seeks to establish different working groups and distribute all relevant information about plant phenotyping in a web-based platform. For more information, visit: www.plant-phenotyping.org.

IWYP

The International Wheat Yield Partnership (IWYP), established under the Wheat Initiative is a new and unique coordinated international research initiative with a goal to generate the breakthroughs that will raise the genetic yield potential of wheat by up to 50% in the next two decades with the ultimate goal of generating significant yield improvements in farmers' fields. IWYP is a voluntary consortium of international public funders, research organizations and private industry partners who share the common goal of significantly increasing the genetic yield potential of wheat in the near future. IWYP embodies an integrated, multi-disciplinary, international research program involving a combination of public and private funded research, in-kind support, and where possible, aligned with other current relevant research programs worldwide. For more information, visit: www.wheatinitiative.org.

MasAgro

MasAgro is a CIMMYT-led project sponsored by Mexico's Agriculture Department (SAGARPA) that strengthens agricultural productivity at all levels of Mexico's maize and wheat value chains. MasAgro develops molecular atlases of maize and wheat to help researchers identify genetic traits that determine grain yield and adaptation capacity, and transfer those traits to the varieties farmers and consumers demand. MasAgro has established networks with public research institutions and Mexican seed companies that evaluate and exchange pre-commercial non-transgenic seed of maize varieties and hybrids adapted to tropical and subtropical production areas and to the Mexican Highlands. More than 300,000 farmers (21 percent are women) who participate in MasAgro apply sustainable conservation agriculture practices on more than 970,000 hectares sown to maize, wheat and associated crop varieties. MasAgro trains and provides technical advice to farmers who produce maize, wheat and associated crops so they can sow improved seed, reduce costs and increase productivity and income sustainably. For more information, visit: masagro.mx.

SAGARPA

The Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food, is a unit from the Federal Executive Branch of the Government of Mexico, which has among its objectives promoting the execution of a policy of support, which allows producers to improve their production practices, utilizing in a more efficient manner the competitive advantages from our agricultural, livestock and fisheries sectors, and integrating the economic activities from rural areas into larger productive chains, encouraging the participation of organizations of producers with economic projects on their own, as well as with the proposal of goals and objectives for the agricultural sector within the National Development Plan. For more information, visit: www.gob.mx/sagarpa.

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About the International Plant Phenotyping Symposium

Phenotyping is key to understanding the physiological and genetic bases of plant growth and adaptation and their application for crop improvement. A revolution in phenomics is taking place, using non-invasive technologies based on spectral reflectance from plant tissue. Automated proximal sensing in controlled environments has sufficient resolution for detailed genetic and physiological dissection in model plant species. Aerial imaging using manned or unmanned low-flying vehicles or even satellites is transforming field phenotyping, at scales ranging from individual plots for breeding to entire fields to characterize agro-ecosystems.

The 4th annual Symposium focuses on three themes:

Advances in Plant Phenotyping Technologies to explore the frontiers of what can be sensed remotely and other technological breakthroughs.

Phenotyping for Crop Improvement to consider the application of phenotyping technologies for crop improvement (breeding, crop husbandry, and estimating the productivity of agro-ecosystems).

Adding Value to Phenotypic Data to review how phenomics and genomics can combine to improve crop simulation models and breeding methodologies (e.g., genomic selection).

For more information, go to www.cimmyt.org/event/4th-international-plant-phenotyping-symposium.

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Tuesday 13th December (Morning)

ADVANCES IN PLANT PHENOTYPING TECHNOLOGIES**Comprehensive data harvesting for field phenomics and knowledge discovery**

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Recently data on yield and rate of fertilizer application in the field can be collected automatically as geolocation data measured by RTK-GPS. However, such snapshot data are not sufficient, since plants grow dynamically under a diversity of environmental conditions and symbiotic microorganisms. We are developing Data-Harvester to collect time-series data in the field as well as a Knowledge Discovery Support System (KDSS). Data-Harvester consists of sensor networks, drones and walking robots and collects time-series data such as air temperature, soil moisture and hourly/daily images of plants. KDSS provides services such as data management, 3D-reconstruction from 2D-images, machine learning, pattern recognition, data assimilation and meta-computing. Ideally Data-Harvester should also automatically collect time-series data on microscopic phenotypes, microorganisms and symptom expression, so the walking robots are equipped with manipulators. As for practical applications, collected big data can be mined for simple knowledge. However, for the first time we are tackling difficult problems such as the elucidation of hybrid vigor to evaluate the capabilities of Data-Harvester and KDSS. We will improve them by appending required functions to solve problems quickly with artificial intelligence, crowd intelligence, etc. This work is supported by CREST, JST.

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

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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 and other crop genotypes under managed water deficit. Located in the Beauce Region in France, it consists of eight 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 data from a fully automated gantry, along with continuous monitoring of plot micro-meteorology. RGB and multispectral acquisition of the full platform is regularly performed from unmanned aerial vehicles to complement spatial and temporal sampling. A team of specialists in instrumentation, signal processing, plant modeling, plant physiology and applied agronomy is running the experiment and transforming raw measurements into pertinent traits to be directly used by breeders.

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 the physiological traits underpinning it. In 2016, PhénoField welcomed a comprehensive panel representing the last 25 years of wheat breeding in the framework of the BREEDWHEAT project. This trial should provide insight into the genetic and physiological architecture of the drought response of wheat. The first instrumental results and methodological studies will be presented along with a testimony of our experience during the commissioning of such semi-controlled field platform, including calibration constraints, real-time campaign monitoring, data management and signal validation for agronomic use.

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 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 visualization. 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, maximizing plant productivity is vital. Therefore, visualization 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 visualize a growing root within an undisturbed soil core. The X-ray CT technique enables non-destructive 3D investigations into root:soil interactions at the micro-scale. By imaging the actual 3D geometries of soil structure and visualizing the interfaces between roots, soil, water and air-filled pores, an accurate representation of water movement and root growth in soil is achieved. Destructive methods such as root washing that were commonly employed for root studies could not provide detailed information on root architecture, including branching characteristics and extension rate, which are inherently linked to conditions within the soil matrix. In contrast, this technique enables root phenotyping of different crop species and varieties in soil. This information is crucial if laboratory research is to be translated into an understanding of responses under field conditions.

Automated segmentation of potato tuber computed tomography measurements for non-destructive biomass determination

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The reconstructed volume data set of X-ray computed tomography (CT) consists of gray levels which represent information about X-ray attenuation. This attenuation coefficient depends on the density distribution of the material, the elemental composition of the X-ray source and its spectral distribution. A 3D image segmentation is needed to study the below-ground growth of tubers. Increasing the dimensionality from 2D to real 3D enhances the complexity of image segmentation algorithms. At the same time, volume information is accessible for direct correlation with volumetric traits, for example, tuber volumes or form factors. Additionally, the calculated X-ray attenuation coefficient of the segmented tubers can be used to track density changes within the tuber. Automatic segmentation and analytical routines are needed to create comparable results between the different points and between different sampling times.

We will present the image segmentation principles and the gathered data on volumetric tuber growth of different varieties. Using this method, it is possible to monitor the growth of each individual tuber. Additionally, the destructive sampling of a subset of all measured pots allowed us to correlate fresh and dry weight with absorption and volumetric parameters from the X-ray measurement. Using the presented principle of biomass determination from X-ray CT data, the same correlation can be obtained for all kinds of plant materials using different segmentation algorithms.

3D imaging approaches in quantitative plant phenotyping: Application scenarios in the lab and in the field

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In plant phenotyping, 3D imaging approaches are increasingly used to study the impact of genetic variability and environmental factors that influence leaf angles and light interception resulting in varying canopy architecture. The non-invasive acquisition of 3D structure of different plant organs such as leaves, roots, fruits or seeds requires using different methodological approaches. Apart from active methods, which make use of an artificial light source (e.g., Light Detection and Ranging [LIDAR], Light Sectioning or Structured Light), camera-based methods are widely used and substantially differ in terms of image data processing. Here we give an overview of 3D imaging approaches, which were developed at the Institute of Biosciences (IBG-2, Forschungszentrum Juelich, Germany) with a focus on developing and benchmarking measurements as part of the two German project clusters Crop.Sense.net (www.cropsense.uni-bonn.de) and the German Plant Phenotyping Network (DPPN; www.dppn.de). We will demonstrate the use of different sensor techniques, ranging from structured light methods in the lab up to light-sectioning approaches and stereo-imaging in different application scenarios in the lab and

in the field, thereby covering the scale of small plant populations to smaller scales of single plants. Using structured light, we were able to resolve and quantitatively characterize single leaves up to a size of 2 mm. We will highlight the application of multi-camera setups under natural environmental conditions at the scale of experimental plots (up to 2 m²) along with new image processing pipelines to estimate leaf area and leaf angle distribution in sugar beet experiments. Here plants were analyzed on the basis of individual leaf 3D models from segmented stereo images.

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

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Understanding the genetic control of biomass production and yield is a major challenge in the context of climate change. We developed a suite of methods to measure light interception, interception efficiency and radiation-use efficiency (RUE) in thousands of plants using a high-throughput phenotyping platform. Different models were interfaced to calculate: (i) the spatial distribution of incident light, as experienced by hundreds of plants in a greenhouse, by simulating sunbeam trajectories through glasshouse structures every day of the year; (ii) the amount of light intercepted by plants (I_{PPFD}) via a functional-structural model using 3D reconstructions of each plant placed in a virtual scene reproducing the canopy in the greenhouse; (iii) RUE calculated as the ratio of plant biomass to I_{PPFD} . Inputs for these models were (i) images of each plant taken every 30° every day for estimating leaf area, biomass and architecture; and (ii) environmental data collected every 15 min at 8 sites within the greenhouse. These methods were tested using the PhenoArch plant phenotyping platform (www6.montpellier.inra.fr/lepse/M3P) during six experiments with panels of 1,680 maize (*Zea mays* L.) hybrids or lines grown in different seasons (contrasting light and evaporative demand) and different soil water potentials. Each of the studied traits displayed large genotypic variability. By calculating "hidden variables" encapsulating spatial and temporal environmental variations such as RUE, we identified heritable physiological traits usable in genetic analyses and crop modeling that are currently applied in field studies, thereby opening the way for large-scale genetic analyses of plant performance components.

Holistic and component-based dynamic vegetative-stage plant phenotyping analysis

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Extracting meaningful numerical phenotypes from plant images remains a critical bottleneck in automated plant phenotyping. We classify image-based phenotyping approaches into holistic and component-based. Holistic analyses consider the whole plant as a single object and measure its

attributes, whereas component-based phenotyping analyzes individual plant parts, e.g., leaves and stems. Two novel holistic phenotypic components are introduced: bi-angular convex-hull area ratio and plant aspect ratio. *Bi-angular convex-hull area ratio* is defined as the ratio of the area of the convex-hull of the plant when viewed from the side at a particular angle and the area of the convex-hull of the same plant when viewed at a rotation of 90°. It provides information about temporal changes in phyllotaxy, i.e., the arrangement of leaves around a stem. *Plant aspect ratio* is defined as the ratio of the height of the bounding rectangle of the plant from the side view and the diameter of the minimum enclosing circle from the top view. It characterizes the canopy architecture that is generated by the crop accessions in the field. The following component-based phenotypes are computed: leaf count, leaf size, stem angle and leaf curvature. The growth of plant is best interpreted by the number of leaves and the size of each leaf during the plant's life cycle. Thus, we introduce an algorithm for leaf-count and leaf-size measurement. The stem angle, i.e., the angle between the stem and the horizontal axis, away from vertical, can be an early signal that a given plant is going to be susceptible to lodging. Yield losses due to lodging reduce the US maize harvest between 5-25% a year (\$2.4-12 billion at 2015 maize prices). Leaf curvature is measured to provide information on leaf drooping. A plant-component tracking algorithm is introduced to study the temporal variation of these component-based phenotypes in maize using our newly introduced benchmark dataset called Component Phenocorn Dataset.

Using LIDAR to measure forage yield of perennial ryegrass (*Lolium perenne* L.) field plots

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A LIDAR-based tool for non-invasive estimation of plant biomass in perennial ryegrass field plots was developed. This included designing and making a prototype of a machine for LIDAR data collection, and developing algorithms for data processing. The biomass estimates were validated with regression analysis against harvest data. The project was implemented in three phases. In phase 1, a prototype-carrying frame and a light-excluding cover was constructed for the LIDAR scanner. An algorithm was developed for grass plot segmentation, ground surface detection and estimation of plant biomass. Phase 2 focused on developing the prototype tool further, including application-specific real-time capture end-user software for data capture and analysis. This included testing the algorithm and in-field testing of the software. An experiment was also conducted to study how the variation in ground level between different scans affected the measurement. It was found that the variation of ground level was significant (more than 20 mm) between adjacent scans and within each segment. An improved method with correction for soil surface variation was developed to estimate the ground level of each scan and increase the accuracy of biomass estimation. In phase 3, 86 segments in replicated field plots of a perennial ryegrass cultivar trial in Canterbury, New Zealand, were scanned with LIDAR at early, mid and late time points, with mechanical harvest and yield data collection at the late growth stage. Significant ($P < 0.0005$) correlations were observed between processed LIDAR data and fresh and dry weights of

plant foliage biomass with R^2 values of 0.78 and 0.76, respectively. The late-growth calibrated data were used to explore ryegrass growth dynamics using LIDAR scans at early growth and mid-growth stages.

Plant phenotyping reveals genetic and physiological factors of plant performance

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Knowledge of the structural and functional genetic architecture of agricultural traits is a prerequisite for the systematic exploration and utilization of plant genetic resources in plant breeding. To uncover mechanistic links between genetic variation, physiological factors and whole plant performance for developing novel crop improvement strategies, Arabidopsis, maize, and rapeseed were studied using integrating genotyping, transcript and metabolite profiling, and automated plant phenotyping with dedicated platforms. The three high-throughput plant phenotyping facilities at IPK support automated whole-plant analyses for small, medium and large plants including cultivation, transport (plant-to-sensor) and imaging of plants in climate controlled phytotron/glasshouse cabins. They were equipped with cameras and illumination systems for visible, fluorescence and near-infrared imaging using top view and side views. Furthermore, 3D laser scanners and LED panels and CCD cameras for functional chlorophyll fluorescence detection and a broad range of environmental sensors were installed. The value of repeated non-invasive/non-destructive monitoring of large plant populations is highlighted by results of the analysis of a collection of 261 maize dent lines using a specifically optimized cultivation and phenotyping setup characterized for biomass accumulation and water consumption, and thus, for water use efficiency. Combined with 50k SNP information, these data have been used to identify QTL of these traits and of the growth dynamics by genome-wide association testing. Twelve main-effect QTL and 6 pairs of epistatic interactions were detected that displayed different expression patterns at several individual developmental time points. A subset also showed significant effects on relative growth rates at different intervals. Using nonparametric functional mapping and multivariate mapping approaches, four additional QTL affecting growth dynamics were detected. Our results demonstrate that plant biomass accumulation is a complex trait governed by many small-effect loci, most of which act at certain restricted developmental stages. This highlights the need to detect and investigate stage-specific growth control genes operating at different developmental phases. Integrated metabolome analysis and whole plant phenotyping performed in Arabidopsis revealed direct links between a promoter InDel polymorphism of the FUM2 gene, its mRNA expression, fumarase enzyme activity, and fumarate:malate ratio in leaves. The promoter InDel that coincided with a fumarate QTL found in Arabidopsis Col-0/C24

RILs and ILs was also significantly associated with the fumarate:malate ratio, with malate and fumarate levels, and with dry weight in 174 natural accessions at 15 days after sowing (DAS) and was associated with biomass production in another 251 accessions (at 22 DAS). This supports a role of the cytosolic FUM2, which specifically occurs in Brassicaceae, in diurnal carbon storage and indicates a growth advantage of accessions carrying the *FUM2* Col-0 allele occurring mostly in colder climates.

Tracking short-term growth response to environmental variables during wheat stem elongation

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The duration of stem elongation (SE) in wheat is critical for yield and is driven by temperature. Wheat growth response to temperature during this phase is therefore an important trait in terms of crop adaptation to different environments and yield. Accurate plant height measurements in high temporal resolution are required to quantify SE growth dynamics and their response to temperature. Using traditional measuring tools is tedious and relies on a subjective choice of a few individual plants to be measured. Terrestrial 3D laser scanning (TLS) has recently been presented as a novel tool for measuring plant height in the field, based on assessing the height of the whole upper canopy.

In this study, we used TLS with a scanner mounted on the ETH field phenotyping platform (FIP) to measure canopy height at 3Day intervals during the whole stem elongation period in a field trial comprising 340 winter wheat varieties. The FIP is a novel multisensory phenotyping platform that covers a 1-ha field by means of a sensor head hanging from a rope. The aims were to test the measurement system's capability to measure canopy height in terms of accuracy and throughput and investigate genotypic differences in growth dynamics and in growth response to temperature during SE.

The TLS approach proved promising for canopy height measurements ($R^2 = 0.99$ compared to manual measurements) at high precision and throughput. A total of 714 plots could be measured within 3.5 hours, which allows for very high measurement frequency (i.e., twice a day). In this way, growth can be captured in high temporal resolution. Genotypes differed significantly in days to final height as well as in days to 50% final height. Daily growth rates differed markedly between measurement intervals and among genotypes. The analysis of variance suggested significant genotypic effects in short-term growth response to temperature and vapor pressure deficit ($p < 0.001$), with heritabilities of 0.4 and 0.6, respectively. The measurement approach presented generates new possibilities for monitoring wheat growth during SE in terms of accuracy and throughput. The results suggest genotype-specific growth habits are responsive to environmental factors, providing insights for further investigation into plant growth response to a changing environment during SE.

Tuesday 13th December (Afternoon)
ADDING VALUE TO PHENOTYPIC DATA

Will high-throughput phenotyping and genotyping techniques help us to better predict GxE interactions? Perspectives from statistics and crop growth modeling

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Predictions of phenotypic traits for diverse sets of genotypes across broad ranges of environmental conditions are at the basis of attempts to maximize selection responses in plant breeding programs. For the last decade, multi-environment trials provided the information for building such prediction models. These prediction models were mostly linear mixed models (LMMs). LMMs are a flexible class of models with facilities for modeling genetic and environmental correlations between traits and environments and allow for heterogeneity of genetic and environmental variances. In addition, on the genotypic side of prediction models, LMMs provide the possibility to include marker and sequence information as genotypic covariates to improve phenotypic prediction, which is then called genomic prediction. On the environmental side, LMMs can be extended by including environmental characterizations as environmental covariates to improve prediction. Recently, the use of phenotyping platforms has led to an additional source of information that may be useful to improve prediction. In the context of LMMs, this information enters the prediction model as additional genotypic covariates.

Models for phenotypic prediction need to address genotype by environment interactions (GxE). The statistical objective is to find functions of genotypic and environmental covariates that can predict GxE. Statistical criteria for choosing appropriate genotype-to-phenotype functions (linear, non-linear, parametric, non-parametric, univariate, multivariate, networks, graphical models) and selecting genotypic (markers, sequences, platform characterizations, resistances, tolerances) and environmental (environmental characterizations, sensors, crop growth model outputs, stress indices) covariates may be insufficiently clear to guide the model-building process and may not lead to accurate phenotypic predictions.

As a complement to purely statistical approaches to phenotypic prediction, crop growth models have been proposed. On the positive side, crop growth models provide a causal ordering and identification of physiological parameters, component traits and target traits (yield, resistance, tolerance, quality) that can help streamline the process of phenotypic prediction, whereas statistical models may at best select and estimate approximate trait configurations and orderings from the data itself. Crop growth models also make explicit the environmental information that is required for phenotypic prediction. On the negative side, crop growth models often contain parameters and inputs that are hard to obtain and measure in practice on diverse sets of genotypes.

We have been studying various ways of hybridizing statistical prediction models with crop growth models. A straightforward hybrid method of prediction inserts genomic predictions for component traits in crop growth models for yield and other target traits. More sophisticated hybridizations are possible and will be presented. Points of consideration are the design and analysis of individual phenotyping trials, both in field trials and on platforms, the choice of genotype-to-phenotype models, and the selection of genotypic and environmental covariates. Special attention will be given to the added value of high-throughput genotyping, phenotyping and envirotyping information for prediction.

Phenotyping for crop improvement in a diversity of climatic scenarios

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The plant science community has to design new genotypes able to cope with diverse environmental conditions, in particular those linked to climate change. A major issue is to combine "black box" strategies, such as genomic selection, with the knowledge originated from phenotyping. The main comparative advantage of the latter is to help deal with the environment-dependent variability of allelic effects. We adopted a multi-scale multi-environment approach, which, in the field, consists of (i) clustering time courses of environmental variables simulated by a crop model in 60 sites x 30 years under current and future conditions into six temperature and water deficit scenarios experienced by plants; (ii) performing field experiments in contrasting environmental conditions across Europe with a panel of maize hybrids; (iii) assigning individual experiments to previously defined scenarios based on environmental conditions measured in each field; and (iv) analyzing the genetic variation of plant performance for each environmental scenario via genome-wide association studies (GWAS). Large variations of QTL effects depending on environmental scenarios resulted in a pattern associated with each QTL, defined by the scenarios in which it had positive, negative or no effect. In a phenotyping platform (Phenoarch), we estimated intercepted light and radiation use efficiency of each hybrid in the same panel via a functional-structural model using 3D reconstructions of each plant. We also estimated the sensitivity of growth to water deficit of each hybrid via a joint analysis of several experiments with contrasting light, evaporative demand and soil water potential. As a whole, the combination of field and platform steps results in a dataset that allows identifying genomic regions associated with tolerance in specific heat and drought scenarios, and with traits associated with these genomic regions. Finally, models allow identifying geographical regions in which a given combination of alleles is likely to have comparative advantages. This approach can be used for assessing the genotypic performance and the contribution of genomic regions under current and future stress situations and thus for accelerating crop breeding for diverse environments.

Strategies to handle root hydraulic architecture at multiple scales

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The importance of a deep root system has been widely advocated as a contributing component to drought adaptation. Nevertheless, recent results and modelling studies indicate that, beyond the depth of the root system, the dynamics of soil exploration and the spatial distribution of root water uptake may have a strong influence on short term plant transpiration and long-term soil water availability. In particular, hydraulic properties of individual root segments play a key role in determining the distribution of water uptake throughout the root system. We have recently developed a detailed model of water flux across roots that streamlines the integration of molecular (e.g. aquaporin expression), anatomical and histological data to predict macroscopic root segment hydraulic properties. The latter feed into a soil-plant model of soil water dynamics (R-SWMS) which scales up local properties to plant- and plot-level water uptake. We are currently developing an analysis frame based on (1) observed standard root system architectures obtained with high throughput root phenotyping platform (using aeroponics as unconstrained growing system), (2) predicted root segment hydraulic properties (guided from molecular- and organ-scale data of hypotheses), (3) simulations of a standard water uptake under spatially uniform soil moisture and, (4) analysis of the deviations from the standard uptake in conditions of heterogeneous and variable soil moisture.

Using structural models to validate and improve root image analysis pipelines

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Many structural root models have been developed, either generic or for specific species, and these have repeatedly been shown to faithfully represent the root system structure, as well as being able to output ground-truthed data for every simulation and image, independent of root system size. However, they have almost never been used as validation tools for image analysis procedure. Here we will show that structural root models can be used in combination with image analysis pipelines to assess and improve their overall performance. First, we will show that an in-depth analysis of root image analysis pipelines using such models reveals strong limitations in their ability to measure complex root systems. Secondly, we will present an innovative strategy that combines root models and machine-learning algorithms (random-forests), that has the ability to increase the measurement accuracy.

Combining high-throughput phenotyping and QTL mapping to reveal the dynamic genetic architecture of maize plant growth

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With the increasing demands in crop breeding for novel traits, the plant research community has to quantitatively analyze the structure and function of large numbers of plants. A clear goal of high-throughput phenotyping is to bridge the gap between genomics and phenomics. In this study, we obtained 106 traits from a maize recombinant inbred line population across 16 development stages using the automatic phenotyping platform. The results showed that the exponential model had better prediction ability for biomass accumulation, even in early plant growth stages. QTL mapping with a high-density genetic linkage map was used to uncover the genetic basis of these complex agronomic traits, and it identified 2249 QTLs for all investigated traits. We also conducted traits-loci network analysis and three housekeeping loci were detected in the hub points for two traits. These results reveal the dynamic genetic architecture of maize plant growth, which will, in the near future, enhance the maize ideotype used in breeding.

Use of high-throughput phenotyping at CIMMYT: New models, challenges and perspectives

Juan Burgueño, José Crossa, Sam Trachsel, Suchismita Mondal

International Maize and Wheat Improvement Center (CIMMYT)

CIMMYT annually establishes a large number of maize and wheat trials at sites in Mexico and with partners from the public and private sector in developing countries in Africa, Asia and Central America. One of the biggest challenges is to generate data of consistently high quality from hundreds of experiments per year because these experiments are conducted in several countries under different environmental and agronomic conditions. Commercial availability of unmanned aerial vehicles (UAV) and the continued improvement of cameras (multi-spectral, hyper-spectral and thermal) have opened new opportunities for the development and adoption of HPP in large-scale multi-environment trials. These technologies obtain high quality data quickly and at relative low cost. Adoption of these technologies will enable field-level genetic testing at a scale that was not previously possible. CIMMYT has been introducing some of these new technologies in field phenotyping since the early 2000s. Modern high-resolution cameras can provide reflectance data at hundreds of wavelengths. This information can be used to derive vegetation indices (VI) that are correlated with important agronomic

and physiological traits. However, the data generated by high-resolution cameras is richer than what can be summarized in a VI. To make full use of rich reflectance data, we compare the predictive performance of several VI with that of prediction equations that use information from the entire available spectrum. We considered three statistical methods: ordinary least squares (OLS), partial least squares (PLS, a dimension reduction method) and a Bayesian shrinkage/variable selection procedure (BayesB, which has been used in genomic prediction but here is used to select the most important spectral bands). Correlations between 0.3 and 0.6 were found when predicting maize yield in a set of 12 experiments. Also, we found that using the complete spectral bands produces higher correlations with grain yield than using some vegetative indexes. Simultaneously, genetic evaluation of several vegetative indexes by calculating genetic variability, heritability and other related measurements showed high variability between trials with repeatability ranging from 0 to 0.9. Results are promising but more research is required to increase data quality and precision at the lowest possible cost and to improve analytical methodologies.

Using high-throughput phenotyping to improve accuracy in genomic prediction: Examples for crop simulated traits

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Prediction of the final state of a target complex and composite trait (yield) can be improved by adding information on the dynamics of this trait and its constituent components. High-throughput phenotyping techniques provide information on the dynamics of target and component traits. This information can be summarized by parametric or semi-parametric statistical models for growth. Statistical parameters characterizing growth over time are expected to have greater heritability and offer a better integration of plant responses to environmental conditions than single time measurements on the same traits and should be useful as correlated traits in multi-trait genomic prediction models.

Multi-trait genomic prediction models are preferred over single-trait models for the following scenarios: (1) predicting a target trait from measurements of component traits at early growth stages, thereby reducing the selection cycle; and (2) increasing accuracy for prediction of a target trait by using multi-trait prediction models that combine the target trait with its components. The success of these two prediction scenarios depends on the heritabilities of all traits involved and the correlation structure of the target trait with its components.

To study the above prediction scenarios, we simulated a RIL population with segregating QTLs for the APSIM-Wheat parameters that regulate phenology, biomass partitioning and the ability to capture environmental resources. The physiological parameter values defining the segregating population were

used to simulate yield, yield components and phenology during the growing season. We considered different experimental and measurement error scenarios. Low heritability of single time points was compensated by using phenotypes at multiple time points simultaneously (h^2 increased from 0.2 to 0.6). Incorporating trait dynamics over time in a multi-trait genomic prediction model showed higher accuracy, compared to single trait prediction and could potentially be used to shorten the selection cycle by producing yield predictions early in the growing season. APSIM-Wheat with genotype-dependent parameters proved to be a valuable tool to assess the convenience of investing in more precise field measurements, or to determine the number and timing of measurements required to best characterize component traits.

Poster Session:

Advances in Plant Phenotyping Technologies

Automated measurement tool for phenotyping root and hypocotyl growth of Arabidopsis plants

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Plant growth responses to various environmental conditions or chemical treatments can be quantitatively assayed by measuring hypocotyl or root lengths of *Arabidopsis thaliana* seedlings. These measurements can be employed to further understand a biological process of interest—for example, how a class of plant steroid hormones called Brassinosteroids regulate thousands of genes to control plant growth. In this research, we automated the process of measuring hypocotyl and root lengths in *Arabidopsis* and applied the developed tool to gain further insight into the Brassinosteroid pathway by performing measurements in the presence or absence of Brassinazole, a Brassinosteroid biosynthesis inhibitor. Recent advances in phenotyping techniques are rapidly encouraging the ubiquity of large-scale imaging of plants, while hardware-based phenotyping methods by robots now require embedding of intelligent algorithms. The automated measurement tool proposed is an enhanced version of the hierarchical structure-based polyline simplification algorithm that has proven successful in cartographic applications. It was improved so that it could determine the sorted coordinates of the vertices between two nodes more robustly than the morphological operation, thinning, which fails with multi-angular foreground disturbance crossing the desired objects.

Our forward-backward evaluation algorithm adjusts the shortest path objective function of the bi-directional search method to marching from one identified tissue point to another on the foreground object in a blind path detection manner. It significantly reduces the number of vertices that needs to be specified on each plant to only those of the end points with higher resolution, while the currently applied 'ImageJ' tool requires more coordinates. It was deployed and tested on several images in a semi-automatic, human-in-the-loop manner for the measurements. We compare its results with those of the ImageJ measurement tool.

High-throughput screening techniques for stomatal response to ABA based on chlorophyll fluorescence under non-photorespiratory conditions

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Fine regulation of stomata aperture is required to allow sufficient CO₂ uptake for photosynthesis, while preventing excessive water loss through transpiration. Although stomatal movements are controlled by a strong and complicated system, this control system can be disturbed under certain environmental conditions, causing changes in closure response of the stomata. Since analysis of stomatal responses has been mainly limited to microscopic and gas exchange measurements, limiting the scale of screening, here we used chlorophyll fluorescence imaging under non-photorespiratory conditions (20 mmol mol⁻¹ O₂) to analyze stomatal responses to exogenous ABA. A decrease in photosystem II photochemical efficiency (Φ_{PSII}) is closely related to stomatal closure when photorespiration is inhibited through exposure to a decreased O₂ environment, but this relationship is not always linear. To confirm that decreased Φ_{PSII} was due to stomatal closure, at the end of Φ_{PSII} imaging samples were kept in an atmosphere with high CO₂ (20 mmol mol⁻¹ O₂, 50,000 μ mol mol⁻¹ CO₂) for 5 minutes to test the recovery of Φ_{PSII} . Closure of stomata results in scarce CO₂ in the stomatal cavity. In this situation, when decreased Φ_{PSII} is due to lack of internal CO₂, very high CO₂ concentration will diffuse into the stomatal cavity and restore the Φ_{PSII} . To screen for stomatal responses to ABA, leaf discs were prepared from different leaves. The leaf discs were put with their abaxial surface up in petri dishes filled with stomata-opening media of different ABA concentrations. To enable fast and uniform uptake of the solutions, 3 min vacuum infiltration was used. After vacuum infiltration, the leaf discs were pre-incubated in the above-mentioned ABA-solutions at 20°C and 40 μ mol m⁻² s⁻¹ irradiance. Thereafter the petri dishes were placed in a flow-through cuvette under a chlorophyll fluorescence imaging system. Just 10 minutes exposure to non-photorespiratory conditions plus 5 minutes exposure to high CO₂ concentration were enough to analyze the stomatal responses of more than 40 samples. With this technique, not only were huge numbers of stomata analyzed in a very short period of time, but heterogeneity in stomatal response over the leaf surface was also visible.

Advanced mathematical algorithms to characterize olive varieties through morphological parameters

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The morphological analysis of olive fruits, leaves and stones may represent an efficient tool for the characterization and discrimination of varieties and the establishment of phenotypic relationships among them. In recent years, much attention has been focused on the application of DNA molecular markers due to their high capacity to efficiently and reliably discriminate cultivars. In this talk, we will present a semi-automatic methodology of detecting various morphological parameters based on image analysis tools. A number of morphological parameters have been used to characterize olive germplasm collections from different countries. The data obtained could complement olive databases comprised of genetic, molecular and morphological data supporting efforts to efficiently discriminate them and infer either genetic and/or morphological relations. With the aid of computing and image analysis software, we created semi-automatic algorithms applying intuitive mathematical descriptors that quantify many fruit, leaf and endocarp features. In particular, we examined quantitative and qualitative characters such as size, shape, symmetry, surface roughness and the presence of additional structures (nipple, petiole, etc.). Finally, in this talk we will present the efficiency and robustness of the proposed methodology for the description of other crop morphologies such as tomatoes, pears, grapes, etc.

AIRPHEN: A multispectral camera dedicated to field phenotyping from drone observations

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The AIRPHEN multispectral camera was specifically designed for accessing canopy biophysical variables from drone observations within field phenotyping experiments. The camera weighs about 200 g, records GPS and IMU information and allows triggering companion devices including a thermal infrared or RGB high resolution camera. Six narrow wavebands selected to sample the chlorophyll specific absorption coefficient record the reflected radiation at a resolution of 1,280 x 960 pixels. The 8-mm focal length provides resolutions at ground level ranging from a few millimeters to a few centimeters, depending on

flight altitude. One of the six cameras is equipped with a 4.2-mm focal length lens to ease the image alignment process and generate more accurate 3D point clouds, while providing the increased throughput allowed by the larger swath of the camera in this band. A pipeline called 'phenoscript' was developed to process the raw images of the AIRPHEN camera, compute as outputs a multispectral ortho-image, and extract the microplots from the original images and the corresponding 3D dense point cloud. The extracts can later be used to compute vegetation indices, measure canopy height, and derive a few biophysical variables from either empirical approaches or radiative transfer model inversion.

The use of the AIRPHEN camera is illustrated in several experiments conducted on a range of species including wheat, maize, sugar beet and potatoes. The dynamics of the vegetation indices show a very good consistency of the measurements over time. The accuracy of estimates of several biophysical variables including the green fraction (GF), the Green Area Index (GAI), the fraction of intercepted radiation (FIPAR) and canopy (CCC) or leaf (LCC) chlorophyll content is presented. The capacity to combine the multispectral camera with thermal infrared imagery is also illustrated by the characterization of water stress. Further developments on the use of the AIRPHEN camera are discussed, including advanced interpretation of the images based on 3D canopy structure models, installation on other vectors such as the phenomobile for working in active mode, or a handheld system dedicated to small and low-cost experiments.

Deriving canopy height from drone observations: Overview of the expected accuracy and main influential factors

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Drones allow collecting images with large overlaps so that the same point on the ground can be seen in several images and thus from different angles. Photogrammetric techniques are then applied to exploit this property and derive a dense 3D point cloud from which the macro-structure (i.e., the convex hull of the vegetation) and the corresponding canopy height can be computed. This canopy characteristic is very appealing in the context of high-throughput plant phenotyping under field conditions. Although this technique is becoming relatively common, the expected accuracy and the main influential factors are not precisely known. The objective of this study was to quantify the accuracy with which canopy height can be estimated and to identify the main factors that have to be accounted for to achieve the best performance.

A series of field phenotyping trials were conducted on sugar beet, wheat and maize in 2015 and 2016. A hexacopter was flown carrying either a Sony Alpha high-resolution camera (24 Mpixels) or an AIRPHEN multispectral camera (1.3 Mpixels). Ground measurements of canopy height were concurrently completed using either visual notations or values extracted from LIDAR observations. The images from the cameras aboard the drone were processed using Agisoft PhotoScan software that aligns images and

generates a dense 3D point cloud from which the vertical distribution can be computed. A percentile of the distribution is used to determine the corresponding canopy height.

Image-based canopy height was in good agreement with visual notations of canopy height, with an uncertainty of around 7 cm (sugar beet) to 15 cm (maize). More detailed results gathered on wheat using LIDAR showed that most of these uncertainties are attributable to the precision of the visual notations. Several factors potentially affecting the accuracy of canopy height estimation from UAV observations were analyzed. One of the most important factors is the way the soil reference altitude is computed. In dense canopies where the soil can hardly be seen, it is advisable to use a previous reference 3D point cloud corresponding to bare soil. Other factors include the ground resolution relative to the size of the vegetation elements, the number of images from which each point on the ground is seen, the field of view and the range of directions available. The influence of the parameters used in Agisoft PhotoScan for generating the 3D point cloud was also evaluated. Finally, an optimal procedure is presented that should ensure accurate estimates of canopy height that can be used for genotype characterization in phenotyping experiments.

A wireless environmental data collection system for high-throughput field phenotyping

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The plant research community needs to combine the environmental conditions with plant phenotyping to have a complete picture of the growing process. The data provided by state-level weather stations do not accurately represent field conditions. In addition, other environmental conditions around the plants are not readily available without a field trip. As a result, there is a need for a remote data collection in the field for environmental conditions.

This article describes the design of a local environment station to be installed in a field for phenotyping. The system includes various sensors, a microcontroller, a wireless module, a wireless internet, and a cloud data base. The above ground sensors include air temperature, relative humidity, sunlight, leaf moisture, CO² level, wind speed, wind direction, and rain fall. The soil water content, soil temperature, and pH sensors are currently used to measure underground conditions. All sensor levels are collected by a microcontroller which converts the signals into environmental data. The data are stored every 10 minutes (or at a time interval determined by the users) in a secure digital card and also sent to a host data base in the cloud as the system has an Internet of Things feature built-in. Updated data can be viewed and retrieved any time on a website. The system is also equipped with a local wireless sensor network to have more detailed environment data in a range of 1,000 m in diameter. The system requires no maintenance during operation and has its own solar charging. Relocation of the system does not require any set-up as the GPS will automatically update the location, date, and time to the website with coordinates on Google Map. Three systems were built and successfully collected data in the spring.

Deciphering the phenotypic code: From lab to field-scale

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The Book of Nature is written in the language of mathematics, as Galileo Galilei aptly formulated almost 400 years ago. Having the latest inventions in biotechnology at hand, scientists started deciphering the molecular code of life. Although tremendous progress in molecular biology has been achieved, the translation of genotypes into phenotypes is poorly understood. Nowadays, progress in non-invasive imaging sensors provides a framework to look into phenotypes at the subcellular (e.g., microscope) and field (e.g., airborne imagery) levels. The amount and diversity of data such as 1D sensor readings, 2D images or 3D point clouds have specific storage and analytical demands.

The challenge is to translate data into information related to the biological object or process. Therefore, data analysis is one part of the phenotyping bottleneck, which is being addressed by various software tools. Making these tools easily accessible to researchers and breeders is one of LemnaTec's main objectives. In contrast to code-based image analysis approaches, LemnaTec provides a graphical programming interface called LemnaGrid. In other words, no line of code needs to be written. LemnaGrid contains various toolboxes for 2D image analysis, hyperspectral data or 3D point clouds. The output delivers dimensions, morphologies, and spectral reflectance of the measured objects. LemnaGrid is currently applied starting from microscopy images in the laboratory, whole-plant images in the greenhouse, and canopy images in the field. Phenotyping solutions are designed to work for related experiments and to be shared among users.

The application range of LemnaTec software is constantly being broadened, and new platforms such as the new Field Scanalyzer are being developed. Recent results from multi-sensor field measurements delivered data on canopy properties of cereals. Linking information derived from such RGB, hyperspectral, laser scanning and fluorescence data to the crops' biological and agronomical properties will enable comprehensive field phenotyping.

Studies of root system architecture in soybean using computer vision and machine learning

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With the advent of computer vision, there is renewed interest in uncovering "the hidden half" of plants, using measurements of root system architecture (RSA) in conjunction with machine learning to discover trait correlations within and between genotypes and phenotypes. This study included more than 300 diverse soybean accessions under 2D (under controlled conditions) and 3D (field tests) imaging platforms, supported by processing and data analytic tools to deep phenotype for important RSA traits using an in-house imaging software, ARIA. Both 2D and 3D imaging platforms revealed tremendous

genetic variability for traits such as root shape, length, mass and angle. The 3D imaging platform developed makes it possible to phenotype hundreds of genotypes and extract numerous root system traits. The 2D platform is non-destructive, adding observations of seedling root growth rates. We are focusing on use of machine learning algorithms to unveil relationships between RSA and correlate them with above-ground performance traits, primarily seed yield and parameters related to the Monteith equation. Building on the imaging platform developed, we are now pursuing deep genome-wide studies.

Recent advancement in development of autonomous mobile robots for plant phenotyping

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Considering the current needs of the world population to increase the rate of crop production, there is a strong need for research on screening crops by use of technology for high-throughput phenotyping (HTP). The objective of our research is to develop a field-based HTP mobile platform for rapid assessment of multiple quantitative plant traits with a focus on wheat, canola, and lentil. The field platform will be designed, fabricated, and tested in a laboratory setting and evaluated in crop breeding nurseries in Saskatchewan. Many imaging technologies are well developed and sophisticated, but not necessarily optimal for in situ high-throughput plant characterization. Which technologies are appropriate for field plot scale advanced characterization of plants in the field, and how can these be delivered in a “location independent method” are some of the questions to be answered. Which sensors are most important for plant characterization in a breeding program, and is it possible to characterize plants below ground in the field? A literature review of devices for plant phenotyping and the state of research in developing an autonomous mobile robot for plant phenotyping will be presented.

An automated soybean multi-stress detection framework using deep convolutional neural networks

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Significant crop yield losses are caused by biotic and abiotic stresses. To mitigate potential crop losses from such stresses, a high-throughput and early detection system for regular and widespread monitoring is of vital importance. Traditionally, soybean abiotic/biotic stress detection methods have relied on subjective symptom-based assessment by agricultural scouts. Machine learning-based automated stress detection using readily available sensors or smartphone cameras can have a transformative impact on soybean farming and scouting. The goal of this study was to develop an automated framework to identify abiotic/biotic stresses on soybean [*Glycine max* (L.) Merr.] under field conditions, based on image classification, using Deep Convolutional Neural Networks (DCNN). More

than 25,000 leaflet images were collected from Iowa (USA) fields for five biotic stresses (bacterial leaf blight, bacterial pustule, frogeye leaf spot, Septoria brown spot and sudden death syndrome) and three abiotic stresses (iron deficiency chlorosis, potassium deficiency and herbicide injury) as well as healthy soybean leaflets using a standard imaging protocol. After processing these images further, a dataset comprised of 16,210 leaflet images from all the collected images was used to develop the DCNN model. For our initial experiment, the model was trained based on a training set of 11,345 images, which was then validated on 3,242 images and finally tested on 1,630 images. Preliminary results show that the trained model is able to efficiently differentiate between the eight different soybean stresses under consideration as well as healthy leaflets. The trained model can be easily exported to a mobile platform utilizing visual sensors in a dedicated low-cost imaging device or even smartphone cameras to perform efficient and robust online detection of soybean stresses in real time.

High-throughput rice phenotyping facility: Strategies and challenges

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The advent of next-generation sequencing technology has had a major impact on genomics in a short period of time. However, phenomics, a new discipline involving the characterization of the full set of phenotypes of a given species, still lags far behind genomics. Traditional phenotyping tools, which inefficiently measure a limited set of phenotypes, have become a bottleneck in functional genomics and plant breeding studies. To relieve the bottleneck, we developed a high-throughput rice phenotyping facility that includes rice automatic phenotyping (RAP), yield traits scorer (YTS), high-throughput leaf scorer (HLS), and high-throughput hyperspectral imaging system (HHIS). Equipped with multidisciplinary techniques, including photonics, automatics, computers, and mechanics, the integrated facility can extract numerous morphology-related traits, biomass-related traits, yield-related traits and physiological traits. When continuously operated, the total throughput of our facility is 1,920 pot-grown rice plants out of a total greenhouse capacity of 5,472 pots. In addition, we discuss the future challenges of rice phenotyping.

Field Scanzlyzer: High precision phenotyping of field crops

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Current phenotyping takes place in a state of tension between throughput and accuracy, also described as screening versus deep phenotyping. Moreover, the importance of field data is becoming increasingly prevalent, as most indoor cultivation environments lack comparability to field growth situations. Deep phenotyping at the field level requires sensor fusion that allows collecting various types of data on the plants, far beyond taking RGB images. Sensor fusion for field phenotyping brings together laser scanning and thermal, fluorescence, and spectral imaging combined with sensors for environmental factors. High-precision positioning allows moving each of the sensors to the same spot of vegetation, thereby collecting the whole range of data types for each individual in the field. Moreover, it enables time-resolved data to be recorded. The Field Scanzlyzer is a deep phenotyping platform consisting of high precision sensors that monitor experimental plots day by day, and even several times a day. 3D laser scanners provide measurements of the volume of the plant material, which is highly correlated to the biomass. Hyperspectral cameras allow measuring vegetation indices that are correlated to physiological parameters such as growth, water and fertilizer, or the plants' health status. Furthermore, the analysis of fluorescence and thermal images delivers parameters that can be linked to the plants' photosynthesis and transpiration via dedicated models. By recording environmental data at each measurement and linking position data of all measurements to genomic information about the plants growing at a given location, the method allows analyzing linkages between phenotypes, genotypes and environment in high spatial and temporal resolution.

Integrated analysis of plant growth and development using high-throughput multi-sensor platforms at IPK

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IPK runs three phenotyping facilities for high-throughput imaging of whole plants of small size (such as *Arabidopsis thaliana*), medium size (such as *Hordeum vulgare*) and large size (such as *Zea mays*). In each system, plant images are acquired in near-infrared (NIR) and visible spectra (for RGB and fluorescence imaging) from top and side views. Imaging is performed repeatedly and thus follows plant growth dynamics. The Image Analysis Platform (IAP) automatically extracts plant architectural traits (height and width, projected leaf area, estimated volume) and color-related and physiological traits (static fluorescence, moisture content-related parameters). The phenotyping facilities have been extended for functional chlorophyll fluorescence analysis using pulsed amplitude-modulated chlorophyll fluorescence imaging systems and for acquiring 3D height profiles of plants and plant stands. These systems are integrated into phenotyping procedures, allowing simultaneous acquisition of multiple complementary plant traits. Co-registration of feature data derived from all the different sensors (sensor fusion) will be

used to deduce novel and refined information on plant architectural and physiological traits and provide the basis for multi-trait association studies. Opportunities and challenges of integrated analyses will be discussed using different use cases. Furthermore, current developments in phenomics data management (e.g., MIAPPE compliant standardized metadata representation for persistent documentation of high-throughput plant phenotyping experiments) will be introduced.

Developing the Enviratron: A facility for automated phenotyping of plants growing under varied conditions

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The Enviratron is a new concept and design in plant phenotyping with the purpose of testing plant performance under different environmental conditions. In contrast to current phenotyping facilities, the Enviratron is designed to analyze plant growth and performance under up to eight different environmental conditions in one experiment. Also unlike current phenotyping facilities, plants are not conveyed to a central analyzing station, instead a mobile robotic analyzer (rover) equipped with a sensor array visits plants, minimizing disturbances in the growth environment. In analyzing the relationship between G x E, most phenotyping facilities are equipped to vary G. The Enviratron's special feature is its capacity to vary E. The Enviratron consists of eight growth chambers that each can be programmed to a unique environment that can vary slightly in a single parameter or by different climate scenarios, including temperature, CO₂, humidity, water, and light (duration and intensity). The rover's sensor array includes a holographic camera, hyperspectral sensor, fluorescence detector, infrared detector, and Raman scattering spectrometer. Individual pots are designed to have soil water potential sensors with a watering system to maintain chosen soil water potentials. To enable downstream discovery and reuse of diverse data collected using the Enviratron system, a MIAPPE-compliant metadata collection and reporting mechanism is under development. Learn more about Enviratron online at <http://enviratron.iastate.edu/>.

Hyperspectral imaging system, individual rice plants, and accurate prediction of above-ground biomass, green leaf area and chlorophyll

Feng Hui et al.

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Biomass, green leaf area and chlorophyll are important components of plant phenomics and the existing methods for estimation of these on individual plants are either destructive or lack accuracy. Hyperspectral imaging is an emerging and nondestructive technology that can acquire spectral and spatial information simultaneously for plant phenotyping. The system has the potential to characterize cereal plants.

Comparing robots and drones as phenotyping tools in field trials

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New approaches are necessary to meet the goals of increased food production. Plant breeding can play a key role by developing cultivars with higher yield potential and better adaptation to stress. Progress in breeding depends on the ability to design crosses with complementary traits, and then perform effective selection among the offspring. This requires precise and cost-effective methods to evaluate large numbers of plants across relevant environments and stresses. Traditional data capture based on low-throughput and manual methods is labor-intensive and prone to human error. While the costs of sequencing and genotyping have dropped dramatically over the last decade, the labor cost has increased and phenotyping has now become the biggest bottleneck for realizing the full potential of genomics in plant breeding.

To address these challenges, a strategic collaboration has been initiated at the Norwegian University of Life Sciences, where research groups across departments and disciplines are joining forces to test new phenotyping technologies in plant breeding research. This includes developing and testing multispectral imaging in visible and near infrared wavelengths of plants in field trials, using cameras mounted on an autonomous field robot and on an Unmanned Aerial Vehicle (UAV). This equipment is part of a common sensor lab that has been established at the university, which also serves many other research purposes.

Based on pilot projects run during the summer of 2016, there are plans to upgrade the field research station with new facilities for high-throughput field phenotyping. Examples of ongoing research and some preliminary results from a pilot experiment comparing the use of a robot and drones in a wheat yield trial will be presented.

The need to account for directional effects and the presence of reproductive organs on canopy reflectance and temperature recorded from drones

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After the flowering stage, several crops grow ears (wheat), male panicles (maize) or flowers (sunflower) at the top of the canopy that have particular structures and optical properties. This significantly impacts the radiation regime, which may induce discontinuity from reflectance and bias the estimates of canopy characteristics. The objective of this study was to quantify the influence of these reproductive organs on the estimation of selected canopy characteristics. Specific attention was paid to the directional variation of this effect.

Three dedicated experiments were conducted in 2016 on wheat, maize and sunflower at specific times after flowering, when green leaves still represent the main contribution of the green area index. The target sites were squares measuring about 10 m per side. The reproductive organs on one half of each square were eliminated, while the other half was left undisturbed. A hexacopter equipped with an AIRPHEN multispectral camera and a FLIR tau2 thermal infrared camera was flown several times over each experiment to capture the variation of the signal depending on the sun's position. In the middle of the square, a reference panel was positioned horizontally to calibrate the signal recorded by the AIRPHEN camera. The brightness temperature of the reference panel was also recorded continuously using a thermoradiometer connected to a data logger to calibrate the FLIR camera. For each elevation, the drone sampled the view directions by flying in 4 to 6 concentric circles at a range of altitudes. The diameter and altitude of the circles were defined to keep the same distance from the reference panel at the center of the 10m x 10m square and maintain approximately the same spatial resolution. The AIRPHEN and FLIR cameras on the gimbal always pointed towards the reference panel. After the experiment, the vegetation structure (silhouette photos, area of organs) and optical properties were measured.

Results show that canopy reflectance and temperature are strongly dependent on the view and sun directions, with a maximum in the backscattering direction corresponding to the hotspot. Reflectance and brightness temperature show similar patterns, mainly explained by the fraction of shadow observed. By comparing the area where the reproductive organs were eliminated with the undisturbed area, we found significant variation of canopy reflectance and brightness temperature. The impact of current vegetation indices as well as estimates of vegetation state variables using radiative transfer model inversion was assessed.

Precision phenotyping: Wheat disco to foster yield potential

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Future increases in wheat yield potential will rely largely on improved biomass production boosted by higher radiation use efficiency (RUE). Recent studies using a recombinant doubled haploid wheat population derived from the cross between a cultivar with high grain number (Bacanora) and one with high grain weight (Weebil) showed spectacular gains in grain yield (from 23 to 31%) over the parental cultivars associated with higher RUE. However, the same population studied in northwestern Mexico only showed a modest yield increase. At the level of plant growth and development, a more optimal balance between source and sink is expected to improve overall RUE. The main objective was to provide extra illumination during the rapid spike growth phase (from booting initiation until seven days after anthesis) and analyze the effect on source-sink related traits.

Field phenotyping: Quantifying dynamic plant traits across scales in the field

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Phenotyping in the field is an essential step in the phenotyping chain from well-defined and controlled conditions in the laboratory and greenhouse to the heterogeneous and fluctuating environment in the field. Field measurements are a significant reference for the relevance of laboratory and greenhouse approaches and an important source of information on potential mechanisms and constraints for plant performance to be tested under controlled conditions. Here we present a range of methods focusing on plant architecture, photosynthesis and water relations, that are being deployed within the German Plant Phenotyping Network (DPPN, www.dppn.de). Specialized field platforms are established (a) to test innovative phenotyping technologies; (b) to provide access to semi-controlled field installation to support breeding approaches for future CO₂ concentrations (breed-FACE); and (c) to study the translation of phenotypic properties from controlled environments to stands in the field. Here we report that stereo imaging enables the quantification of canopy structure; active thermography estimates leaf water content and provides information on transpiration conditions; and sun-induced (SIF) and light-induced fluorescence transient (LIFT) techniques allow remote estimating of photosynthesis at the canopy and leaf-to-plant levels, respectively. For photosynthesis, SIF will be measured by the next European Space Agency satellite Earth Explorer mission. All methods will be further tested and incorporated in (semi-)automated systems that position sensors in the field. A promising portfolio will be introduced to measure plant traits for field phenotyping and to enhance our understanding of relevant traits under natural conditions.

3D point cloud-based monitoring of soybean growth in early stages

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Currently, high-resolution point cloud data can be acquired easily and cost-effectively. For example, a pipeline using Structure from Motion (SfM) and Multi-View Stereo (MVS), which is a promising technique for reconstructing a 3D surface as point cloud data from a series of 2D images taken from different angles, has been implemented in several libraries and software products. In this study, we developed a workflow for measuring the canopy growth of three soybean cultivars to compare the growth speed of the canopies based on point cloud data acquired from the SfM and MVS pipeline. First, we took multi-view 2D images of soybean plants growing in a field with a digital camera (EOS 60D, Canon, Tokyo). The images of plants in a single plot were taken from around 40 different directions. To scale the images and calibrate camera parameters, we used Calibrated Photogrammetric Scale Bars (Cultural Heritage Imaging). From these multi-view 2D images, point cloud data of soybean canopies were reconstructed using the SfM and MVS pipeline. Second, we segmented the point cloud of plants and their leaves for each plot from the reconstructed data. Finally, we fitted several models to the point cloud data for estimating phenotypic values of plant organs constituting the canopy architecture (e.g., leaf area, leaf shape, curvature). For example, we reconstructed 3D surfaces of leaves from the point cloud data with the penalized B-spline surface fitting by regarding a leaf as a 2D closed surface embedded in the Euclidean space \mathbb{R}^3 . Using the workflow, we successfully acquired high-resolution point cloud data of canopies of soybean plants growing in the field. Even when leaves slightly overlapped each other, a single leaf was successfully distinguished from the point cloud. When field conditions were not desirable (e.g., wind, change in light conditions) for acquiring corresponding points among multi-view images, incomplete point cloud data were reconstructed. A photogrammetric system that allows us to take multi-view images simultaneously will be necessary to obtain high-resolution point clouds of plants growing in a field.

Greenhouse, field and root phenotyping infrastructure at the Department of Plant and Environmental Sciences of the University of Copenhagen

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The Department of Plant and Environmental Sciences at the University of Copenhagen has established a broad range of state-of-the-art facilities and equipment for plant phenotyping. Specialized and experienced technicians, gardeners and researchers ensure optimal and qualified use for both internal and external collaborators. PhenoField and drones are available for field phenotyping. PhenoField is a mobile, closed, multi-spectral imaging system that shuts out wind and sunlight to ensure the highest possible precision. Images are acquired with a top centered monochromatic CCD camera 2 m above ground covering an area of 1 m x 1 m, and a spatial resolution of 0.43 mm/pixel and 9 narrowband LEDs

as the active light source. Three rotary-wing unmanned aerial vehicles (UAVs), two hexacopters and one octocopter equipped with true color (RGB) and color-infrared (CIR) cameras are used for measuring crop coverage and vegetation indices. An in-house semi-automated process to handle and analyze images of plots in field experiments is used.

Roots can be studied in root towers, tube rhizotrons, minirhizotrons and the Radimax facility. With minirhizotrons, roots can be studied to a depth of 2.7 m under field conditions by single cameras, which can be used in combination with isotope placement and irrigation systems. The tube rhizotrons can be used for visual inspection in root studies up to 2 m under greenhouse or outdoor conditions. Twelve root towers consisting of two compartments separated in east-west orientation allow visual inspection for root studies up to a depth of 4 m. The RadiMax facility consists of 4 individual pits with an area of 400 m² each with moveable rainout shelters, a sophisticated under-watering-system and 150 fixed-installed rhizotrons per pit for semi-automated multi-camera systems allowing root studies of up to 150 different lines at a time down to a depth of 3 m.

The automated, conveyor-band based, high-throughput phenotyping platform PhenoLab is installed in a temperature controlled greenhouse for monitoring and caring for up to 468 pots. The pots can be randomized, rotated, measured and weighed, and the system is able to manage plants individually. Soil water potential is measured continuously in each pot and individual irrigation is adjusted accordingly. It also has multi-reflectance and multi-fluorescence imaging systems, and thermal cameras inside the imaging station as well as outside under cultivation light conditions. The sensor-based imaging techniques are complemented by various platforms for advanced physiological phenotyping for photosynthetic activity and gas exchange measurements and the determination of activity signatures of key enzymes of carbohydrate and antioxidant metabolism, antioxidant capacity and phytohormone profiles.

Morphometrics for genomic prediction of plant morphological traits: Its application to genetically dissect sorghum grain shape

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Recent advances in imaging and remote sensing allow the collection of large datasets of images for the genetic dissection of plant morphological traits. In addition to size (length, area) and color, which are common characteristics measured in image analysis, shape is important, but has high-dimensional complexity. Since morphometric methods can reduce complexity without serious loss of information, they can be useful for genetically dissecting plant morphological traits. Grain shape is an important breeding target in cereal crops. We applied two morphometric methods — i.e., elliptic Fourier analysis (EFA) and generalized Procrustes analysis (GPA) — to genetically dissect the remarkable diversity of grain shape in sorghum (*Sorghum bicolor* (L.) Moench). EFA treats the shape of a contour as a sum of

waves, and describes it with elliptic Fourier descriptors (EFDs). GPA superimposes contours to minimize the Procrustes distance between them, and obtains superimposed landmark points (SLPs). Principal component (PC) analysis of EFDs and SLPs identified and quantified shape characteristics, which were difficult to capture by lengths and their ratios. In GWAS, a highly significant association was found in the second PC of SLPs, while no significant association was detected in lengths and their ratios. The association detected in the PC of SLPs was confirmed in a bi-parental segregating population. In genomic prediction, the contour shape of a grain was predicted from genome-wide marker genotypes by using kernel partial least squares (PLS) regression of EFDs. The predicted shape showed close similarity to the observed shape except in a few cultivars, suggesting the high accuracy of genomic selection of grain shape. The results suggest the important role morphometrics play in the genetic dissection of plant morphological traits, whose data will be more accessible via high-throughput imaging and remote sensing.

Image-based phenotyping and machine learning to advance genome-wide association and prediction analysis in soybean

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Traditional visual evaluation of crop biotic and abiotic stresses is time-consuming and labor-intensive, and limits the ability to dissect the genetic basis of quantitative traits. To address these constraints, a machine learning (ML)-enabled image-phenotyping pipeline was developed and applied to genome-wide studies of iron deficiency chlorosis (IDC), one of the leading abiotic factors lowering soybean (*Glycine max*) yield in the United States. The pipeline consisted of a multi-stage procedure: (1) optimized image capture across plant canopies; (2) canopy identification and registration from cluttered backgrounds; (3) accurately represented IDC expression by extracting domain expert informed features from the processed images; and (4) supervised ML-based classification and severity assessment of canopy images. The genotype panel consisted of 461 diverse accessions. Genotypes were grown in a field suitable for IDC testing, and imaged using an RGB camera. Statistical analysis was done in R using appropriate packages. Genome-wide association studies identified a previously reported locus. Additionally, a novel locus harboring a gene homolog involved in iron acquisition from low bioavailability sources was identified, indicating the reliability and advantage of ML-enabled image-phenotyping. Genomic prediction analysis using surrogate traits in the prediction model had higher prediction accuracy than marker alone model(s), suggesting a promising path for further increase of genetic gains via integrating this pipeline into breeding programs. This study provides a systematic framework that enables quicker robust phenotyping of leaf symptoms in soybean (and other crops).

Proposal for evaluating plant stress via steady state fluorescence

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Chlorophyll fluorescence analysis is a powerful tool to estimate photosystem II (PSII) performance under standard or biotic or abiotic stress conditions. An important limiting factor when measuring chlorophyll fluorescence parameters in a high-throughput phenomics system is the availability of a flashing light source (used to block the photosynthetic apparatus), which is large and homogeneous enough for whole plants. Until recently, such systems were limited to smaller plants such as Arabidopsis. Larger phenomic systems were, and often still are, built with imaging chambers that measured chlorophyll fluorescence under steady state conditions. Under these conditions, a single fluorescence value for each image pixel is measured and quantum efficiencies cannot be calculated. In this study, we investigate the use of the hue component of the HSI (hue, saturation, intensity) color space, which is analogous to the light spectrum, from plant chlorophyll fluorescence light as a parameter to measure plant stress and compare it to parameters derived from traditional chlorophyll fluorescence kinetics. Tomato plants were subjected to heat or drought stress; subsequently chlorophyll fluorescence kinetic measurements were taken, as well as images at steady state fluorescence. By analyzing the chlorophyll fluorescence hue channel, we were able to detect differences from control plants in both the heat-shocked and drought-affected plants, while the standard PSII yield measurement was only capable of measuring differences in the heat-shocked plants. These results appear to suggest that the analysis of chlorophyll fluorescence light in the hue channel is capable of identifying perturbations due to abiotic stress in tomato plants. Currently we are continuing to investigate whether this method can be applied for more plant species and abiotic stresses.

Development of high-throughput phenotyping software for plant breeding, genetics and physiology studies

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Efficient, reliable and high-throughput phenotyping is time-consuming and sometimes difficult. To solve these problems, we have been developing high-throughput phenotyping software using digital imaging and information technologies. This includes (1) image analysis software to measure crop or plant organ sizes; we are developing an image analysis method that recognizes outlines of crop and plant organs from digital images based on color segmentation and measure shape parameters (length, area and so on); we also developed the software SmartGrain for automatic seed size measurements; and (2) an iOS program for supporting field phenotyping. Field phenotyping focuses on scoring target traits. We are developing an application for recording tasks via functions of tapping the displayed numbers or items, feeding short notes and shooting pictures. For greatest efficiency, we need to make customized software for specific measurement aims. Team site: <http://www.kazusa.or.jp/phenotyping/>

National Science Foundation programs that address areas with high impact on food security

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The National Science Foundation (NSF) was established in 1950 and is the only US federal agency dedicated to supporting fundamental research and education in all scientific and engineering disciplines, which are represented in its seven directorates. PGRP is in the Division of Integrative Organismal Systems in the BIO Directorate. PGRP supports basic research in economically important plants. Among the focus areas are the study of the structure and function of plant genomes and their interactions with other genomes, responses to the environment, and the development of tools to connect the genotype to the phenotype. The tools and resources developed by PGRP-sponsored projects are expected to transform and modernize agriculture for the betterment of society. Several PGRP-sponsored projects have active international collaborations. The Innovations at the Nexus of Food, Energy, and Water Systems (INFEWS) Program has the participation of most NSF Directorates. The Division of Chemical, Bioengineering, Environmental, and Transport Systems (CBET) in the ENG Directorate is one of the principal contributors to the INFEWS initiative along with the Geological Sciences (GEO) Directorate; the NSF-wide INFEWS Working Group is co-chaired by CBET and GEO. This initiative was developed at NSF in response to recognized threats posed by climate change and population growth, which can have a significant impact on the adequate supply of water, energy and food. INFEWS supports projects that increase our understanding of the food-energy-water supply system through modeling approaches.

Projects in this program address the development of technological solutions that provide robust decision support capabilities. An important component of NSF programs is the education, training and development of human resources. NSF is interested in facilitating opportunities for scientists in the US who are funded by NSF projects to collaborate with their counterparts in other countries.

Plant health monitoring using multispectral imaging and volatile analysis for space and terrestrial applications

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Plant phenotyping technology has the potential to advance basic scientific knowledge and save resources in crop production not only on planet Earth, but also on the Moon and Mars. Interestingly, developing plant phenotyping technology for such seemingly distant space applications has the potential to improve terrestrial technology even before any equipment is shipped in a rocket. If humans are to explore deep space, food has to be produced on site using minimal resources. Currently, however, our basic understanding of how plants and crops will behave under a gravitational pull different from the terrestrial one is still very limited. The only way to grow plants under simulated Lunar or Martian gravity is on board the International Space Station (ISS) using what is known as the European Modular Cultivation System (EMCS). However useful, the EMCS now needs updates and improvements, including its plant phenotyping capabilities. Within the *Time Scale* project, we are developing a plant health monitoring system consisting of multispectral imaging and volatile monitoring systems. The imaging system is able to perform chlorophyll fluorescence kinetics and monitor visible, near-infrared (NIR) and long-wave infrared (LWIR) spectra. The volatile analysis system is based on Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), which is able to detect and quantify in real time complex mixtures of biogenic volatile organic compounds (BVOCs). The use of these systems should allow the identification of novel stress markers for monitoring crops. Additionally, the lessons learned from the design, construction and use of this terrestrial system will guide the miniaturization and simplification of the technology so it will fit into a plant monitoring system prototype to update the EMCS' plant phenotyping capacity on board the ISS.

High-throughput field-based phenotyping in breeding with UAV platforms

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As field measurement of massive germplasm for complex traits in breeding is challenging, there is a strong demand for real-time, rapid and non-destructive phenotyping to accelerate breeding efficiency. Remote sensing using unmanned aerial platforms can be applied to rapidly and cost-effectively phenotype large numbers of plots and field trials. In recent years, strategies for high-throughput field-based phenotyping in breeding were investigated by the National Engineering Research Center for Information Technology in Agriculture (coordinated by NERCITA), where proximal remote sensing is performed by deploying sensors using aerial platforms. Strategies include the following: (1) selection and specification of UAV platforms for applying phenotyping in breeding; (2) rapid processing of multi-source remote sensing data for high-throughput phenotyping; (3) analysis of phenotypic information on soybean, maize and wheat in breeding (over 10,000 plots) by proximal remote sensing at different growth periods; (4) determining the optimal growth stage, indices and algorithm model for crop yield prediction; (5) validation of the phenotypic information resolution and yield prediction using agricultural UAV for breeding plots to ascertain its stability and accuracy; and (6) genome-wide association study of morphological indicators and maize genotypes, and identification of candidate genes.

Capabilities of the Field Phenotyping Platform FIP demonstrated by characterization of the time series of canopy cover in wheat as related to G x E interactions

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Advances in plant phenotyping technologies enable us to measure plant traits and genotype-by-environment (G×E) interactions non-destructively at high temporal resolution, which thus allows for continuously quantifying traits and dissecting G×E interactions. However, under uncontrolled environmental conditions, it is hard to separate effects of multiple environmental factors such as multiple biotic stresses. In this case, significant bias is likely introduced when interpreting the cause-effect relationships between phenotypic/complex traits and environments. Here, we propose a model for characterizing the trait “time series of canopy cover” as a response to multiple factors under natural environments, which suggests promising potential in predicting G×E interactions for future climate change scenarios, taking advantage of the ETH Field Phenotyping Platform (FIP), a 2013-2016 field experiment comprising more than 300 wheat varieties at the ETH Lindau-Eschikon experimental station, Switzerland. An integrated, multi-sensor platform, FIP allows automated measurement of multiple traits, including canopy cover (CC), temperature, height and multispectral properties. To evaluate the model, CC was measured every 3-4 days throughout two growing seasons and a time-series of CC-based growth rate was calculated as the response to seasonally fluctuating environments. The response could be visualized as a plane constructed in 3D by two environmental factors, for instance, the growing degree days (GDD) and vapor pressure deficit (VPD), against which the CC-based growth rate was plotted. The response was quantified as three variables: the slopes on GDD and VPD and the intercept

on the growth rate axes. Linear mixed models were employed for the best linear unbiased predictions (BLUPs) and results showed that heritability was 0.75, 0.40 and 0.75 in terms of the three variables, respectively. Results confirmed that GDD and VPD have a large influence on crop growth rates in natural environments. Additionally, this model provides a plausible way to weight the effects of different environmental factors on crop growth and thus interpret G×E interactions. Preliminary results also suggest that this multi-dimensional model improves prediction of G×E effects, which will be further validated in multiple years with the FIP as well as collaboratively at multiple sites.

Next-generation maize field phenotyping approaches using innovative sensing techniques for improved breeding efficiency

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Good phenotyping is one of the most critical aspects of a successful breeding program. Phenotyping methods/platforms are therefore expected to provide the concepts and tools required to increase efficiency in breeding for crop improvement. Unfortunately, phenotyping methods/tools for abiotic stresses have rarely been implemented by breeding programs. Due to the limitations of manually performed phenotyping measurements, recent advances in imaging and aerial technologies are expected to enable better integration of phenotyping approaches into breeding programs by helping to extract more value from every research plot and improve the precision and reliability of phenotypic data. We present the use of sensor technologies that provide opportunities to advance toward next-generation phenotyping, which is more compatible with maize breeders' needs and will significantly minimize selection costs while maximizing selection efficiency and accelerating the process to deliver better genetics to farmers.

Phenotyping leaf traits in wheat genotypes based on hyperspectral data

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Hyperspectral analysis has been introduced as an alternative technology to characterize the different properties of crop canopies, including applications in field phenotyping of genetic resources. Wheat germplasm is characterized by having broad phenotypical diversity, including leaf traits. In this respect, the open question is how reliable are hyperspectral indices for estimating leaf properties when applied to a broad spectra of genotypes differing in plant and leaf morphology, anatomy and chemical composition of leaves. To answer this question, hyperspectral field records and, subsequently, leaf analyses were conducted on more than 100 wheat genotypes from the Slovak National Genebank collection. The traits of fully developed flag leaves (chlorophyll and carotenoid content per leaf area and per dry mass unit, chlorophyll a to b ratio, chlorophyll to carotenoid ratio, and leaf thickness measured as specific leaf weight, leaf area, SPAD value, etc.) were correlated with 132 hyperspectral indices developed to estimate different properties of aboveground crop biomass. The selected genotypes provided relatively high diversity in all observed traits (thick vs. thin leaves, high vs. low chlorophyll concentration, very small vs. very large leaves), providing good background for correlation analyses. The results indicated that numerous parameters designed for estimating specific leaf traits (e.g., chlorophyll content, canopy structure) showed poor correlation with measured data. We also identified the parameters that had relatively good correlation across the entire collection of wheat genotypes, which can be regarded as more reliable and universal, and useful for phenotyping wheat genetic resources. The study represents one of the initial steps of the program aimed at phenotyping wheat germplasm, and at developing methodological approaches to assess the genotypes, including traits related to adaptability, plasticity and tolerance to abiotic stress factors. The study was supported by national grants APVV-15-0721 and APW-15-0562 and bilateral project with China SK-CN-2015-0005.

Poster session:
Adding value to phenotypic data

Finding a needle in a haystack – Using Zegami to visualize phenotypic data sets

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High-throughput phenotyping facilities, such as the Australian Plant Phenomics Facility, generate large phenotypic datasets, with thousands of images. These data sets are often too large and overwhelming for plant scientists to work with and make sense of. As a consequence, data analysis takes a lot longer than the actual experiment and has become a new bottleneck. To reconnect plant scientists with their data, The Plant Accelerator and Zegami Ltd (UK) collaborated to customize Zegami software to the specific needs of researchers in plant phenomics.

Zegami is an easy-to-use interactive tool that allows users to browse their image collections, create graphs and plots on the fly and extract subsets of images. Users can use the functionalities to detect outliers, find patterns within the data and explore novel phenotypic traits. Importantly, plant scientists can come up with new research questions to be further explored in collaboration with statisticians. All datasets generated at The Plant Accelerator will be uploaded to Zegami for private user access, in the first instance, and public access, once datasets have been published.

(<https://zegami.plantphenomics.org.au/>)

Taking the next hurdle: Management of phenotypic data with PIPPA

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While digital phenotyping plays a central role in many plant research projects, phenotypic data are being produced at high speed and in high quantity. High-throughput phenotyping platforms continuously generate plant images with several modalities. Nowadays, the same system can acquire RGB, thermal infrared, fluorescence and hyperspectral images and store environmental, weighing and irrigation data. The next hurdle is to be able to properly manage the high amounts of raw and derived data. At VIB, we developed PIPPA as a central database and web interface with image and data visualization and analysis functions. Several automated WIWAM phenotyping platforms, ranging from an XY table for controlled irrigation and imaging of Arabidopsis to a conveyor belt system for full life-cycle analysis of maize plants, have been integrated into PIPPA. The interface allows scientists to set up and analyze their own experiments, while keeping all data together in a structured database that takes care of data management and integration, linking images, metadata, environmental data, and image analysis and

measurement results. As the software package was developed as a web interface, the tool is available on every computer within the department. Pre-processing 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. Current and future developments focus on the interoperability of image processing tools and public accessibility of raw phenotypic data to enable community-based 'big data' analysis initiatives.

OPENSIMROOT: Computational functional plant modeling and simulation

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We introduce OPENSIMROOT, a three-dimensional functional structural plant model and use it to integrate different root traits into a whole plant root architecture, and crop “rootopy”. We also show that we can study the importance of a trait and integrated phenotypes under multiple resource constraints with our recently open-sourced software OPENSIMROOT. The phenotyping revolution in plant sciences provides ever increasing amounts of data which need to be interpreted not only in a genetic context but also in a functional context. Functional understanding reflects the strategy for adapting to different environments. Characterization of soil-root interaction in a functional model is one step towards understanding how different traits work together to improve plant growth under natural or agricultural conditions.

With modeling tools like OPENSIMROOT, we help support the decision cycle of plant performance analysis where driving processes are identified. That can help determine on which uncertain input parameters we should spend resources to gain the biggest reduction in uncertainty, by closing the loop of experiments and modeling. Experimental and simulation studies show, for example, that steeper seminal and crown roots improve nitrate capture in coarse soil and that root proliferation increases uptake of locally placed phosphorus fertilizer. Synergism between long root hairs and shallow rooting (and other effects) can be verified by functional modeling. This is a challenging task and we need to integrate phenotypic information, different environmental factors and the plasticity of the plant to those factors. Models may aid in establishing dose response curves, and studying trait or GxE interactions.

Determination of differential pathogen sensitivity of barley cultivars by multi-reflectance and -fluorescence imaging in combination with deep physiological phenotyping

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Great advances have been made in cost-efficient and high-throughput analysis of genetic information and non-invasive phenotyping, but large-scale analysis of the underlying physiological mechanisms is lagging. Phenotype is determined by the sum of the complex interactions between metabolic pathways and intracellular regulatory networks reflected in an internal, physiological and biochemical phenotype. These various scales of dynamic physiological responses need to be considered, and genotyping and non-invasive phenotyping must be linked to physiology at the cellular and tissue level. Thus high-dimensional physiological phenotyping across scales is needed that integrates the precise characterization of the internal phenotype into high-throughput phenotyping of whole plants and canopies. Thus complex traits can be broken down into individual components of physiological traits. Since the higher resolution of physiological phenotyping by wet chemistry is inherently limited in throughput, high-throughput non-invasive phenotyping needs to be validated and verified across scales to be used as a proxy for underlying processes. As a case study, we addressed the infection of four barley cultivars by two fungal pathogens with distinctly different lifestyles, the obligate biotroph *Blumeria graminisor* and the necrotroph *Drechslera teres*, using a multidimensional, holistic phenotyping approach. Non-invasive phenotyping by multispectral and fluorescence imaging in the novel high-throughput phenotyping facility PhenoLab was complemented by metabolic fingerprinting via determination of activity signatures of key enzymes of carbohydrate and antioxidative metabolism, phytohormone profiles and determination of specific defense marker genes. The differential sensitivity of the tested barley genotypes to both pathogens was detected by sensor-based imaging techniques and further validated and verified by distinct physiological responses and defense reactions. This study proves that in such an interdisciplinary and multi-dimensional phenomics approach, plant physiology, non-invasive phenotyping and functional genomics will complement each other, ultimately enabling the *in-silico* assessment of responses in defined environments with advanced crop physiology models. This will allow the generation of robust physiological predictors for complex traits to bridge the knowledge gap between genotypes and phenotypes for applications in breeding, precision farming and basic research.

Maize Phenomap1 and Phenomap2 datasets: Integration with genomes to fields

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Throughout most of the history of plant genetics, which phenotypes were measured depended largely on what could be easily scored. For example, 8 of the 10 most widely cited maize genes produce observable, segregating phenotypes at the kernel stage. In principle, both hyperspectral cameras and computer vision algorithms make it possible to quantify hundreds if not thousands of individual plant phenotypes, as well as second-order and derived phenotypes (such as ratios of different measurements, or the rate of change of a given measurement over time). However, it is not currently known which phenotypes, if any, measured in a controlled environment have value for predicting how maize and other panicoid crop plants will perform under field conditions.

Two datasets were generated using the automated greenhouse phenotyping facility at the University of Nebraska-Lincoln. The first, Maize PhenoMap1, included 31 maize inbreds imaged from germination to late vegetative development (39 DAP). These same inbreds were grown and phenotyped under conventional agronomic conditions at 19 locations across the US and Canada in 2014 and 24 locations in 2015. The second, Maize PhenoMap2, included 140 maize hybrids generated using recently off-patent inbred parents generated by major seed companies, making these lines as close to the material grown by North American farmers as legally possible. Plants were again imaged at the automated phenotyping greenhouse, to a height of approximately 2.5 meters over 63 days, enabling the observation of the onset of reproductive development, as well as under field conditions near Mead, NE.

These datasets make it possible to develop and test new computer vision-based algorithms for extracting novel phenotypes from RGB or hyperspectral image data on plants, and to rapidly assess the heritability of newly defined phenotypes, so that traits not under significant levels of genetic control can be efficiently triaged. Future efforts will focus on component-based phenotyping of reproductive structures (ears and tassels) as well as extending the phenomap concept from maize to sorghum.

Low-cost 3D imaging systems for high-throughput field phenotyping

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Given the high demand to support and accelerate breeding for novel traits, the crop research community faces the need to accurately measure increasingly large numbers of plants and plant parameters. A variety of imaging methodologies is being used to gather data for quantitative studies of complex traits. These techniques (such as visible imaging, imaging spectroscopy, thermal infrared imaging and fluorescence imaging) provide quantitative morphological measurements. When applied to a large number of plants, however, complete 3D image acquisition is time consuming for high-throughput phenotyping with a huge amount of data. In some contexts, it may not be necessary to fully rebuild entire plants. Low-cost depth imaging systems can be useful to produce a smaller amount of data per plant. We propose using a low-cost depth camera called Time-of-Flight (ToF) to have videos and pictures of the plant in 3D. A software program was written to display the plant in 3D and estimate certain traits.

We also compared the use of a Microsoft Kinect V2 camera, a digital camera and the ToF Agros P100 camera to provide 3D images of wheat and canola. The Kinect Fusion system reconstructs a single dense surface model with smooth surfaces by integrating the depth data from multiple viewpoints. The Kinect V2 emits high-frequency modulated light and measures the phase shift of the return signal to provide a 3D depth image. However, its ambient light rejection is not strong enough for outdoor use due to the wide divergence of illumination energy both horizontally and vertically.

For the digital camera, 2D images were taken and 3D model software (Agisoft photoscan) was used to construct the 3D images. Although this approach can be useful in a greenhouse, it is also highly affected by the illumination level. A low-cost depth camera with ToF technology, Agros3D P100, was used in the comparison. This smart 3D camera simultaneously delivers depth information and gray value image data for each pixel. By using active IR illumination, the sensor captures 3D and 2D information at a resolution of 160x120 pixels with up to 160 frames per second independently of ambient light. Based on these characteristics, it is suitable for use in a greenhouse setting or in the field. Using the 3D images from this low-cost ToF camera, the height of the wheat and canola was estimated using the developed algorithm; the number of canola branches was also correctly counted. Of the three cameras, the ToF seems suitable for high-throughput phenotyping in the field. The drawback of this camera is its resolution, which was not sufficient to count the number of seeds in canola or wheat in our experiments. A combination of 2D and 3D images is being investigated to improve the resolution of the current ToF camera in order to have a better biomass estimation and identify other phenotypic characteristics.

Wednesday 14th December (Morning)
PHENOTYPING FOR CROP IMPROVEMENT

Phenotyping for root-based gains in crop productivity

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The amazing production increases of the 20th century were achieved with soil and soil input management (e.g., phosphorus fertilizer, lime for soil acidity, rhizobia with legumes, nitrogen fertilizer, rotations to control root diseases, irrigation, and plowing management). Meanwhile, new varieties have been selected based on shoot phenotypes largely by eye in the field (e.g., canopy architecture, height, disease resistance and abiotic stress tolerance based on leaves, and flowering time), combined with grain weight and quality destructive measures. Roots are not generally targeted by management or breeding despite their potential to increase water and fertilizer uptake, harvest indices, and adaptations to land conservation and soil amendments, and despite the fact that the 21st century population will see reductions in land, water and fertilizers available for food production. We will present notable examples of where root phenotypes were directly selected during pre-breeding activities and transferred to breeding programs and discuss how phenotyping technologies allow holistic selection of root and shoot traits allows products to be delivered more quickly and directly to farmers.

Multi-modality remote sensing and data analysis for high throughput phenotyping

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Increases in global population, coupled with challenges of climate change require development of technologies to support increased food production throughout the entire supply chain – from plant breeding to delivery of agricultural products. Developments in remote sensing from space-based, airborne, and proximal sensing platforms, coupled with advances in computational capability and data analytics, are providing new opportunities for contributing solutions to address grand challenges related to food, energy, and water. Space-borne platforms carrying new active and passive sensors are moving from complex, multi-purpose missions to lower cost, measurement specific constellations of small satellites. Advances in materials are leading to miniaturization and mass production of sensors and supporting instrumentation, resulting in advanced sensing from affordable autonomous vehicles. New algorithms to exploit the massive, multi-modality data sets and provide actionable information for agricultural applications from phenotyping to crop mapping and monitoring are being developed. An overview of recent advances in high resolution multiple modality remote sensing, as well opportunities and challenges for data science in analysis of multi-temporal, multi-scale remotely-sensed data focused on high-throughput phenotyping will be presented.

Phenotyping at Bayer HyperCare farms

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Bayer, Crop Science division

The Crop Efficiency research programs of Bayer Crop Science are designed to enhance crop productivity by preserving and maximizing yield, with the primary focus on wheat, but also pursuing projects for soybean, corn and canola. These programs are using various technologies to modulate plant metabolism or improve plant nutrition. Through understanding yield formation in wheat, researchers work cross-functionally to deliver integrated solutions to farmers with yield increase and abiotic stress tolerance as prime targets. Several enabling innovative technologies are supporting the different R&D programs, and one of them is the HyperCare Farm concept, focusing on in-field precision phenotyping activities. The HyperCare Farms are field stations that have been upgraded in terms of precision phenotyping equipment. The main purpose is to measure, in various R&D trials, in a precise, automated and non-invasive manner, plant parameters (e.g. spectral reflectance, canopy temperature) as proxies for different phenotypical traits (e.g. biomass and water stress) and to evaluate the utility of new sensors under field operating conditions. Several HyperCare Farms have been established around the world. Examples of the high precision capabilities available in the HyperCare Farms are the PhenoTracker, a vehicle conceived as a mobile lab equipped with high resolution cameras, scanners and reflectance sensors and the PhenoTower, a camera system, measuring canopy temperature of the field plots. Via interpretation of all these precision phenotyped data, researchers can draw conclusions concerning the effects of the applied technologies on crop development and performance.

Reducing lodging in irrigated wheat: Focus on stem and root characteristics and HTP methods

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In Australia, wheat breeding efforts have focused largely on improving crop yields for dryland environments. But wheat germplasm with high yield potential and reduced lodging, well-suited to irrigated systems and high rainfall environments/years where lodging likelihood can be high, is also needed. This study was designed to evaluate traits and phenotyping methods for improved lodging tolerance. The main objectives were to: (1) evaluate the variation and robustness of traits proposed to improve lodging tolerance, (2) establish the relationships of traits with lodging expression and with yield, and (3) develop a high-throughput phenotyping method for root anchorage characteristics. Up to 50 bread wheat genotypes, identified as high-yielding and contrasting for lodging in multi-environment trials, were grown under irrigation at Gatton, NE region of the Australian wheat belt, during two consecutive years. Characteristics of stems (lower internode maximum breaking force and length) and of roots (crown root plate-spread and depth) displayed high and repeatable variation. Lines with greater lodging had weaker and thinner stems and narrower root plates. High yields were associated with greater specific stem weight, stronger stems and wider and deeper crown roots. The contribution of root characteristics to anchorage was further studied in the field, via root length evaluation in the top

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50 cm, and in an experiment under controlled conditions. The latter was set-up in an effort to develop a high-throughput phenotyping method for nodal roots to support germplasm screening and molecular marker development. Results are discussed in relation to field observations.

LeasyScan re-loaded: 3D scanning plus seamless monitoring of crop canopy and water use

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In this presentation, an update is given on the use and upgrade of the LeasyScan platform, installed at ICRISAT in 2014. LeasyScan is based on a novel 3D scanning technique to capture leaf area development continuously, and a scanner-to-plant concept to increase imaging throughput. The initial idea for its development was to assess canopy traits affecting water use (leaf area index, rate of development), which can reveal crop fitness to specific water stress scenarios. We describe how the technology functions, how data are visualized via a web-based interface and show validation data on the close relationships between scanned and observed leaf area data of individual plants of different crops (R^2 between 0.86 and 0.94), or of scanned and observed area of plants cultivated at densities reflecting field conditions (R^2 between 0.80 and 0.96). Examples of the first applications of the platform are presented: (1) to compare the leaf area development pattern of pearl millet breeding material targeted to different agro-ecological zones; (2) for the mapping of QTLs for vigor-related traits in chickpea, shown to co-map with a “drought tolerance” QTL reported earlier; and (3) for the mapping of leaf area development in pearl millet. Recently, the capacity to measure transpiration seamlessly and at a high rate was added to the platform with a set of 1,488 analytical scales. Examples of the monitoring of plant transpiration by the analytical scales are presented as part of the assessment of the transpiration response to high vapor pressure deficit (VPD) in sorghum and pearl millet. The new large-scale setup using large trays reflecting field conditions is presented. This new platform has the potential to phenotype at a high rate and with precision for traits controlling plant water use, which are of critical importance for drought adaptation and provide the opportunity to harness their genetics for breeding improved varieties.

Extending the phenotype: Integration of field and glasshouse phenotyping with crop modeling

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Crop models approximate plant processes in order to compute phenotypes of interest. Some of these phenotypes (for example, seasonal water use) are difficult to observe directly over the season. However, proximal sensing of crop canopy and root characteristics and their response to the environment starts to provide information to 'correct' the model and allow prediction, or at least genotype ranking, for these 'unobservable phenotypes'. Remote data capture from fixed, ground and aerial sensors provides information that can be extended by models. Different levels of models may be developed for short/long time frames and for incorporating greater or lesser mechanistic detail. A key point to consider is that models and the informing of them can come from multiple types of experiments, including plant phenotyping in glasshouses, and still be used to contribute to 'disentangling' the phenotypes of economic interest.

Phenotyping and GWAS for rice improvement: A strategy and partial results towards multi-trait ideotype construction by genome editing

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The GRiSP Global Rice Phenotyping Network generated extensive phenomics data on subspecies diversity panels, including morpho-physiological, phenological and chemical traits conferring yield potential, lodging resistance and adaptation to abiotic stresses. Genome-wide association studies (GWAS) identified many loci and candidate genes that are potentially relevant to breeding. Key examples will be presented. However, using these resources in molecular breeding requires many time-consuming QTL validation, introgression and selection steps. We therefore propose a shortcut strategy that uses direct, multi-trait engineering of improved plants through germline genome editing, such as improved CRISPR/Cas9 or similar technologies. We developed optimized rice ideotype blueprints by *in-silico* virtual breeding based on the SAMARA crop model, which simulates trait-trait compensations and trait-environment interactions (phenotypic plasticity), and present the results. As a next step, guided by a new, higher-yielding, lodging resistant ideotype, major causative DNA polymorphisms discovered by GWAS will be genome-edited into elite genetic backgrounds. Ideally, the resulting prototype plants will

require only few further breeding steps. The work presented here will continue in the successor program of the Global Rice Science Partnership (GRiSP), called RICE-CRP, from 2017 onwards. An effort to phenotype the 3,000 available sequenced rice genomes is under way. Success of the proposed strategy will hinge partly on improved methodologies to edit targeted base-pair or gene substitutions into genomes. If the approach is successful, we predict that libraries of causative, high-effect SNPs will become a much sought-after resource for crop improvement. This, in turn, will affect phenotyping strategies in the future.

Scalable in-field phenotyping platforms for dynamic measurement of performance-related traits in bread wheat

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Automated in-field phenotyping can provide continuous and precise measures of adaptation and performance traits that are key to today's breeding pipelines and agricultural practices. In our talk, we will introduce our integrated field phenotyping systems at John Innes Centre and Earlham Institute, including unmanned aerial vehicles (UAV), a 3D scanning crop phenotyping platform (Phenospex), cost-effective CropQuant workstations and other novel hardware/software solutions that facilitate high-resolution and high-frequency crop phenotyping. In particular, to empower the assessment of genes controlling yield potential and environmental adaptation, we will talk about our CropQuant system, a cost-effective Internet of Things (IoT) in agriculture platform (<http://www.earlham.ac.uk/cropquant-next-generation-phenomics>) designed to enable next-generation automated crop phenotyping. CropQuant incorporates networked sensors, single-board computers, in-field wireless communication and open high-throughput analysis algorithms to process field experimentation data. Besides crop trait analysis, we have established machine-learning based models to explore and predict the dynamics between genotype, phenotype and environment (GxPxE). A proof-of-principle example based on near-isogenic lines (NILs) of wheat such as *Ppd-1* (loss of function), *Ppd-D1a* (photoperiod insensitivity), *Rht-D1b* (semi dwarfing), stay green induced mutants, *Lr19* (hypersensitive response to the pathogen), and *Paragon* wild type will be discussed in our talk.

Effective delivery of phenomics in commercial breeding is more a question of what and when, not how

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Breeding for improved adaptation to water-limited environments has been successful but at rates of genetic gain below that of favorable environments. In less favorable environments, reduced genetic variance together with large genotype × environment interaction reduces repeatability of genotype performance and, hence, confidence in selection. In populations containing appropriate traits for improved water productivity, an opportunity exists to complement traditional selection for yield with advanced phenotyping technologies to improve response to selection in challenging environments. New high-throughput phenotyping technologies promise much benefit to breeders but also pose challenges. Due to fixed breeding capacity constraints, application of additional phenomics tools requires the displacement of tried and tested assessments related to breeding goals. As such, breeders have required strong evidence of the value of these complementary technologies in delivering genetic gain before deployment.

The evidence of the value of these new high-throughput technologies is accumulating with the breeder now faced with the question of not ‘how’ to phenotype but ‘when’. Yield in large plots is still the best predictor of yield, and can be readily achieved with a plot header and a balance. Enriching early generations in a breeding cycle for key alleles genetically correlated with performance significantly increases the likelihood of identifying superior genotypes in later, more expensive stages of multi-environment testing. It is at this early stage in the breeding cycle, where small plots or spaced-rows are typical, that implementation of new phenomics tools holds most promise. Cost-reducing hyperspectral imaging and LiDAR (Light Detection and Ranging), coupled with more traditional RGB and NDVI, allow routine data capture on portable, self-propelled platforms. When combined with tools such as aerial infrared thermo-imaging, biomass, leaf area, dry matter composition and water-use/stomatal conductance can be readily estimated.

To drive genetic gains for water-limited environments per unit cost, we propose that careful multistage selection, beginning with high-throughput phenotyping in early generations and culminating in later assessment in managed environments, be readily integrated with genomic prediction modeling to target selection under specific, high-frequency environment types. Together, repeatability and confidence should increase to produce correlated increases in genetic gain at reduced cost or cycle time to breeding programs. With breeder-led engagement that includes close collaboration with physiologists, geneticists and modelers, long-term genetic progress will be enhanced through assessment and identification of new traits and trait combinations in elite and diverse genetic resources, allowing targeted deployment into adapted breeding gene pools.

Plant disease phenomics: Identification of quantitative resistance in crop plants using phenomic approaches

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Plant pests and diseases account for up to 40% of total agricultural losses worldwide. One route to reducing these losses is to use phenomic methods to identify quantitative resistance to infection in crop varieties. Quantitative responses result from the interaction of multiple genes and thus provide a sustainable and durable route to crop protection. We are using a range of phenotyping approaches to examine the impact of both foliar and below-ground diseases on host physiology and disease development in diversity panels of crop plants. Physical (developmental), physiological (photosynthesis, water use) and biochemical (pigmentation and metabolic profiling) methods have been assessed for their ability to quantify host responses to disease. In addition, we are exploring novel, low-cost approaches such as multispectral imaging and Electrical Impedance Tomography (EIT) to quantify disease response. As an example, Brassicas from a diversity panel were inoculated with *Plasmodiophora brassicae*, the causative agent of clubroot disease. Multiple phenotypic parameters were measured to identify those that enabled disease progression to be quantified in a non-destructive manner. While photosynthetic measurements obtained using quantitative analysis of chlorophyll fluorescence quenching changed only relatively late during the infection process, water relationships were altered much earlier. Both evapotranspiration (measured using thermal imaging of leaves) and water use (as determined by automated pot weighing) showed a marked impact of infection on the host plant. Above-ground developmental differences were also observed, but these were highly dependent upon environmental conditions. The selected parameters are currently being used to examine a larger population of Brassica varieties so that the disease phenotypes can be correlated with genetic markers. Preliminary measurements using EIT have also shown promise as a cost-effective way to visualize below-ground responses directly.

Strategies for crop field-based high-throughput phenotyping (FB-HTP) in breeding using UAV platforms

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Rapid and non-destructive measurements for field-based phenotyping play an important role in accelerating breeding efficiency. Unmanned aerial vehicle (UAV) platforms have been used for high-

throughput phenotyping in recent years, because they rapidly and cost-effectively provide crop physiological traits to crop breeders. Given that complex traits such as plant height, biomass, lodging resistance and yield are controlled by multiple loci, large-scale multi-environmental phenotypic information analysis is necessary for locating the genetic loci that are closely related to these traits. The objective of this study was to investigate a method for field phenotyping of soybean in breeding plots by proximal sensing during different growth periods and determine the best model for yield prediction of massive germplasm.

Field experiments on 100 soybean breeding materials were conducted in 2014 and 2015. An unmanned aerial platform equipped with a digital camera, a multi spectral camera, a hyper-spectrometer and a thermal imager was used for field-based high-throughput phenotyping of soybean in breeding plots. Ten vegetation indices combining algorithms including partial least-squares regression (PLSR), principal component analysis, multiple regression, exponential regression, an artificial neural network, a support vector machine and a digital elevation model were adopted for estimating four field-based soybean phenotypes in breeding plots: height, leaf area index (LAI), biomass and canopy temperature. Plant height of soybean in breeding was estimated accurately, with a correlation coefficient between observed and estimated values of 0.92. The PLSR approach performed best for predicting soybean yield and estimating LAI and biomass with correlation coefficients of 0.85, 0.91 and 0.84, respectively. The correlation coefficient between canopy temperature detected by the UAV-based Infrared Thermal Imager and observed by hand-held Infrared radiometer was 0.91, which showed good stability and consistency.

The UAV-based field phenotyping platform is a rapid and affordable method for performing crop phenotypic information analysis in breeding. A UAV platform equipped with multi-sensors was able to identify the differences in phenotypic estimates among soybean cultivars. Combining proximal sensing data and crop physiological traits can improve the accuracy of yield prediction in soybean breeding. The UAV-based proximal sensing platform provided novel insights in accelerating the breeding efficiency.

Wednesday 14th December (Afternoon)
PHENOTYPING FOR CROP IMPROVEMENT cont'd

Remote sensing for crop improvement: From research to industry

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Plant breeding is costly and time-consuming, and any technology that can help predict the performance of each genotype while reducing the cost and amount of testing greatly improves the overall rate of genetic gain. In response to this problem, we developed a prototype remote-sensing-based system to characterize the response of wheat varieties to nitrogen. It is underpinned by the knowledge of how nitrogen affects wheat canopy characteristics, such as leaf area and angle, and chlorophyll content. We

installed RGB (red, green, blue) cameras and multispectral reflectance spectrometers on a tractor, which enabled us to screen the dynamic response of 220 wheat varieties to different nitrogen treatments. Using these data, we showed that such systems can provide results relevant for identifying genes and their functions, and for screening in plant breeding. We identified aspects of the wheat genome that affect canopy reflectance, chlorophyll content, biomass and yield. We showed that these measurements are sufficiently repeatable and well linked to the tolerance of some varieties to nitrogen deficiency. Hence, we were able to use reflectance traits at specific stages of the wheat crop to identify superior wheat varieties for nitrogen deficit tolerance. We were then able to scale up the system from trials of around 100 plots to 1000, which helped us to specify new systems developed within the scope of Phenome, a plant phenotyping network based in France. We implemented these prototypes on an autonomous ground vehicle.

More recently, an airborne system was implemented and is now available to plant breeders and geneticists. It is used to assess a wide range of relevant traits linked to drought stress tolerance in wheat and maize.

Testing the efficacy of large-scale field phenotyping in genomic selection to accelerate wheat breeding

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The process of breeding new plant varieties can be accelerated and made less costly by applying new methods of genomic selection (GS). GS uses high-density molecular markers and relatively inexpensive genotyping, in combination with phenotypic and pedigree data, to increase the selection intensity and decrease the cycle time between initial crosses and finished varieties. Increased precision comes from both exploiting genetic relationships between traits and reducing the effect of environmental influences. However, a bottleneck that occurs when applying GS is the cost of phenotyping the requisite training population, which for commercial breeding programs must be large (several thousand individuals). Robust prediction algorithms developed from the training population can be applied to reference populations without needing to repeat the phenotyping process. While yield is the fundamental phenotypic variable of interest, we test the idea that additional physiological and morphological traits can increase the predictive power of algorithms, akin to increasing the number of genetic markers. The scale and cost of phenotyping can be reduced by using remote sensing methods, largely based on spectral reflectance from crop surfaces, which have been used in agriculture research for decades. In a collaborative project with four commercial breeding companies, we are testing these principles using a UAV platform to supplement ground-based measurements to derive phenotypic values in a large population of 3000 elite wheat lines planted in two field locations in Cambridgeshire in 2016. Typical vegetation indices based on reflectance in the visible and near infrared and high resolution RGB images will be used to estimate canopy growth and senescence, crop height, etc. Hyperspectral and LiDAR data (e.g., to estimate stem soluble carbohydrate concentrations and biomass), will supplement data derived from multispectral cameras. Further precision can be obtained by taking into account environmental covariates that vary spatially across the trial area, such as differences in soil texture and

moisture content, obtained using methods such as electromagnetic induction. Field trials on the same materials will be conducted again in 2017.

Phenotyping for abiotic stress tolerance in crops: Indian initiatives

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Tolerance to abiotic stress in crops is crucial for Indian agriculture as the food security of millions will continue to depend on new crop cultivars that yield more grain and fodder in both favorable and unfavorable agro-ecosystems. Water-deficit agricultural areas are predicted to expand due to imminent competition between different sectors and the adverse effects of climate change. At present, 68% of Indian agriculture is rainfed and highly prone to drought. High temperatures limit the productivity of winter crops like wheat. The predicted rise in temperature could have more impact on crop productivity in the absence of sufficient soil moisture. Increased demand for food could extend crop cultivation to degraded soils with edaphic stresses such as salinity, waterlogging and nutrient imbalance. Wheat genotypes selected based on yield alone may not be sufficient to address these challenges, as evident from the marginal genetic gains in crop productivity in the recent past. Hence traits contributing to resilience of crops to abiotic stresses will be essential for future food production. Rapidly evolving molecular approaches are widening the scope for genetic improvement and can be effective if the association between the genes to be introgressed and drought tolerance traits is established with greater precision. These concerns and emerging opportunities for investigating and phenotyping traits recently prompted the Indian Council of Agricultural Research to establish high-throughput phenomics platforms for cereals, pulses and other crops. Efforts have been made to employ phenomics tools for assessing responses of different crops to abiotic stresses such as drought. This includes assessing the utility of techniques such as chlorophyll fluorescence and thermal imaging systems for differentiating stress tolerant and intolerant crop genotypes; methods for identifying mungbean genotypes that produce more biomass with less water relative to locally adapted cultivars; and phenotyping photosynthetic efficiency in spikes. Efforts to associate stress resilience traits and genes are expected to accelerate if these phenomics tools are adopted along with a field-lab-field approach at different experimental sites. This review will also briefly describe existing and emerging opportunities for research collaboration to enhance crop resilience to climate extremes through international networks.

Improving the precision of phenotypic data using UAV-based imagery

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Physiological breeding complements conventional breeding approaches by designing strategic crosses that aim to foster complex and complementary traits in plants in a wide range of environments. Effective screening for these traits in genetic resources and further screening of the progeny are key elements of this approach. Remote sensing tools provide an excellent opportunity for high-throughput phenotyping under field conditions, enhancing the value of physiological breeding. In this context, unmanned aerial vehicles (UAVs) have been implemented by the Wheat Physiology group at CIMMYT resulting in a suitable and effective platform to remotely measure traits, such as NDVI and canopy temperature, in thousands of wheat lines. Aerial imagery enables fast collection of spatially resolved data, potentially improving the precision of trait measurement and the elimination of confounding factors such as environmental fluctuations or non-vegetation elements within the plot. By selecting pixels within each plot using statistical outlier analysis, we improved the prediction of biomass and yield for various genotypes compared to ground-based measurements. This suggests that higher-resolution images could result in more precise measurements of remotely-sensed traits. However, this also implies more complex images showing a higher degree of heterogeneity which needs to be understood. In this presentation, we describe our latest advances in the analysis of UAV-based imaging for screening genetic resources within the context of physiological breeding.

Phenotyping for breeding and physiological pre-breeding

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Phenotyping has been the cornerstone of plant breeding but, until the advent of remote sensing (RS) tools, selection was largely restricted to heritable traits — phenology, height, kernel size, disease resistance, and non-negotiable complex traits including end-use quality and yield itself. RS allows the evaluation of integrative traits — by definition genetically complex — on a scale that somewhat overcomes the disadvantages of their relatively low heritability. In particular canopy temperature is a good predictor of yield and root function under abiotic stress, and a number of spectral indices, including NDVI and water index, also show predictive power.

Recent research has shown that the improved precision of aerial phenotyping platforms leads to increased heritability and therefore greater predictive power. Additional traits that lend themselves to aerial high-throughput screening are pigments and other indices associated with photosynthesis and photo-inhibition and water-related indices associated with tissue hydration status. Innovations in the pipeline include use of image analysis to estimate agronomic traits and the application of vegetative indices to correct for spatial variation in large trials. By virtue of being high-throughput, many of the RS indices mentioned lend themselves to large-scale genetic resource screening as well as genetic analysis.

Integrative traits have less power to predict performance under high yield environments. There, physiological breeding focuses on potential parents' phenotypes to design complementary crosses, though on a smaller scale. A good example is spike photosynthesis (SPS), which is difficult to measure and has thus been largely ignored, despite the fact that spikes intercept up to half of incident radiation during grain-filling. Three innovations have been applied to better characterize SPS including a 360 degree LED illumination chamber for gas exchange measurement, long-duration spike shading treatments and use of stable carbon isotopes analysis of grain; both of the latter help to estimate the integrated contribution of SPS to yield.

DOCUMENTING ADVANCES and IDEAS IN PLANT PHENOTYPING

Harmonizing efforts among phenotyping initiatives: Bottlenecks and opportunities

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Phenotyping is the main bottleneck to the application of new molecular techniques and breeding progress in general, raising the interest of the global scientific community in collaborative initiatives, such as the IPPN and the Wheat Initiative, which reflect the varied perceptions, priorities, experiences or sense of opportunity of their promoters. In some cases, emphasis is on the development of first-rate facilities to phenotype for special traits — for example, root architecture and functionality — under controlled conditions or featuring permanently deployed “field platforms”. Although these facilities may help to advance the research frontier within the discipline, the consumers — the breeding community — are still sceptical. A recent survey by the Wheat Initiative's Expert Working Group on Phenotyping identified data management and annotation as the biggest immediate issues for the “phenotyping community” to address, whereas accessing reliable phenotypic data, together with feeding high-throughput phenotyping results into breeding programs, will be among the biggest issues in one decade. In that context, harmonizing efforts across international initiatives, together with other relevant actors (CGIAR centers, seed companies and national agricultural programs), with a view to breeders' real needs, may pave the way for the future of crop phenotyping.

Poster session:
Phenotyping for crop improvement

Optimizing wheat root architecture by exploiting diverse germplasm

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Root architecture is key for efficient nutrient uptake and thus has a direct effect on yield. However, wheat breeding and selection programs often do not directly consider root architecture due to the practical difficulties of measuring it. Preliminary analyses point to a reservoir of genetic variation in key root traits among wheat landraces and wild relatives that can be introduced into modern cultivars to increase the genetic variation in modern breeding programs for key agronomic traits. A population of *Amblyopyrum muticum* double haploid introgression lines produced by the Nottingham/BBSRC Wheat Research Centre has been phenotyped using high-throughput, low-cost pipelines for both root and aerial traits. The phenotyping methodology and preliminary data from these lines will be presented.

Water stress field phenotyping and PHENOMOBILE-LV monitoring of wheat

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The PHENOMOBILE-LV is a fully automated robot designed for high-precision, high-throughput field phenotyping. It is equipped with several sensors including RGB cameras, spectroradiometers working in the visible and near infrared, and LIDARs. All these measurements are performed from nadir and inclined directions to gather complementary information on canopy structure. The sensors run in active mode using synchronized flashes to produce measurements that are fully independent from natural illumination conditions. They are automatically following the acquisition scenarios. In 2016, a large field experiment comparing 220 wheat genotypes under both water-stressed and irrigated conditions was conducted. Using a set of sensors, several biophysical variables were estimated: crop height, green fraction, the fraction of intercepted radiation (fIPAR), green area index (GAI) and chlorophyll content. Compared with destructive measurements taken on a subset of micro-plots, this was in agreement with a relative precision of around 10% for GAI and chlorophyll content. Our main interest in these non-destructive measurements was to analyze their dynamics. Each micro-plot was phenotyped on 10 different dates during the growing season. The time and intensity of the stress experienced by each genotype were therefore quantified by comparing the changes in canopy structure and leaf characteristics between both modalities. The possible impact of soil heterogeneity was accounted for in

data interpretation by using a map of available soil water content, previously derived by combining a soil electrical resistivity map and local soil analyses. After describing the main functions of the PHENOMOBILE-LV, the methods and performance of the algorithms developed to estimate the biophysical variables are presented, followed by their correlations with the target variables (yield and grain protein content) and their heritability. Finally, the most pertinent traits derived from PHENOMOBILE-LV non-destructive monitoring are identified based on Indirect Selection Efficiency for tolerance to water stress, which combines heritability and correlation with productivity and quality.

High-throughput phenotyping of early vigor in wheat: Rapid measures of leaf area as proxy of early plant growth parameters and yield

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In semi-arid Mediterranean-like environments, improving early vigor of wheat varieties is an important breeding target. Genotypes with early vigor will establish better and complete ground cover faster, which reduces water loss via evaporation. Phenotypic parameters such as coleoptile length, leaf area and relative emergence are associated with early vigor and can be measured on single-plants, and a field Septometer can be used to monitor leaf area index (LAI) in larger experimental plots with commercial field stands. However, those methods, as well as biomass sampling, are time-consuming and have technical constraints that limit the number of plants that can be efficiently screened in a wheat breeding program. Easy Leaf Area is free, open-source software that rapidly measures leaf area in digital images (ordinary photographs or scanner images). This software uses the RGB value of each pixel to identify leaf and scale regions in each image and to calculate percentage of leaf ground cover. Our hypothesis is that % cover could serve as a rapid low-cost method to measure early vigor and biomass accumulation in wheat. In the current study, we aim to assess the correlation between % cover to: (i) phenotypic parameters of early vigor, (ii) biomass accumulation and agronomic traits. Phenotyping of % cover, early vigor and biomass parameters were recorded in five independent field experiments at three different scales: experiment-(1-2) Rehovot 2014-16: near-isogenic lines (NILs) in a 1-m single row; experiment-(3) Bet-Dagan 2015-16: landraces in 0.5 m²; and experiment-(4-5) Gilat 2014-16: modern cultivars in microplots (40 m²). In all experiments, high significant correlations were observed between % cover, early vigor parameters and biomass accumulation. In experiment-1, % cover 21 days after emergence was positively correlated with initial grain size ($r=0.85$, $p<.0001$) and 3rd leaf area ($r=0.57$, $p=0.02$). It was also positively correlated with biomass ($r=0.67$, $p=0.004$) and grain yield ($r=0.69$, $p=0.003$). In experiment-2, % cover 28 days and 42 days after emergence was positively correlated with 3rd leaf area ($r=0.72$, $p<.008$) and fast emergence rate. In addition, % cover on both dates was also positively correlated with flag leaf area and grain yield ($r=0.6$, $p<.0038$). In experiment-4, % cover was positively correlated with LAI measurements both 50 and 70 days after emergence ($r=0.6-0.65$, $p<.0001$). PCA analysis of experiment-4 (explaining 47.5% of the experimental variance) showed high association between % cover at 30 and 50 days and biomass accumulation (on a degree-day basis), height and final biomass (mechanical harvest 130 days after emergence). These results show the potential of % cover

monitoring as a good phenotypic indicator of both early vigor and biomass accumulation at the vegetative stage and, in some cases, final dry matter and grain yield. Further large plot experiments are needed to assess whether, or to what extent, % cover could serve as a practical, high-throughput alternative to LAI.

An efficient tool to describe olive varieties through strictly mathematically defined morphological parameters

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Morphological analyses of olive fruits, leaves and stones may represent efficient tools for characterizing and distinguishing varieties and for establishing phenotypic relationships among them. In this study, we present a further step towards the development of integrated automated methodologies for describing fruit, leaf and endocarp morphologies. Our approach differs from the trends and tools currently used to describe the morphology of various organs, since there is no need for prior time-consuming manual techniques or prerequisites regarding the color of the image background or the position of the described organ. Here we present biologically meaningful morphological traits in order to describe fruit, leaf or endocarp morphologies. The developed parameters have been defined strictly mathematically, making our methodology more robust and efficient. We examined quantitative and qualitative morphological traits such as size, shape, symmetry, surface roughness and presence of additional structures (nipple, petiole, etc.).

Some morphological parameters appear to be slightly influenced by the environment and the proposed methodology can be a useful and rapid tool for identifying and distinguishing olive cultivars. We hope that our methodology is useful and will lead to morphological analyses of crop species such as tomatoes, pears, grapes and, especially, olives.

High-throughput laboratory phenotyping of lettuce to assess drought tolerance

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Lettuce (*Lactuca sativa* L.) is one of the most important salad vegetables in the world. It is characterized by variable morphological forms and interesting nutritional content of health-promoting substances. Many different forms of lettuce pose a challenge when selecting suitable genotypes for cultivation. The classic destructive analysis of plant material is slow, time-consuming and often expensive. Currently, modern plant phenotyping approaches based on optical signals from plants provide opportunities for fast phenotyping and selection of appropriate biological material. The adaptive responses of 12 different lettuce genotypes to progressive drought were studied in a pot experiment under environmentally controlled conditions using the PlantScreen™ phenotyping platform in the AgroBioTech Research Center of the Slovak University of Agriculture in Nitra, Slovakia. Genotype profiling was complemented by measuring water content and biochemical compounds (chlorophylls, carotenoids, anthocyanins, polyphenols and proline content). A high-throughput phenotyping method based on automated digital image analysis of fluorescence, RGB and hyperspectral signals was used during the entire vegetative period to accurately measure the growth and physiological and nutritional responses of lettuce genotypes to water shortage. Non-invasive automatic RGB and VNIR hyperspectral imaging of plants allowed dividing genotypes based on leaf coloring into three groups (light green, dark green and red leaves). Morphometrical analysis showed water shortage reduced plant growth measured as planar plant area with significant genotypic differences. On a physiological level, plant water stress was accompanied by a pronounced reduction in leaf water content and proline accumulation. Maximal and actual photochemical efficiency of PSII was higher in red lettuce genotypes. This observation was confirmed by the evaluation level of the photochemical reflectance index. Scanning of optical signals from plants has the potential to discriminate lettuce cultivars with different colors and physiological traits and can be successfully used for basic automatic genotype selection. This study was supported by national grants APVV-15-0721 and APW-15-0562 and bilateral project with China SK-CN-2015-0005.

An image-based automated pipeline for maize ear and silk detection in a high-throughput phenotyping platform

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Water deficit strongly impacts silk growth and silk emergence in maize (*Zea mays* L.), which in turn determine the final number of ovaries that develop grains. However, phenotyping silk growth and silk expansion is difficult at throughput needed for genetic analyses. We developed an image-based automated pipeline for maize ear and silk detection in a high-throughput phenotyping platform. The first step consists of selecting the best whole plant side-view images containing maximum information for each plant and day, as that containing most leaves and whole stem, based on top-view images. In a second step, the best side images are segmented and skeletonized, and potential ear positions are

determined based on changes in stem widths. The x y z ear position identified in this way serves to pilot the movement of a mobile camera able to take a detailed picture at 30 cm from the ear, with the final aim of determining silk emergence and silk growth duration. These methods were tested using the PhenoArch plant phenotyping platform (www6.montpellier.inra.fr/lepse/M3P) on a panel of 300 maize hybrids. The first results showed that in >80% of cases, ears were successfully detected before silking and the duration of silk expansion significantly correlated with visual scores. The image pipeline presented here opens the way for large-scale genetic analyses of control of reproductive growth to changes in environmental conditions in reproductive structures.

Identification of resistance to ramulosis caused by *Colletotrichum gossypii* var. *cephalosporioides* in advanced cotton breeding lines and monitoring of ramulosis disease by RGB-image analysis

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Cotton growing regions in South America are affected by ramulosis, a disease caused by *Colletotrichum gossypii* var. *cephalosporioides* (Cgc). The most severe epidemics cause considerable yield reduction linked to meristem necrosis, oversprouting, branching and stunting. The Sinu Valley, the biggest cotton producer in Colombia, is heavily affected by this disease. Rainfall was identified as the main driver of ramulosis development in the Sinu Valley in crops planted at the beginning of the rainy season. Fifty-five advanced breeding lines (ABLs) were assessed for ramulosis field resistance. Nine ABLs exhibited high levels of partial resistance (< 10% of plants exhibited sprouting). To optimize the accuracy of disease assessment and breeding for ramulosis resistance, we evaluated the use of Red-Green-Blue (RGB) images for automated assessment of ramulosis symptoms. Indices obtained from RGB digital images have been proposed as affordable high-throughput phenotyping tools for estimating plant disease resistance, crop growth and yield. RGB images obtained from inoculated plots were analyzed by Breedpix software for extracting indices. The accuracy of RGB indices for assessing ramulosis incidence and crop growth was compared with visual assessment of plant disease severity, leaf area index and plant biomass. RGB-based indices are accurate predictors of cotton growth, yield and ramulosis incidence and cost-effective tools for cotton phenotyping based on automation of RGB-image assessment and affordable RGB cameras.

Predicting yellow rust and *Septoria tritici* blotch in wheat by hyperspectral phenotyping and machine learning

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Various foliar diseases affect bread wheat (*Triticum aestivum* L.), thus significantly reducing its grain yields. Yellow rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst) is one of the most devastating fungal diseases of wheat in major parts of the world. In Sweden, yellow rust has become increasingly common in southern and central parts of the country and has become a major concern. *Septoria tritici* blotch (STB) caused by *Zymoseptoria tritici* is also a damaging disease of wheat causing yield losses when left without control. Hence, early detection of the outbreak of these diseases is needed to reduce the extent of the damage. Hyperspectral phenotyping with proximal sensors or sensors mounted on unmanned aerial vehicles (UAVs) can record the canopy reflectance spectra over a wide range of visible and near-infrared spectrum. The hyperspectral reflectance data can generate enormous amount of information on the physiological and biochemical traits of the plants. Any deviations in these traits during the growth cycle suggest abiotic or biotic stress factors affecting the plant. Thus, hyperspectral phenotyping can be extremely useful in predicting foliar disease outbreak during various stages of the plant growth.

The aim of this project is to evaluate the possibility of predicting yellow rust and STB incidence in wheat by hyperspectral phenotyping and machine learning. Thus, hyperspectral phenotyping (350-1150 nm) was performed with a handheld hyperspectral sensor Apogee PS-100 (Apogee Instruments, Inc., USA) at the milk development stage (Zadoks 71-77) of two bi-parental doubled haploid winter wheat populations in fields of southern Sweden. The two populations were scored for yellow rust at the booting (Zadoks 40-49) and dough development stages (Zadoks 80-87) and STB at the booting stage. Over 80 previously known indices were estimated from the obtained hyperspectral data using a custom R package. Thereafter, random forest (RF) prediction models were built from the first bi-parental population as the training population and separately for yellow rust and STB. The second bi-parental population was used as an independent test set to evaluate the obtained prediction model. The yellow rust RF model explained 66% and 71% of the phenotypic variance in the training and the test population respectively. The coefficient for prediction (R^2) was 0.74 in the test population. Ongoing work will include second year of field trials and further refinement of the models. Based on these preliminary results, hyperspectral phenotyping combined with machine learning approaches seems to be a very promising approach for predicting foliar diseases incidence levels in wheat.

Exploring the potential of spectral reflectance for detection of organ and canopy properties in wheat

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Our work aims to obtain high-throughput information on key biochemical components of crop organs, without costly and time-consuming destructive sampling of single organs or crop canopies, with hyperspectral imaging. In this paper, we explore the potential of this tool for determining spike, grain and stem properties during grain-filling. To take measurements at the organ level, a system consisting of artificial illumination, hyperspectral cameras and a moving flatbed was designed to scan spikes, grains and stems. Hyperspectral data and wet chemistry are combined to develop and evaluate different predictive algorithms for the detection and discrimination of concentration of carbohydrates, protein and water content. A similar effort is being carried out in field experiments, combining reflectance with LiDAR information.

Improving nitrogen use efficiency: Can better phenomics make a difference?

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Nitrogen (N) fertilization is fundamental for achieving crop yields but the inefficient use of nitrogen fertilizer leads to major economic and environmental costs. Over the last 20 years, there has been major public and private investment in improving plants' nitrogen use efficiency (NUE), but with limited success. The reasons behind the inability to improve NUE are becoming clearer and technological advances are paving the way for addressing these issues and making real progress. A major reason for the lack of progress in improving NUE is that what determines NUE in a crop like wheat differs greatly depending on environment and management, both of which vary greatly. Even within Australia, improving wheat NUE means very different things for winter rainfall crops in the deep sandy soils of Western Australia compared with wheat crops in the deep clays of Southern Queensland which rely on stored summer rainfall, and around the world there are many different environment and management scenarios. Global efforts, such as the Wheat Initiative, are cataloguing different wheat NUE scenarios to look for common targets, thus enabling better coordination of global activities. The impact of environment and management means that good phenomics, both in controlled environments and in the field, is essential to make real progress in NUE. Advanced field-based phenotyping platforms that allow detailed measurement of biomass and nitrogen status throughout the season can facilitate the unravelling of genetic x environment x management interactions. Improved controlled environment

phenotyping systems and non-destructive measurement of growth and tissue N offer the potential to further dissect the genetic basis of NUE. Controlled environment phenotyping has to be relevant to field performance and this can be helped by better matching of controlled environment conditions to the known environment and management scenarios that most effect NUE.

Identifying wheat root traits and regulatory genes that control nitrogen uptake efficiency

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Wheat (*Triticum* spp.) is a particularly important crop for food security, providing 20% of worldwide calorie intake. Wheat yields are not meeting an increasing global demand of 2.4% per annum. Improvement of resource capture in wheat could help meet this yield demand. Nitrogen is an essential macronutrient for plant growth and development; however, only 50-60% of the N in fertilizers is taken up by the plants. Domestication of modern varieties of wheat may have caused potentially beneficial agronomic traits, particularly in the root system, to be lost. Optimization of root system architecture (RSA) could thus profoundly improve nitrogen uptake efficiency (NUpE), which could in turn increase yield potential. Ancestral wheat germplasm and doubled haploid (DH) mapping lines can be used to identify and breed desirable traits back into commercial wheat varieties to increase yield potential. Using a new root phenotyping system, X-ray Micro Computed Tomography (μ CT), a three-dimensional representation of wheat roots can now be imaged in soil. Seedling root QTL have been previously identified using a Savannah X Rialto (SXR) doubled haploid mapping population under replete N hydroponic conditions. This mapping population has now been screened under low N conditions, and N treatment specific and nonspecific QTL identified. A subset of SXR lines was used for 3D μ CT analysis based on 2D RSA and field NUpE parameters to identify promising root traits in seedlings and mature plants.

Can high-throughput phenotyping help predict soybean yield in contrasting environments?

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Improving seed yield is the most important objective in soybean [*Glycine max* L. (Merr.)] breeding programs. The objective of this study is to find the physio-genetic parameters that drive soybean yield through a critical assessment of physiological traits at multiple crop growth stages. This study included 32 genotypes from the soybean nested association mapping (SoyNAM) panel consisting of maturity groups II and III with indeterminate and semi-determinate growth habits, and ancestries ranging from Plant Introduction accessions to modern elite cultivars. Three contrasting planting densities (50K, 140K, 230K per acre) were treated as fixed factors to study the physio-genetics of yield under different crop management conditions. Experiments were grown in replicated plots in five environments in Iowa, USA.

Physiological parameters collected included leaf area index (LAI), intercepted photosynthetically active radiation (iPAR), canopy temperature, SPAD chlorophyll content, and multiple hyperspectral remote sensing vegetation indices through spectroradiometer via ground truthing. Physiological traits were collected over multiple growth stages throughout the growing season to determine plant response. Seed yield was collected per yield plot. Yield prediction equations were built using step-wise regression equations, and several common indices were identified as best predictors of yield in different environments and/or density treatments at different crop growth stages. On-going research is attempting to determine the genetic factors related to spectral indices using a SoyNAM mapping population sub-set through phenotyping based on spectroradiometer and aerial multi-spectral cameras.

High-throughput field phenotyping of wheat plant height and growth rate in field plot trials using UAV remote sensing

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There is a growing need to increase global crop yields, while minimizing use of resources such as land, fertilizers and water. Agricultural researchers use ground-based observations to identify, select and develop crops with favorable genotypes and phenotypes; however, the inability to collect rapid, high quality and high volume phenotypic data in open fields is restricting this. This study develops and assesses a method for deriving crop height and growth rate rapidly and repeatedly using multi-temporal, very high spatial resolution (1cm/pixel), 3D digital surface models of crop field trials produced from imagery collected by Unmanned Aerial Vehicle (UAV) flights via Structure from Motion (SfM) photogrammetry. We compared UAV SfM modeled crop heights to those derived from terrestrial laser scanner (TLS) and to the standard field measurement of crop height conducted using a 2-m rule. The best UAV-derived surface model and the TLS both achieved a Root Mean Squared Error (RMSE) of 0.03m compared to the rule. The optimized UAV method was then applied to the growing season of a winter wheat field phenotyping experiment containing 25 different varieties grown in 27 m² plots and subject to four different nitrogen fertilizer treatments. Accuracy assessments at different stages of crop growth produced consistently low RMSE values (0.07, 0.00 and 0.03m for May, June and July respectively), enabling crop growth rate to be derived from the multi-temporal surface models. We found growth rates ranged from -13 mm/day to 17 mm/day. Our results clearly show the impact of variable nitrogen fertilizer rates on crop growth. The digital surface models produced novel spatial mapping of crop height variation both at the field scale and within individual plots. This study proves UAV-based SfM has the potential to become the new standard for high-throughput phenotyping of in-field crop height.

Phenotyping traits to improve drought tolerance in wheat

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Since wheat is grown mainly in rainfed and low irrigation regions, drought is one of the most important stresses worldwide. As drought may occur at every growth stage to various degrees, different traits are involved in drought tolerance at different stages. Drought tolerance is thus a complex quantitative trait. To improve drought tolerance, we used various traits, such as coleoptile length for seedling emergence, early vigor for quick seedling population establishment, higher transpiration efficiency (CID) for leaf structure and canopy temperature for the root system. We also evaluated the effects of GA-responsive dwarfing genes, Rht4, Rht12, Rht13 and Rht18 on drought related and agronomic traits and found that those genes could improve coleoptile length, early seedling vigor and the root system in general, while reducing plant height to some extent, compared with the GA-insensitive dwarfing genes RhtB1b and RhtD1b; however, their effects varied greatly with the different genetic backgrounds.

Hyperspectral disease signatures for detecting charcoal rot in soybean

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Soybean [*Glycine max* (L.) Merr] yield is under constant threat by biotic factors, especially diseases including charcoal rot caused by *Macrophomina phaseolina*, which is an important fungal pathogen that affects soybeans in warm, dry environments, and is moving northward into the primary soybean growing regions of the United States. As this disease can cause up to 30% yield loss, breeding programs strive to produce new soybean varieties that include disease resistance traits to combat yield losses. However, current phenotyping methods used to judge disease severity often include time- and labor-intensive visual ratings that are subject to human error and bias. Hyperspectral imaging (HSI) technology offers an alternative solution to the phenotyping bottleneck as it has the ability to collect precise and accurate phenotypes and can detect minor differences in disease expression. In addition, HSI captures spectral reflectance from regions of the electromagnetic spectrum beyond human vision and therefore has the potential for detecting symptoms not yet visible to the human eye. The purpose of this research is to determine the spectral reflectance signatures of charcoal rot using HSI for future implementation in high-throughput phenotyping platforms. The study utilized hyperspectral imaging to examine the spectral reflectance patterns of two resistant and two susceptible soybean genotypes that were mock-inoculated and inoculated with charcoal rot using a cut-stem inoculation protocol in a growth chamber. Preliminary results indicate that HSI successfully differentiated symptoms on plant parts that were not distinguishable through visual assessment.

Identification of water use strategies at early growth stages in durum wheat using modern shoot image phenotyping

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Modern imaging technology provides new approaches to plant phenotyping for traits relevant to crop yield and resource efficiency. The greenhouse phenotyping facility at the Jülich Research Center was used to investigate water use strategies at early growth stages in durum wheat genetic resources combined with physiological measurements. 12 durum landraces from different pedo-climatic origin were compared to three modern check cultivars in a greenhouse pot experiment under well-watered (75% plant available water) and drought (25% plant available water) conditions to identify different water-use strategies at early vegetative stages. During early growth, genotypes with large leaf area had high dry-matter accumulation under both well-watered and drought conditions compared to genotypes with compact stature (Nakhforoosh et al. 2016). However, high stomata conductance was the basis to achieve high dry matter per unit leaf area, indicating high assimilation capacity as a key for productivity in modern cultivars. In independent experiments we analyzed root architecture changes in the same contrasting panel to reveal below-ground responses. We conclude that the identified water use strategies based on early growth shoot and root phenotyping combined with detailed gas exchange analysis provide a framework for identifying water-use strategies and developing targeted selection of distinct pre-breeding material putatively adapted to different scenarios of water-limited environments.

Tackling a major epidemic of maize lethal necrosis in eastern Africa

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The sudden outbreak of maize lethal necrosis (MLN) in 2011 in Kenya, followed by confirmed reports of MLN in D.R. Congo, Ethiopia, Rwanda, Tanzania and Uganda, is causing serious concern to stakeholders in SSA. In eastern Africa, MLN is caused mainly by synergistic interaction between two viruses, maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV). In Kenya, field observations suggested that the disease was affecting almost all commercial maize varieties, causing estimated yield losses of 30-100% depending on the stage of disease onset and severity. Responding rapidly to the MLN epidemic, CIMMYT, in partnership with the Kenya Agricultural and Livestock Research Organization (KALRO), established a centralized MLN Screening Facility at Naivasha in September 2013. We have optimized the protocols for artificial inoculation of maize germplasm against MLN, as well as for the individual viruses. The facility also offers MLN phenotyping service to national agricultural research institutions (free of charge) and seed companies (on a cost recovery basis). Since the establishment of the MLN Screening Facility in 2013, more than 60,000 germplasm entries (more than 100,000 rows)

have been screened for MLN. Germplasm entries included inbreds, open-pollinated varieties, pre-commercial hybrids, commercial hybrids, mapping populations and landraces. Five first-generation MLN-tolerant (CIMMYT-derived) hybrids have been identified and released in Kenya (H12ML and H13ML), Tanzania (HB607) and Uganda (UH5354 and UH5358), and are being scaled up by seed company partners for commercialization in 2016-17. We are now testing 15 second-generation MLN-tolerant/resistant hybrids in National Performance Trials in Kenya, Tanzania and Uganda. Several more promising MLN resistant hybrids are in the pipeline. The MLN Screening Facility also serves as a platform to understand the genetic basis of MLN resistance, as well as to undertake research on seed transmission of MLN-causing viruses.

The Maize Genomes to Fields (G2F) initiative: Data management and availability

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The multi-institutional Genotype by Environment (GXE) subproject of the maize Genomes to Fields (G2F) Initiative aims to assess the impacts of genotype and environmental effects on the performance of a large collection of maize hybrids. Toward that aim, we have collected and analyzed genotypic, phenotypic, and environmental data from more than 30 North American field locations across 3 years (a total of 86 environments). These data comprise 14 core phenotypic traits and weather measurements combined with genotypic data, and for a subset of locations, image data (at scales from individual plant to individual field). To assist in the management of these diverse data types, we have developed and deployed a robust yet flexible data management and analysis pipeline that meets the project's needs but is also extensible to the broad plant breeding community. In this poster, we present progress made over the past year working with partners at CyVerse and describe methods for G2F data access and analysis. For more information, visit <http://www.genomes2fields.org/>.

NSF research traineeship – P3, predictive plant phenomics

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New methods to increase crop productivity are required to meet anticipated demands for food, feed, fiber and fuel. Using modern sensors and data analysis techniques, it is now feasible to develop methods to predict plant growth and productivity based on information about their genome and environment. However, doing so requires expertise in plant sciences as well as computational sciences and engineering. Through P3, we bring together students with diverse backgrounds, including plant sciences, statistics, and engineering, and provide them with data-enabled science and engineering training. The collaborative spirit required for students to thrive in this unique intellectual environment will be strengthened through the establishment of a community of practice to support collective learning. This traineeship anticipates preparing 48 doctoral students, including 28 NRT funded doctoral students, with the understanding and tools to design and construct crops with desired traits that can thrive in a changing environment.

Analysis of water limitation effects on the *phenome* and *ionome* of Arabidopsis at the Plant Imaging Consortium

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Understanding plant responses and adaptations to limited water availability is key to maintaining or improving crop yield; this is even more critical considering the different projections of climate change. This is an NSF-funded collaborative effort between the Mendoza and Lorence groups as part of the activities of the Plant Imaging Consortium (<http://plantimaging.cast.uark.edu/>). In this work, we combined two high-throughput *-omic* platforms (*phenomics* and *ionomics*) to begin dissecting time-dependent effects of water limitation in Arabidopsis leaves and, ultimately, seed yield. As proof of concept, we acquired high-resolution images with visible, fluorescence and near-infrared cameras and used commercial and open-source algorithms to extract the information contained in those images. At a defined point, samples were also taken for elemental profiling. Our results show that growth, biomass and photosynthetic efficiency were most affected under severe water limitation regimes, and these differences were exacerbated at later developmental stages. The elemental composition and seed yield, however, changed across the different water regimes tested and these changes included under- and over-accumulation of elements compared to well-watered plants. Our results demonstrate that this combination of phenotyping techniques can be successfully used to identify specific bottlenecks during plant development that could compromise biomass, yield and the nutritional quality of plants.

High-throughput phenotypic exploration of genetic variation from wild relatives for breeding high biomass and yield in wheat

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With the global population set to reach nine billion by 2050, there is an urgent need to increase food production by at least 60%. Wheat production has plateaued in many areas of the world due to a lack of novel genetic variation for agronomically important traits, compounded by the effects of climate change. Distant relatives and landraces offer a valuable source of phenotypic traits lost in the evolution of modern varieties as the result of severe genetic bottlenecks. Recent advances in the ability to detect and characterize genetic material from crosses between distant relatives and elite lines (introgressions) provide powerful tools to rapidly introduce greater genome-wide variation. Rapid phenotyping of these introgressed lines is a vital step in identifying variation in relevant traits that can be used for breeding and pre-breeding into elite varieties for improved biomass and yield. There is now recognition that improved grain yields of major crops require enhanced total dry weight production which must arise mostly from an improvement of radiation-use efficiency (RUE). Raising RUE requires a higher leaf and canopy photosynthesis rate and this remains an important target to underpin future yield progress. Working with the current Wheat Improvement Strategic Programme (WISP), the objective of this collaboration is to develop a rapid phenotyping screen to identify improved photosynthetic traits in new accessions of distant relatives of wheat (e.g., *Ae. mutica* and *T. urartu* accessions) and introgressed populations from WISP, with the aim of backcrossing these traits into modern wheat varieties for improved biomass and yield in the field.

High-throughput phenotyping of canopy development in soybean

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The Soybean Breeding program at Purdue University previously showed that high-throughput UAS-based phenotyping of canopy development can predict the yield potential of soybean lines, and early-season canopy development has a strong genetic association to yield. Several aspects of canopy development were evaluated and average canopy coverage (ACC) had the highest correlation with yield. The overall goal of our project is to select high yield potential soybean lines using yield performance data supplemented by drone-based canopy development data, to demonstrate whether new sources of data and analytical approaches can improve selection efficiency. A specific objective is to evaluate the response of ACC after selection. Weekly measurements of canopy and yield were acquired for 4,640 progeny rows and 13 checks and 673 lines and 6 checks in the 2015 preliminary yield trial (PYT). The canopy trait was measured by percentage of canopy coverage using UAS with RGB cameras. Breeding

values were estimated and lines were selected using three selection criteria: yield, ACC and yield given ACC. The selected lines were grown in 2016 and canopy measurements were performed as described above, but with one additional flight a week. For the progeny row derived lines, the response to selection of ACC was 21.6% and the correlated response of ACC with selection based on yield and yield given ACC were 21.6% and 20.4%, respectively. For PYT derived lines the response to selection of ACC was 16.8% and the correlated response of ACC with selection based on yield and yield given ACC were 17.7% and 17.7%, respectively.

Mechanism of heat tolerance evaluation in wheat through phenotypic parameters

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A set of 20 genotypes of wheat (*Triticum aestivum* L.) differing in their performance under heat stress was evaluated under normal and late sown conditions. Data on days to heading (DH), yield, days to maturity (DM), the tetrazolium triphenyle chloride test, leaf membrane stability (%) and grain yield were recorded. Heat stress had a significant impact on days to heading, as the mean heading date was about 11 days earlier under heat stress (90.48 ± 0.83 days) than under normal (102.86 ± 0.74 days) conditions. Genotypes WH 283 and HD 2329 escaped heat stress because of their early heading in both environments. But genotypes PBW 343 (87), WH 730(87) and UP 2425 (85) escaped heat stress by accelerating their life cycle. Heat stress had comparatively little influence on days to heading in genotypes Kanchan, PBW 373, NIAW34, Pastor, GW 173 and Kauz, which also excelled in grain yield and/or heat tolerance parameters under heat stress. This may be attributed to their ability either to avoid heat stress by absorbing more water from the soil for cooling through transpiration or by other heat-protective mechanisms. Significant Heat Susceptibility Index (HSI) values and Heat Response Index (HRI) values for genotypes WH 730, WH 533, Nesser, Raj 3765 and Kauz suggested that the heat stress performance of these varieties may not only result from heat tolerance, but also from heat escape and high yield potential. Similarly, high grain yields of Seri and HUW 234 under heat stress may be attributed only to HRI, while high grain yield of PBW 373 may only be due to high yield potential, because this variety did not manifest any escape mechanism.

Identification of root traits that can serve as suitable selection targets to enhance winter wheat production in the Southern Great Plains of the USA

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Agriculture in the Southern Great Plains of the USA consists predominantly of beef cattle production systems, supported by a variety of forages species and, during fall to early spring, limited grazing supplemented by hay. The major goal of plant breeders is to develop forage cultivars that are tolerant to different biotic/abiotic stresses that allow increased grazing time. We initiated a study to determine root

traits that contribute to enhanced forage productivity under the low-input agricultural systems of the Southern Great Plains. One approach has been to compare root architectural traits between commonly used winter wheat cultivars (e.g. Duster) and those that perform poorly (e.g. Cheyenne). Root studies in the laboratory and greenhouse showed that Duster had lower total root biomass due to slower seminal and primary root growth rates. Duster also had more shallow seminal and crown root angles than Cheyenne, whose roots grew more steeply. Our results indicate that wheat cultivars with reduced root biomass, shallow seminal and crown root angles and slow seminal root growth merit consideration as selection targets. The lower root biomass observed in Duster is consistent with previous hypotheses that plants that reduce the metabolic cost of soil exploration improve their ability to acquire limited soil resources. The shallow seminal and crown root angle also could enable plants to use available surface water and immobile phosphate pools more efficiently during early seedling establishment. We are currently verifying our results using a range of root phenotyping systems in the lab, greenhouse and field, and have expanded our studies to 200 hard winter wheat cultivars from the Triticeae Coordinated Agricultural Project (TCAP) plus 47 lines from other breeding programs. Our preliminary results show large variability in seedling root growth rates and angles among the wheat cultivars. Using cultivars with extreme root phenotypes, our future studies will aim to correlate seedling root growth rate and angle with field performance to determine if these root traits can serve as surrogate selection targets for small grains breeding efforts in the Southern Great Plains.

High-throughput screening tools for identification of traits contributing to salinity tolerance

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Soil salinity is a key stress affecting the world's agricultural lands and plants suffer rapid growth reduction including slower leaf emergence when their roots are exposed to salt stress in early growth phases. Our work provides quantitative insights into the early phase of salinity response and a robust protocol for high-throughput, image-based analysis of phenotypic traits associated with the early phase of salinity response. We tested a non-invasive protocol based on technology from Photon Systems Instruments (PSI, Czech Republic) and automated integrative analysis of photosynthetic performance, growth and color index at the onset and early phase of salinity stress response in soil-grown *Arabidopsis thaliana* ecotypes. Salinity stress rapidly and significantly affected photosystem II efficiency and impacted the growth and greening index of *Arabidopsis* plants. The PlantScreen™ platform provides a powerful tool to detect and select morphological, physiological and biochemical parameters to identify components that underlie early plant responses to diverse environmental conditions.

Customizing soybean cultivar development through aerial and ground phenotyping

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As agronomic practices continue to evolve, physio-genetic parameters (i.e., light interception, energy conversion, and biomass partitioning) must be customized in tandem to improve the rate of yield gain. The objective of this research was to identify the “drivers” of yield in multiple environments and genotypes using aerial and ground-based high-throughput phenotyping for physiological traits (LAI, iPAR, canopy temperature and hyperspectral indices). Thirty-two diverse soybean genotypes were planted in two row-to-row spacing treatments of 15” and 30”. Genotypes varied in origin (plant introductions, diverse and elite genotypes), growth habit (indeterminate and semi-determinate) and maturity groups (II and III). Three types of optimal yield responses were observed: fit for 15”, fit for 30” and fit for 15” and 30” row spacing; these differences could be attributed to the physiological traits. Unlike traditional ‘Black Box’ statistical approaches, we deployed a Probabilistic Graphical Modeling methodology to link explanatory variables with yield. Such an approach naturally captures conditional and causal dependencies across spatial and temporal scales, while also providing the critical ability to seamlessly incorporate domain knowledge into the model. We explored how the inference process can be used for predicting and identifying drivers of yield in a more meaningful manner so that it can be applied in selection regimes of cultivar development programs. These approaches enable a systematic and rigorous methodology to customize cultivar development.

Phenotyping and genetic analysis of lodging-related traits

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Lodging is an important problem affecting grain quality and grain yield in wheat. The risk of lodging in wheat will increase with the current strategy of several multinational initiatives aimed at improving wheat productivity by raising yield potential. In this context, increasing resistance of plant support structures such as stems (stem base) and roots (plant anchorage) will be crucial. Standard methods to measure biophysical properties of the stem base and plant anchorage (stem and anchorage strength) are too laborious to be incorporated in breeding programs. The implementation of rapid methodologies that enable plant breeders to select for greater lodging resistance in wheat will be a major breakthrough that will help guarantee future grain yield increases. To set a scale of work that has to be done to

evaluate lodging resistance, it is necessary to mention that, in total, standard methods have to measure 14 variables on 10-15 plants per plot. In addition, each plant has to be extracted from the field and washed in the laboratory to remove soil from roots (crown roots), which means that 100-120 minutes are needed to assess a single plot. Optimizing standard methods by reducing the number of variables and plants measured per plot has reduced the assessment time to 20-30 minutes per plot. This can be further reduced if root variables are measured directly in the field without removing the soil. However, the most promissory strategy to breed for lodging resistance is perhaps to develop reliable molecular markers that can be used in marker-assisted selection (MAS). In this regard, progress has been made by identifying QTLs associated with stem and anchorage strength in UK reference wheat population Avalon x Cadenza. Phenotypic screening of this population using optimized standard protocols indicated wide genetic variation and significant G×E interactions for lodging-related traits. However, stem strength traits can be described as highly heritable, while root or root anchorage strength traits are less heritable. Genetic analysis has identified two QTLs for stem strength on chromosome 3B (explaining 44% of phenotypic variation) and two QTLs for anchorage strength on chromosome 5B (explaining 32% of phenotypic variation). According to the literature and these findings, it seems that regions of chromosomes 3B and 5B have major effects on stem and anchorage strength, respectively. However, further validation and/or fine mapping will be necessary to develop molecular markers.

Phenotyping shows association of high seed yield with stem water soluble carbohydrates and chlorophyll content during anthesis or grain-filling in wheat cultivars under heat stress

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New wheat plant types adapted to heat or drought require cooler canopies for remobilizing assimilates efficiently and translating accumulated above-ground biomass into achievable yield. Phenotyping promises to help dissect genotype-by-environment interaction of genetic resources for these traits. The present study evaluated seven different wheat cultivars for tillering, number of grains per spike, thousand-grain weight, spike density, biomass and grain yield, harvest index and physiological maturity. Phenotyping for canopy temperature, stem water soluble carbohydrates and stay-green traits was performed at anthesis or grain-filling to determine their potential association with yield traits. Cultivars Sehar-2006, Faisalabad-2008, AARI-2011, Inqilab-91, Pasban-90, Lasani-2008 and Shafaq-2006 with restricted maturity and heat tolerance were compared with a temperate sown crop.

Heat stress reduced the number of tillers per plant, thousand-grain weight and days to maturity in the test cultivars but they expressed high seed yield, harvest index, spike index and water soluble carbohydrates at anthesis as compared to the temperate sown crop. However, no differences in biomass yield, number of grains per spike and chlorophyll content during grain-filling were found in both environment types. Under heat stress, cultivars Sehar-2006, Inqilab-91 and Shafaq-2006 had the highest increase in seed yield (20%), harvest index (31%) and spike index (133%) compared to the temperate sown crop. Stem water soluble carbohydrates at anthesis and flag leaf chlorophyll content at grain-filling

increased by 24% and 20%, respectively. These cultivars also matured earlier (15-17 days) and had lower canopy temperature during grain-filling under high heat stress than the temperate sown crop. Nonetheless, the high seed yield observed in cvs. Sehar-2006, Inqilab-91 and Shafaq-2006 was associated with high chlorophyll content ($R^2=0.41$) during grain-filling and stem water soluble carbohydrates ($R^2=0.54$) at anthesis. Likely, association of water soluble carbohydrates at anthesis was positive ($R^2=0.30$) with chlorophyll content at grain-filling. In summary, wheat cultivars (Sehar-2006, Inqilab-91 and Shafaq-2006) with earlier maturity, high seed yield, stem water soluble carbohydrates and stay green traits can be promising ones to be tested for heat adaptation and stability across different heat environments and utilization in physiological breeding for future climate change.

Phenotyping tools and physiological breeding: Optimizing biomass distribution within the plant to increase harvest index in wheat cultivars

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Conversion of intercepted radiation into biomass is a key major physiological process determining productivity in yield potential environments. Current strategies for yield genetic improvement have a strong basis on improving photosynthetic capacity and efficiency for increased biomass production. While allocation of carbon to the developing wheat spike determines grain sink strength, concurrent growth of other plant organs competes for carbon. Therefore, it is crucial to identify 'useful' biomass traits that enable breeders to maximize assimilate partitioning to the grains from existent and further gains in biomass. Genetic variation for structural and non-structural dry matter (DM) partitioning to the stem, sheath and spike seven days after flowering was observed in 26 CIMMYT spring wheat cultivars and advanced lines under high radiation, irrigated conditions in northwestern Mexico during 2011-12 and 2012-13. Furthermore, DM partitioning was assessed in the stem internodes and among the non-grain spike DM (rachis, glume, palea, lemma, awn) at harvest. Results showed that lower structural DM partitioned to the stem was associated with greater spike partitioning index (SPI; spike DM/aboveground DM at anthesis + 7 days) at seven days after anthesis, potentially increasing grain number per unit area at harvest. Results also showed a trend towards a positive association between SPI and harvest index ($R^2 = 0.16$, $P < 0.05$). Moreover, strong negative associations between SPI and spike DM per unit area and stem internode 2 (peduncle -1) and 3 (peduncle -2) DM partitioning were found ($P < 0.05$), but there were no associations with other internodes. Results indicated associations between spike morphological characteristics at harvest and fruiting efficiency (FE; grains per g of spike DM at flowering). According to our results, a value of HI > 0.6 could be achieved in CIMMYT spring wheat by combining the biggest expression for 'useful' biomass traits.

Phenotyping tools to dissect morphological diversity in sweet potato

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The genetic diversity of sweet potato (*Ipomoea batatas* (L.) Lam) and its wild relatives has been collected and conserved in germoplasm collections worldwide and explored by several tools. Characterization of crop and wild-related diversity through morphological and molecular tools produces useful information for identifying genotypes with desirable traits for use in breeding. However, characterization using morphological description presents low levels of polymorphism affecting the estimation of diversity. New phenomic approaches were used to increase efficiency when determining polymorphisms not detected by morphological descriptors. Seventy accessions of sweet potato collected on the northern coast of Colombia were characterized using 49 parameters from sweet potato descriptors (Huamán 1991) and data obtained by RGB imaging and colorimetry analysis. Field description, RGB imaging, colorimetry and their combined databases were analyzed using Gower's general similarity coefficient for clustering in R. Estimation of genotype similarity was significantly improved when quantitative data obtained by RGB imaging and colorimetry analysis were included. Variations in traits such as flesh and periderm color in roots, leaf vein color and leaf shape, which were not detected by field descriptors, were efficiently discriminated by measuring pixel values from images, estimated shape descriptors (circularity, solidity, area) and colorimetry data. As expected, high correlations were found for field parameters (number of lobes, lobe type, central lobe shape) and image data (circularity and solidity). However, visual color parameters showed low correlation with pixel values and color space parameters, confirming their limitations when used solely for morphological characterization. Combining RGB imaging and colorimetry benefits the quality of morphological characterization due to the use of quantitative instead of subjective qualitative measurements, resulting in a cost-effective process able to identify polymorphisms and target traits for diversity estimation and breeding.

NDVI values and grain mineral content predict the potential of synthetic spring wheat and wild relatives

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The dynamics of biomass accumulation as reflected by Normalized Difference Vegetation Index (NDVI) values demonstrate the reaction of genotypes to stress conditions (high temperature, moisture stress, etc.). Our data show that wheat wild relatives do not sharply reduce NDVI under stress and are characterized by less sharp curve during vegetative growth. Modern spring wheat varieties show much higher variability of NDVI values during vegetative growth. Synthetic spring wheat genotypes react more specifically to abiotic stresses than varieties and wild relatives. The criterion for selection using physiological traits would be a high NDVI curve with slow reduction after heading. NDVI values ranged from 0.19 to 0.77 for varieties, wild relatives and synthetics. High NDVI values correlated with grain yield exceeding 5 t/ha in the best synthetic lines.

Evaluation of grain mineral content revealed generally higher values in wild relatives, especially *Ae. ovata* and *Ae. triuncial* compared to modern varieties. The maximum values were recorded for *T. kiharae* (N, Mg, Mn, Fe, Zn), *T. militinae* (N, P, S); *T. compactum* (K, Zn) and *T. petropavlovskye* (Mn, Fe, Zn). Among synthetics, the following lines had the highest mineral content: Kazakhstanskaya 10 x *T. dicoccum* (K, P, Ca, Mg); Kazakhstanskaya 10 x *T. timopheevi* (N, S, Fe, Zn, Mg, Mn); and Kazakhstanskaya rannespelaya x *T. timopheevi* (Fe, S). S content in synthetics does not exceed S content in varieties but line Zhetisu x *T. militinae* had the highest value including for Ca, Fe, Zn and Mn. Top-cross tests indicated these traits were transferred to three breeding lines: Kazakhstanskaya rannespelaya x *Tr.timopheevi*; 6625 x *T. timopheevi* and 6583 x *T. timopheevi*.

Global network for precision field-based wheat phenotyping

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The CGIAR Research Program on WHEAT envisions a network of precision field-based wheat phenotyping platforms developed with co-investing national agricultural research systems. The main goal of this network is to generate high-quality data to assist plant breeders in developing resistant, high yielding wheat varieties with a broad genetic base, by maximizing the potential of new genotyping technologies. To initiate this network, five agreements were signed with national partners to set up facilities and protocols for evaluating heat stress in Sudan, multiple diseases in Uruguay, Septoria tritici blotch in durum wheat in Tunisia, wheat blast in Bolivia and yellow rust in Turkey. Additional platforms are planned to phenotype for Septoria tritici blotch in bread wheat (Ethiopia), wheat rusts (Ethiopia), Helminthosporium leaf blight (Bangladesh/Nepal), Fusarium head blight (China), heat and drought

tolerance in spring wheat (Morocco, India, Pakistan, Bangladesh, Nepal), heat and drought tolerance in facultative/winter wheat (Turkey) and yield potential (China, India, Pakistan, Egypt, Zimbabwe). All platforms will operate in close collaboration with currently functioning platforms in Kenya and Mexico. Interdisciplinary teams will promote the use of precise phenotyping approaches, standardized protocols and novel tools to accelerate superior germplasm development and dissemination.

Root cortical senescence influences metabolic costs and radial water and nutrient transport in barley

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Root cortical senescence (RCS) is a type of programmed cell death in cortical cells of many Poaceae species. The functional implications of RCS formation are poorly understood, but studies suggest that RCS formation confers both benefits and costs. The objectives of this research were to test the hypotheses that: (1) there is genetic variation for RCS; (2) RCS reduces the metabolic cost of root tissue; (3) RCS decreases radial water and nutrient transport. Using a Pitman chamber, radial water and nutrient transport were measured on excised roots of barley using stable and radioactive isotopes. Landraces had greater RCS formation than modern genotypes. Nitrogen- and phosphorus-deficient conditions increased the rate of RCS development in all lines. RCS decreases the metabolic cost of root tissue: RCS reduced root nitrogen content by 66%, phosphorus content by 63%, and respiration by 87% compared to root segments with no RCS of the same length. Older root segments with complete RCS had 90% less radial water transport, 92% less radial nitrate transport, and 84% less radial phosphorus transport compared to younger root segments with no RCS. RCS was associated with 30% greater aliphatic suberin content in the endodermis. RCS may be a useful adaptation to drought by reducing the metabolic costs of soil exploration. As RCS progresses, fewer metabolic resources need to be invested in cortical maintenance, which would permit greater resource allocation to the growth of shoots, other roots, and reproduction. Reduced hydraulic conductivity induced by RCS may also be advantageous under drought conditions because it prevents desiccation of the root tip and surrounding soil. These proposed merits of RCS under edaphic stress need further investigation under field conditions.

Nutrient-related traits for improved growth and grain quality in Indian wheat

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Wheat grain micronutrient content (e.g., iron and zinc) needs to be of sufficient quality for human health, especially in developing countries. It is not clear if further yield and quality improvements can be achieved due to the narrow genetic potential of modern wheat cultivars, and to limited land availability (e.g., many soils are marginal due to salinity, pH, and mineral nutrient imbalances). This study quantified yield, yield component and mineral quality traits in diverse wheat genotypes, including Indian varieties, wild relatives and lines containing wild wheat introgressions. Field experiments were conducted at six diverse sites in India on hostile soils with contrasting mineral stresses (pH 4.5-9.5) over two years (2013/14 and 2014/15). Traits measured included yield and yield components, and grain mineral composition determined using inductively coupled plasma-mass spectrometry (ICP-MS).

Mean grain yield ranged from 1.0 to 5.5 t ha⁻¹ on hostile and non-hostile soils, respectively. The mean grain Fe concentration ranged from 25-39 mg kg⁻¹ and grain Zn concentration ranged from 25-37 mg kg⁻¹ across the six sites. Site (E) had the largest effect on yield and grain mineral composition traits; however, there were significant genotype (G) and genotype × environment (G×E) interactions, which implies there is scope for generic selection within and between major agro-ecological zones, as currently practiced, and also for site-specific selection if the phenotyping pipelines are sufficiently cost-effective.

Large-scale phenotyping for next-generation wheat varietal improvement

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On-farm and genebank wheat diversity represent an important resource to speed advances in yield potential, address climate change effects through breeding, meet expected food demand increases and broaden breeding germplasm pool. Through the CIMMYT-led “Seeds of Discovery” (SeeD) project, we have we have conducted phenotyping and genotypic characterization of the center’s more than 140,000 wheat genebank accessions: 70,000 for heat and/or drought, 20,000 for grain quality, 10,000 for disease

resistance, 2,000 for PUE and 900 synthetic wheats for yield potential. Useful trait donors identified are being incorporated into pre-breeding programs, following a two-tier strategy: (1) incorporating general genetic diversity and (2) mobilizing the identified trait donor accessions. More than 1,000 exotic wheat lines have been mobilized in the breeding pipeline. Advanced pre-breeding sets are being evaluated in Mexico and target environments in India, Iran, and Pakistan. Wheat landrace core subsets have been formulated using large-scale phenotype-genotype data and leveraged with national research programs in Africa, South Asia and the USA.

Using shovelomics to identify rooting traits for improved water uptake under drought in a winter wheat doubled haploid population

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Water capture in wheat under drought depends on root system architecture (RSA) traits that facilitate root foraging in soil. Root phenotyping is being used to study genetic variation for RSA and link this variation to functional utility. Here we describe the root crown phenotyping of a wheat mapping population using a high-throughput shovelomics-based approach.

Field experiments were carried out over three years on a drought prone loamy sand soil at the University of Nottingham farm on 94 lines of the Savannah x Rialto doubled-haploid winter wheat population under irrigated and rainfed conditions. Root crowns were excavated from the field and washed free of soil. The whole root crown was imaged, then split into the main shoot and tiller root systems which were imaged separately. Custom software was used to measure properties such as crown root number, angle, diameter and root system width for all root systems. Soil coring of 16 DH lines validated that root crown phenotypes partially determine root length density at depth. Significant genetic variation was found for RSA under both irrigated and rainfed conditions and QTL were identified.

Shoot and root phenotyping of spring wheat under waterlogged conditions in field and rhizotron experiments

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Improving the waterlogging tolerance of wheat varieties could alleviate yield constraints caused by excessive rain and poor soil drainage. Reliable screening methods and accurate phenotyping protocols are fundamental for achieving genetic improvement. In 2013-2014, 177 wheat genotypes were phenotyped for waterlogging tolerance in Norwegian hillplot field trials with controlled waterlogging

treatments. Tolerance properties were determined using traditional phenotyping methods such as trait scoring and counts combined with principal component analysis. In 2015 and 2016, 12 and 16 genotypes with contrasting tolerance, respectively, were selected for larger scale experiments. Estimates of phenotypic traits from 2015 were analyzed in single, multiple and principal component regression analyses with yield as the response variable. Best model fit was obtained by the percentage of chlorosis ($R^2=0.87$). Image analysis (RGB and NDVI) for tolerance characterization is currently under investigation. Results of the field experiments indicated different adaptation strategies. For further investigation, a randomized split-plot rhizotron experiment including 6 contrasting genotypes was conducted. Images of roots and shoots were taken every second day to study the stress response of waterlogged and control plants prior to, during and after a 7-day waterlogging treatment. Results showed that tolerant genotypes had significantly higher seminal root elongation rate prior to the treatment. Nodal roots of these genotypes also elongated at a significantly higher rate during the treatment. All genotypes resumed seminal root growth post-treatment. The presence of aerenchyma in root samples is currently being examined. Efforts to identify QTL associated with waterlogging tolerance under field conditions are ongoing.

Predicting sorghum biomass using aerial and ground-based phenotypes

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In a crop such as bioenergy or forage sorghum, above-ground plant biomass is considered the measure of yield. However, determining biomass by waiting until the end of the year to destructively harvest plots with a biomass harvester does not allow selection in a breeding program before flowering and crossing, thereby delaying breeding cycles. Early-season destructive sampling is one option that allows selection before the sorghum flowers; however, removing a subset of plants decreases the accuracy of the biomass estimate as well as the number of plants available for breeding (or increases the total experiment size needed to account for these losses), and eliminates the option of later phenotyping on the removed material. Less direct measurements of plant biomass can be calculated non-destructively, such as the approximation of stem volume derived from plant height with top and bottom stem diameters. This approach can provide fairly accurate prediction, demonstrated here across multiple time points in the growing season. These measurements can be time-consuming and labor-intensive to take by hand, but advances in remote sensing technologies allow a large number of varieties to be screened from aerial platforms. We compare and combine results for estimating end-of-season biomass using remotely sensed plant height (derived from RGB point clouds) and hyperspectral features (including Extended Multi-Attribute Profiles, or EMAPs), paired with extensive ground-based phenotyping to provide a benchmark for our results. Overall, this study highlights the usefulness of a combination of platforms for predicting end-of-season biomass in forage or bioenergy crops.

Alterations in root proteome of salt-sensitive and salt-tolerant barley lines under salt stress conditions

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Salinity is one of the most important abiotic stresses that cause significant reductions of crop yields. To gain a better understanding of salinity tolerance mechanisms in barley (*Hordeum vulgare*), we investigated the changes in root proteome of salt-sensitive (DH14) and salt-tolerant (DH187) lines in response to salt stress. The seeds of both barley lines were germinated in water or in 100 mM NaCl for six days. The root proteins were separated by two-dimensional gel electrophoresis. To identify proteins regulated in response to salt stress, MALDI-TOF/ TOF mass spectrometry was applied. It showed that sensitive and tolerant barley lines respond differently to salt stress. The most significant differences concerned proteins involved in signal transduction (annexin, translationally-controlled tumor protein homolog, lipoxygenases), detoxification and protein folding processes (osmotin, vacuolar ATP-ase, protein disulfide isomerase) which were up-regulated only in the tolerant line under salt stress. The results suggest that the enhanced salinity tolerance of line DH187 results mainly from increased activity of signal transduction mechanisms, which eventually leads to the accumulation of stress protective proteins.



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