Proceedings of the II Latin-American Conference on Plant Phenotyping and Phenomics for Plant Breeding

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Proceedings of the II Latin-American Conference on Plant Phenotyping and Phenomics for Plant Breeding

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Foreword

Global agricultural production has been pressured to attend the increasing demand for food, fibers, and energy and to adapt to the unstable weather conditions, such as unexpected long droughts and warmer temperatures. The only way to answer to such demands in time is by breeding more productive crops tolerant to severe weather conditions, especially biotic and abiotic stress. Thus, plant phenotyping is the key to accelerate the development of new species by providing non-invasive tools to understand the physiological and genetics basis of plant growth and adaptation.

While a revolution from the new plant phenotyping methods and phenomics are taking place in Europe, USA, and Oceania, plant phenotyping is still incipient in Latin America, despite being responsible for 16% of global exportations between 2012 and 2014 in food and agriculture. Latin America is a promising market for plant phenotyping not only because of its production potential, but also for its large biodiversity which could lead to new genetic materials.

In this sense, Latin American scientists have been engaged to establish and consolidate the Latin American Plant Phenomics Network and to organize conferences with renowned scientists from other continents in plant phenotyping aiming to promote the conditions for partnership. Thus, it is our great pleasure to welcome all participants of the Second Latin American Conference on Plant Phenotyping for Plant Breeding (II LAPhPB) to Embrapa Instrumentation, São Carlos, SP, Brazil. Embrapa Instrumentation is one of the 47 research centers of Brazilian Agricultural Research Corporation (Embrapa) and was created with the purpose of integrating knowledge areas, such as physics and engineering with agriculture, and developing instrumentation technologies for agribusiness, such as machinery, equipment, sensors, and process automation. Embrapa Instrumentation is fortunate to be located in São Carlos – "he national capital of technology" – a title given because of its high technological research level developed by the University of São Paulo (USP), São Carlos Federal University (UFSCar), and two Embrapa's research centers.

The II LAPhPB continues the discussions initiated during the first conference, which took place at the University of Talca, Chile, in 2015. The II LAPhPB organizing committee made an effort to bring scientists from all over the world to present the current trends in plant phenotyping and to discuss the research performed in Latin America. A demonstration of the use of drones will also take place at a maize field experiment on September 22. During the conference, the organizing committee also expect to strengthen the Latin American Plant Phenomics Network built during the first conference.

The main themes of this conference are: i) Field Plant Phenotyping: use of precision agriculture tools, elaboration of protocols to improve yield, and plant breeding; ii) Novel Non-Invasive Instrumentation Technologies, such as automation, sensors, modeling, bioinformatics, and signal processing for plant phenotyping; and iii) Phenotyping for Crop Improvement and for biotic and abiotic stress.

In total, 57 abstracts were accepted, among which 8 were selected as oral presentation. Another 2 oral presentations were invited from Latin American researcher or scientific committee member. Using the first author's affiliation, 81% of the accepted papers were from Brazil, 12% from Latin America (excluding Brazil), and 7% from Europe. We would like to thank all reviewers for their dedicated work and prompt responses.

Up to the late registration deadline, II LAPhPB had 71 registered participants, a larger number than in the first conference. There were also interests from companies to support the II LAPhPB, with 3 sponsors providing financial and in-kind contributions. We are grateful to Lemnatec, Tecnal, and Pd Instrumentos for their generous support. We also thank Brazilian National Council for Scientific and Technological Development (CNPq), Brazilian Coordination for the Improvement of Personal Higher Education Level (Capes), Forschunszentrum Jülich / IBG-2: Plant Science, Embrapa Labex Europe in France, and Brazilian Society of Vegetables Physiology (SBFV).

We are also honored to have six distinguished scholars as invited speakers, namely, Ulrich Schurr (Germany), Francois Tardieu (France), Jose Luis Araus Ortega (Spain), Uwe Rascher (Germany), Jörg-Peter Schnitzler (Germany) and Michael Schaefer (on behalf of Xavier Sirault) (Australia). We are grateful to their motivating contributions.

II LAPhPB has been organized by Embrapa Instrumentation. We are indebted to a number of people who have contributed generously to the organization of the conference, in particular all members of the technical committee. We also thank II LAPhPB executive committee members and Embrapa Instrumentation directors: João de Mendonça Naime, Wilson Tadeu Lopes da Silva, Odemilson Fernado Sentanin, and Cauê Ribeiro de Oliveira, for their support.

We sincerely hope you find II LAPhPB intellectually inspiring and wish you an enjoyable stay at São Carlos.

Editors

Preface

Human population growth at alarming rates, scarcity of natural resources, climate change, and the complexity of global market increasingly challenge agricultural research as well as food, fiber and bioenergy production chains. In order to keep up with these complex scenarios, agriculture and livestock farms are swiftly becoming production units which are similar to industrial plants in several aspects. These changes include making automated decisions by collecting and transforming data into information which, in turn, is promptly converted into actuation. In the last decades, genetic engineering has been playing a key role in the evolution of productivity of several important crops and animal breeding around the globe. However, evidently, this rapidly ascending curve is close to reaching a saturation level. Nowadays it is mandatory to invest effort to reduce the time frame required to launch a new cultivar in the market. In such process, conventional plant phenotyping is a time-consuming bottleneck as it is a tedious and lengthy process of observing the physical and behavioral traits of the plant. For that reason, more and more R&D institutions worldwide are focusing on the development of plant phenotyping methodologies in order to accelerate the development of new cultivars.

Compared to some regions, rapid plant phenotyping is still a relatively new technique in Latin America – in spite of being the region which will be responsible for the largest percentage of the food production required to feed the world by 2,050. Therefore, it should come as no surprise the importance of the Conferences on Plant Phenotyping for Plant Breeding. The inauguration of the Conference was held at the University of Talca (Chile) in 2015 and it became the initial milestone of the Latin-American Plant Phenomics Network. By means of these events, Latin America presently has the appropriate conditions to boost multi-institutional and multidisciplinary cooperation and, consequently, result in important advances to the knowledge frontier. This can be verified by the excellent quality and the originality of the works present in this proceedings book. The following topics are covered:

- Field plant phenotyping using precision agriculture tools and elaboration of protocols to improve the yield and plant breeding;
- Brand-new non-invasive techniques based on instrumentation, automation, optics and photonics, sensor and biosensor, math modeling, bioinformatics, image and digital signal processing, and high-throughput plant phenotyping;
- Phenotyping for crop improvement and to investigate biotic and abiotic stress.

The present proceedings gather 57 papers authored by 207 researchers from Brazil, Argentina, Mexico, Uruguay, Peru, Chile, France, Belgium and Denmark.

The Brazilian Agricultural Research Corporation (Embrapa) is intensively investing on plant phenotyping since 2012, when a scientific cooperation inaugurated the Forschungszentrum (IBG2) at Jülich and established a Labex (virtual lab of Embrapa abroad) on this specific research theme. The chair of the II Conference, Paulo Herrmann, was the pioneer of Labex in Germany and now is leading a research network financed by Embrapa.

We would like to thank Herrmann for the initiative and leadership, as well as the executive committee, the scientific committee and local organizing committee for the success of the II Conference. We would specially like to thank the invited speakers Ulrich Schurr, François Tardieu, Jose Luis Ortega, Uwe Rascher, Jörg-Peter Schnitzler, and Michael Schaefer (On Behalf Xavier Sirault) for the important contributions they made to the Conference discussions.

João de Mendonça Naime
Director-General of Embrapa Instrumentação

Summary

Field Plant Phenotyping

Keynote

Dr. Uwe Rascher
Chefe do grupo Ecossistemas dinâmicos, Forschungszentrum Juelich / IBG-2: Plant Science, Juelich, NRW, Alemanha

Title:

Measuring and understanding dynamic plants traits in the field – non-invasive plant field phenotyping across scales

A better understanding of the dynamic spatiotemporal adaptation of our crops is needed to provide the basis for crop breeding, management, and protection measures. To scientifically understand the mechanisms behind the dynamic structural and functional adaptation of plant traits, it is mandatory to phenotype plants under their natural conditions in the field. In this presentation, we introduce recent concepts for phenotyping of dynamic plant traits in the field. We give an overview on the most prominent sensors and measurement concepts that are the backbone for plant phenotyping initiatives. We focus on the quantification of most relevant traits, which are related to plant structure, water relations and transpiration, and photosynthetic energy conversion. Ground based sensors are complemented by drone and airborne sensors to better understand the dynamic adaptation of crops across scales. We finally give an outlook into recent developments of satellites that will complement field based phenotyping in the future and will facility to map and understand functional plant traits on the larger scale. New satellite missions such as Europe's Sentinel satellites or the new Earth Explorer mission FLEX will open the path to observe spatio-temporal variations of plant development and photosynthesis globally. Integrated data management will provide the basis to map anthropogenic impact and to better understand gene × environment × management interactions, which ultimately will be the basis for a sustainable and resource-efficient use of our plant resources in a future bio-based economy.

Keynote

Prof. Dr. Jose Luis Araus Ortega
University of Barcelona, Department of Evolutionary Biology, Ecology and Environmental Sciences
Faculty of Biology, Barcelona, Espanha

Title:
High throughput field phenotyping: top tech and low cost are not mutually exclusive

The presentation will deal about low cost approaches for field phenotyping, basically (but not only) related to the use of RGB images and other remote sensing solutions.

Methodology for evaluation of biostimulants effects on maize root system

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Keywords: plant growth promoters, nutrient solution, humic substance, aminoacids

Biostimulants have increasingly been used in modern agriculture as plant growth promoters and can be extracted from several sources. Biostimulants have the potential to stimulate growth, to mitigate biotic and abiotic stresses, and to increase yield. Among agricultural products, biostimulants still do not have a clear definition due to the complexity of their compounds and different responses induced by interactions with the plant, rhizosphere and environmental factors. It is fundamental to establish a robust methodology to evaluate their action in different regulatory and functional systems of plants. In this context, this study aimed to establish a root morphology evaluation protocol for maize seedlings under humic substances (HS) and aminoacid biostimulants. Initially, we evaluated root morphology aspects and dry weight of two maize commercial cultivars, L521236/CMSM036 (Genotype 1) and L521274/CMSM033 (Genotype 2), grown in nutrient solution with biostimulant A (HS), B and C (Aminoacids) in a paper pouch system. This test indicated that that the paper pouch system was not suitable for biostimulants assessment. In later experiments, we used the floating system with various concentrations of biostimulants and treatment times. In addition, we evaluated maize seedlings with an acclimatization period. After different tests, we determined that the most suitable methodology for biostimulants evaluation was an acclimatization of maize plantlets from genotype 1 for seven days with ½ Hoagland´s nutrient solution in a floating system, with the addition of 54.4, 5.0, 5.0 µL L-1 of the biostimulants A, B and C, respectively, leaving the plantlets in the system for more seven days. In this condition, there were an increase in root total surface area, surface area of fine roots and total dry weight. This method will allow the development of molecular and physiological experiments that will shed a light on biostimulants action mechanisms.

Acknowledgments:

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Weed species phenotyping into a culture of maize for the precision application of herbicides

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Keywords: weed phenotyping, weed control, advanced image processing, precision spray.

The weed management in agriculture plays an important role, since the yield of a crop can vary depending on the species of invasive plants involved, and due to its percentage of occupation by area. Since one function of the phenotyping process is the morphological characterization of plants [1], such concept can be used to discriminate between monocot and dicots weed species, i.e., those having narrow-leaves and broad-leaved weeds, groups that can be appropriately controlled by specific herbicides. In fact, efficiency is higher if selective treatment is performed for each type of infestation instead of using a general application of herbicide on the whole agricultural surface. This paper presents a method for weed recognition based on phenotyping processes, i.e., to improve precision spraying based on the control of weed species into a maize culture. The method was developed considering the use of shape modelling techniques, which uses concepts of multi-resolution analysis in both radial and circular directions. Results obtained were compared with a solution presented in the literature, which used the Hu moments [2]. The performances of the classifiers were compared based on the class of error, risk assessment, accuracy and algorithm quality. Besides, for validation both the Moments-Invariant and the Centroid-Radii models were used. The proposed approach has shown robustness characteristics for the recognition of those analyzed weeds. The data set for experimentations consisted of 100 images collected form an agricultural field of maize, considering specific set of data for training and testing, as well as for analyses and comparison purpose. In this context, the results have shown that the consideration of the species and its occupancy rate in the area of culture associated with the herbicide application at variable rates based on zones and precision application can be useful to minimize the economic and environmental impacts, and competitiveness.

Acknowledgments:
This work was supported by the Brazilian Corporation for Agricultural Research (Embrapa Processes' No. 02.11.07.025.00.05, and No. 01.14.09.001.05.05).

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A new approach for plant phenotyping and image segmentation based on contextual information

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Keywords: plant phenotyping, image processing, contextual information, decision making, advanced method.

Plant phenotyping is an area that challenges the image processing field because the identification of plants into complex scenarios requires the use of specialized algorithms in order to prepare the recognition process and to decrease errors [1]. In this context, it is verified that image segmentation is one of the main tasks in image processing. It partitions an image into distinct regions, and the success of image analysis depends on reliability of segmentation which is a challenging problem [2]. This paper presents a new approach to deal with such problem that uses contextual and color information. Given a color image, the component in green tones was used and two regions, one associated with the object of interest (s) and the other associated with the background of the image (t) were selected. In addition, for each pixel the contribution of green tone (G_c) was calculated considering its RGB composition. The graph g was used as an auxiliary structure to classify the pixels associated with s. In the structure, each pixel corresponded to one node and two virtual nodes, s and t, were added in g. The nodes were connected to each other by t- and n-links that carried the information of the degree of relationship between them, called capacity. The n-link capacity considered the contextual information of the pixel and its neighbors and the information of the local gradient. The t-link capacity was defined by means of the use of a Gaussian distribution and C_g was used as way to penalize edges with low contributions of green tone. Finally, the pixels associated with s were obtained by segmenting the image. In order to improve the results, the image was divided using windows. In conclusion, a new approach for plants segmentation based on the contextual and color information was presented. The results have indicated the establishment of a robust method for real applications. For future work, noise's treatment as well as the use of Graphics Processing Unit will be included.

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Study of mortality, growth and initial genetics parameters in *Corymbia* spp. populations

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Keywords: populations, silvicultural performance, breeding.

One of the stages for introducing new species is the implementation of species/provenance trial that measure silvicultural performance and genetic parameters (GPs). In addition, knowledge of such characteristics are complementary parts for phenotyping. This work aimed to evaluate mortality and initial growth of populations of *Corymbia citriodora* subs. variegata, *C. maculata* and *C. henryi*. The populations of *C. citriodora* subs. *variegata*, *C. maculata* and *C. henryi* were implanted in October 2016 in Itatinga, SP, Brazil, with seeds from Australia. The experimental design for the three trials were composed of incomplete randomized blocks with 5 trees per linear plots, both for *C. citriodora* subs. *variegata* and *C. maculata* were composed of 9 blocks with up to 15 treatments per block; *C. henryi* was composed of 10 blocks with up to 5 treatments per block. We used spacing of 3 by 3 m between plants and lines. Plant height (H) and mortality (M) were evaluated at 6 months after planting. The GPs were obtained by the REML/BLUP method using Selegen software (1). *C. citriodora* subs. *variegata*, *C. maculata* and *C. henryi* presented respectively 0.03, 0.02 and 0.03 of M, 171 cm, 160 cm and 174 cm of mean H, 0.72, 0.26 and 0.84 of average heritability of the population mean (H2MP), 0.84, 0.50 and 0.84 of accuracy of population selection (ACPROC) and 0.014, 0.004 and 0.001 of determination coefficient of plot effects (C2). M was considered low for all the species, demonstrating good management of the trials. C2 values indicate low environmental influence in the trials, evidencing precision in genetic selection. Based on the values of H2MP and ACPROC, species *C. citriodora* subs. *variegata* and *C. henryi* presented potential for breeding, but it is still early and this information needs to be confirmed with the results of the coming years.

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Estimation of corn plant population density by digital image analysis and NDVI

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Keywords: Remote sensing; Plant population; Vegetation sensor; NDVI.

Quantification of plant density becomes necessary to guide agricultural management, such as nitrogen fertilization. Thus, one can use remote sensing tools, used in precision agriculture for identification of plant density [1]. The objective was to evaluate a population density of plants capable of promoting variability in the values of the indexes of digital images (crop cover) and NDVI (normalized difference vegetation index) and its relation to productive potential of corn. The experiments were carried out at research station of UFRGS. The experiment was conducted under field conditions in 2014 and 2015 seasons. Treatments used were four population densities (4, 6, 8 and 10 plants m^{-2}). Canopy reflectance (NDVI) was evaluated by Greenseeker® optical sensor, while proximal RGB canopy images (crop cover) were taken by using a Canon Power Shot digital camera [2]. Images were processed by SISCOB software. NDVI and grain yield data were analyzed by regression analysis for each phenological stage. Pearson's linear correlation coefficient was determined between the indices of digital images and NDVI with grain yield. The highest correlation between NDVI and crop cover was observed to V3 and V9 stages for the different population densities. It can be concluded that the use of any of the methodologies is adequate to estimate plant population density of corn under field conditions.

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Canopy sensors for estimating in-season corn grain yield potential

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Keywords: Remote Sensing; Precision agriculture, NDVI; Vegetation sensor.

Estimating corn yield potential during the vegetative phase is crucial for obtaining high grain yield. In order to access spatial variability of nitrogen (N) supply *in situ* one can use tools for estimating in-season corn yield potential by using vegetation sensors for the evaluation of N supply and for identification of in-field N requirements variability [1]. The objective of this work was to evaluate the relationship between normalized difference vegetation index (NDVI), crop cover percentage, leaf chlorophyll content and corn grain yield. The experiments were carried out at research station of UFRGS. Treatments consisted of different dissection times of previous crop (white oat, Avena Sativa L.) before sowing corn. Canopy reflectance (NDVI) was evaluated by Greenseeker® optical sensor, while proximal RGB canopy images were taken by using a Canon Power Shot digital camera. Images were processed by SISCOB software. Relative leaf chlorophyll content (SPAD) was evaluated by SPAD-502® Minolta Chlorophyll meter. All regressions between variables were linear with p-value of the 5%. The highest coefficient of determination (R2=0.81) was observed between NDVI and crop cover. In relation to leaf chlorophyll content, coefficient of determination varied from R2=0.48 between SPAD and crop cover up to R2=0.54 between NDVI and SPAD. All variables correlated well to corn grain yield. Coefficient of determination varied between R2=0.53 for the relationship between crop cover and corn grain yield and R2=0.56 for the relationship between NDVI and grain yield [2]. For the relationship between SPAD and grain yield the regression adjusted was quadratic with coefficient of determination of R2=0.79. The estimation of in-season corn grain yield potential can be used to estimate nutritional status of the crop.

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Phenotyping for drought tolerance of a biparental wheat population, using agronomic traits for validation of spectral and fluorescence sensors

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Keywords: *Triticum aestivum*; Water levels; Gene prospection, High throughput phenotyping.

One of the main reasons for the low productivity of wheat in the Brazilian Cerrado region is the low water supply, that limits the productive potential of this crop. Therefore, the improvement for resistance to drought has great importance for wheat in Central Brazil. The objective of this work was to validate spectral and fluorescence sensors as a tool for phenotyping to select wheat lines for the prospection of genes linked to drought tolerance in the future. A population of 93 wheat double-haploid lines from crosses between MGS-1 Aliança and PF 020062, as well as the parents and MGS Brilhante (materials with known behavior) were evaluated. An irrigation system, consisting of an irrigation bar drawn by a reel at different speeds was used to apply two water levels. . In one treatment irrigation was compensating 100% of the evapotranspiration, recommended for wheat in the Cerrado region, and in the other treatment 50% of the required evapotranspiration, measured in meteorological station adjacent to the experiment. The following agronomic parameters were evaluated: yield, hectoliter weight, height and weight of 1000 grains. The sensor measurements were fluorescence, measured by the Light Induced Fluorescence Transient (LIFT) instrument, from which the photosynthetic efficiency of Photosystem (Fv'/Fm') was calculated and spectral indices (NDVI and PRI) from the LIFT built-in spectrometer. The results showed variability within the population and among the parents, as well as transgressive individuals in relation to the parents. The sensors showed a significant correlation with all parameters evaluated, linked to drought tolerance, and are therefore convenient tools in the phenotyping process both for breeding and for the prospection of genomic regions associated with drought tolerance.

Low-cost thermal field mapping

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Keywords: Thermal map, temperature sensor, Arduino Uno, water stress.

This project uses several MLX90614ACF infrared temperature sensors integrated with an Arduino Uno microcontroller and a SD card to make a thermal scan of the field. This system is attached to a 24 meters horizontal bar that is pulled by a tractor Cardan axis. The infrared sensor can detect the thermal radiation emitted by objects nearby and then determine its temperature. The data acquired is sent through I2C-BUS serial communication to the microcontroller and stored. With all the field data stored, the result is a thermal map of the field. With the generated thermal map, the plants and field status can be evaluated. Therefore, it is possible to show which of the crops is more adapted to water stress. This project is originally designed to be used in rice genetic enhancement fields, but it can be used for other purposes, for example, to verify if any part of the tillage has a disease, when comparing the thermal signature of the species with the thermal map. This technique has great advantages because it is a non-contact, non-invasive and fast way to measure the temperature of crops. Another advantage is its low-cost, the hardware costs less than $ 200.00

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Validation of reflectance and fluorescence sensors for wheat tolerance to drought in field conditions in Cerrado region

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Keywords: high troughput, *Triticum aestivum*, Hydric stress.

Rainfed wheat has had its productivity limited by insufficient rainfall. In this sense, it is necessary to introduce evaluations related to drought tolerance in breeding programs. However, many of these parameters are destructive, which is a problem, especially in segregating generations. This leads to the need to use noninvasive phenotyping tools. A protocol of phenotyping during the dry period was developed at Embrapa Cerrados, which a linear irrigation bar drawn by a reel is used. In this protocol an irrigation gradient is obtained. Four wheat genotypes were evaluated. The objective of this work was to validate reflectance and fluorescence sensors as a phenotyping tool for select wheat genotypes for drought tolerance. The experiment was conducted in the winter due to the absence of precipitation. The validation of the sensors was verified through the correlation of the parameters obtained with the physiological, biometric and agronomic parameters. The sensor measurements were fluorescence, measured by the Light Induced Fluorescence Transient (LIFT) instrument, from which the photosynthetic efficiency of Photosystem (Fv/Fm) was calculated and reflectance indexes (NDVI and PRI) from the LIFT built-in spectrometer. The correlation intensity classification for p <0.01 was considered to be very strong (r + 0.91 to + 1.00), strong (r +0.71 to +0.90), mean (r + 0. 51 to + 0.70) and weak (r + 0.31 to 0.50), according to Gonçalves and Gonçalves (1985), cited by Guerra and Livera (1999). The spectral indexes were positively correlated with the productivity. The correlation of these indexes with the physiological parameters such as photosynthesis, conductance, and internal carbon, was more expressive, with correlation intensity, considered strong. The fluorescence sensor showed a significant, but low correlation with the studied parameters. Thus, these correlation indexes obtained between the parameters validated the use of these spectral sensors in the selection of drought-tolerant genotypes using a non-invasive methodology.

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A protocol for measuring canopy temperature of sugarcane plots in the field using a thermal imager embarked in an UAV

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Keywords: thermography, plant breeding, drought tolerance, remote sensing.

Thermal imagers are the most suitable commercially available equipment for measuring plant canopy temperature in large areas by remote sensing. However, its use under field conditions presupposes overcoming two challenges related to image capture speed and measurement precision: 1. to increase the size of the area and the number of plants simultaneously imaged; 2. to isolate the target object, that is, the plant canopies from possible contaminants in the thermal image. Thus, for measuring the canopy temperature of six sugarcane genotypes aiming at discrimination for drought tolerance under field conditions, a thermal camera was embarked in an unmanned aerial vehicle (UAV). A gimbal was developed for camera support and stabilization. Also, a device was developed for camera focus adjustment and triggering. An equipment that simultaneously generates thermal and RGB images, captured by different sensors was used. The imaging processing consisted in the alignment of both images, followed by segmentation of corresponding pixels to the plants. For this, RGB image was used to produce a color mask which was applied to the thermal image by means of a program developed in Python language. Thus, the pixels presented in the green channel a value 5% higher than the red channel were selected as "plant". Based on this assumption, it was possible to segment the pixels corresponding to plants from those of soil. Finally, canopy temperature of the sugarcane genotypes in each plot could be determined.

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Use of thermal imaging for discrimination of maize genotypes submitted to water deficit stress

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Keywords: thermography, plant breeding, drought tolerance, remote sensing.

Plant responses to drought have been discriminated by using parameters derived from canopy temperature. The principle underlying this practice is based on the observation that, under drought stress, plants close the stomata and, consequently, reduce transpiration. Since water loss through stomatal transpiration is one of the main mechanisms of leaf cooling, restriction to water loss leads to increased leaf temperature. Plants tolerant to drought can regulate canopy temperature more effectively than sensitive ones. Based on these assumptions, plants (pre-flowering stage) of four maize genotypes were submitted to drought by water withholding. These plants were evaluated by the standard method of gas exchange and also by thermal imaging. As expected, the stressed plants dramatically reduced the rates of stomatal conductance, transpiration and net CO_2 assimilation. In parallel, there was an increase in the canopy temperature of the stressed plants in comparison to the control ones. Although one cannot compare data obtained in such a small area (6 cm^2) of the metabolically more active leaf, such as those obtained by gas exchange, with data obtained in the whole canopy of plants, such as thermography, it was expected that there would be some coherence between the two sets of data. However, depending on the selected temperature range or measure of central tendency (arithmetic mean, median or mode) used to express the plants canopy temperature, the discrimination of genotypes based on canopy temperature presented different results. This is a recurrent problem in most studies that use data generated from thermal imaging for discrimination of plant responses to stresses.

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Validation of spectral and fluorescence sensors for soybean tolerance to drought in field conditions, in Brasilian Cerrado, using physiological parameters

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Keywords: productivity, drought, soybean, sensors.

Drought is one of the most limiting events in the yield of crops of agronomic interest and thus the efforts to select plants that increasingly tolerate these events are increasing. The use of hyperspectral reflectance image, fluorescence sensors and portable photosynthesis meters (IRGA), which measure parameters related to efficiency in the conversion of light to photoassimilates, are emphasized. Directly related to the soil cover and the productivity. In this way, this work aimed to evaluate the effect of drought on three soybean genotypes submitted to four water regimes and also, if there is correlation of productivity with data of hyperspectral reflectance image and fluorescence sensors, the data of gaseous exchanges obtained by IRGA. The study was conducted at Embrapa Cerrados, using soybean genotypes NA5909, BRS5980IPRO and BRS7280RR, which were submitted to four decreasing slides of water through the Line Source (modified) system. The experiment was carried out in a randomized block design in subdivided plots with three replicates, the main plot was the cultivar and the subplots the four water levels. Data were submitted to correlation analysis in the SPSS software. A strong (above 0.70) and positive correlation was obtained among the productivity and the data obtained by the hyperspectral sensor namely: NDVI, NDVI_I, MCTI and PRI and also with gas exchange parameters such as photosynthesis (A), stomatal conductance (gs) and transpiration (E). In this way, it was concluded that the decrease in crop productivity was correlated with the lower values of NDVI, NDVI_I, MCTI and PRI found by the hyperspectral sensor, which were more correlated to productivity than the values of A, gs and E, demonstrating that the parameters generated Sensors may better explain the observed increases or decreases in productivity.

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Embrapa, UnB, Instituto Federal Goiano

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Vegetation indexes for management of irrigated wine vine orchard

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Keywords: *Vitis vinifera L.*, NDVI, NDRE, CCCI, zone

Vegetation indexes help the evaluation of plant canopy. The objective of this work was to obtain different leaf coverage indexes of wine vine cv. Chardonnay/Paulsen 1103 (growing season January to May 2017), in a drip irrigated orchard in Espírito Santo do Pinhal, State of São Paulo, Brazil, to evaluate its spatial and temporal behavior, and to determine sites for measurement and sampling of plant attributes. The Crop Circle™ active terrestrial optical sensor was used to measure normalized difference vegetation index (NDVI), normalized difference red-edge (NDRE) and canopy chlorophyll content index (CCCI), with georeferenced walking in all plant rows. The active terrestrial optical sensor ClorofiLOG™ was used to measure relative total chlorophyll index (RTCI), every three plants (georeferenced) in alternating rows. Measurements were made from flowering to the beginning of maturation stages. Data were submitted to descriptive and geostatistical analysis to characterize the spatial dependence of the data and interpolation by ordinary kriging for the NDVI, NDRE and CCCI data, and simple kriging for the RTCI data. The interpolated data were submitted to similarity analysis using the Pearson correlation matrix. Two management zones (higher and lower NDVI and NDRE values) guided the sites for pre-dawn leaf water potential ΨL measurements (using a Scholander chamber), berry sampling for laboratory evaluation (weight, must volume, pH, soluble solids content, and malic acid concentration), counting of number of cluster per vine (NC) and measurement of yield per plant (Y). Higher similarity (> coefficient of correlation) was observed between RTCI and CCCI data on most measurement days. A difference ($p < 5\%$) between Ψ values from both zones were found in days with less soil water availability. More plant tissue water was found in higher NDVI and NDRE zones. Differences ($p < 1\%$) of NC and Y between both zones also occurred. Higher NDVI and NDRE zone presented higher NC and Y. The phenotyping using terrestrial optical sensors proved to be suitable and feasible for guidance of measuring and sampling sites in a vine orchard management based on precision agriculture.

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Validation of spectral and florescence sensors with growth parameters in sugar cane submitted to different water levels

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Keywords: Modified Line Source; Saccharum officinarum; yield.

The Brazilian Cerrado has two well defined seasons: a dry and a rainy seasons. During the dry period, the sugar cane receives only a 40 mm irrigation, called rescue irrigation. Drought tolerance and irrigation responsiveness are desirable attributes for selecting sugar cane genotypes in this region. Therefore, the objective of this work was to validate light induced fluorescence (LIFT) as a tool for phenotyping sugarcane and correlate it with plant growth parameters, under four water depths. The experiment was carried out at Embrapa Cerrados, Planaltina – DF, in three randomized blocks, with a split-plot design. The plots were composed of six genotypes of sugarcane and the subplots by four water depths. The irrigation system was performed by sprinklers coupled to a linear bar, drawn by a spool with sprinklers with four flow rates. The optimum irrigation level was based on 100% of the replacement of evapotranspiration for cane in the Cerrado region. The sensor measurements were fluorescence, measured by the Light Induced Fluorescence Transient (LIFT) instrument, from which the photosynthetic efficiency of Photosystem (Fv'/Fm') was calculated and spectral indices (NDVI and PRI) from the LIFT built-in spectrometer. There was a significant difference among sugar cane genotypes at all irrigation levels, for all measurements evaluated (NDVI, PRI and Fv'/Fm'. Also, there was a significant correlation among NDVI, PRI and Fv'/Fm' with the mean stem weight. In this way, the LIFT can be used as a tool for selecting genotypes more tolerant to water stress.

Use of thermographic sensors to determine the water status of plants in a controlled environment

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Keywords: greenhouse, thermal camera, leaf temperature

The foliar temperature of plants, relative to ambient temperature, can be used to infer the water status of plants, as can be seen in [1]. Both in the field (crops) and in greenhouse, infrared temperature sensors can be used or even thermal cameras to measure leaf temperature without contact with plants. For the case of the greenhouse, Embrapa Rice and Beans created a system, called SITIS (Integrated System of Induced Treatment of Stress). This platform automates the planning and execution of plant irrigation in a greenhouse. One of the variables that this system provides is the water status of the plant. In addition to the leaf temperature sensor MLX90614, which gets punctual data, there is another way to infer about the water status of the plant, which is low-cost thermal camera measurement. It is a Flir Lepton 3 long-wave infrared imager camera integrated with a minicomputer (Toradex Colibri or Apalis, Raspberry Pi, etc.) to produce thermal imaging of crops. The long-wave infrared sensor can detect thermal radiation emitted by objects near the ambient temperature and then determine its temperature. The acquired data is sent through the communication of the serial peripheral interface (SPI) to the computer and with this data a false color image of 160 x 120 pixels is created. With the thermal images generated, the state of the plants can be evaluated. This technique has major advantages because it is a non-invasive, non-contact and non-destructive way of determining the water conditions of crops. This camera can evaluate at least 8 different plants simultaneously and with various temperature values throughout the plant.

Acknowledgments:
To Embrapa Rice and Beans and Universat (universat.com.br) by the resources made available.

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Prediction of sun damage in Granny Smith apple fruit

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Keywords: spectroradiometry, pigment concentration, water potential

Granny Smith apple fruits with medium to severe sunburn are discarded for fresh fruit market, and those with mild burns evolve negatively in cold storage. Sunburn causes changes in pigment concentration, water potential and compatible solutes. Measurements of these variables are destructive, expensive and require a lot of time. Quick and non-destructive measurement of these variables would be a valuable tool to discriminate fruits prior to packing. This study aims to help on early determination of mild burning as a useful tool to improve fruit quality.

In Southern Uruguay (-34.63 S, -56.66 W) sun exposed fruits of adult plants were tagged, with three different initial conditions: incipient sunburn, without sunburn, and with red coloration on their exposed face. At 91, 99 and 154 days after full bloom, measurements of reflectance of the exposed face were performed, estimating Chlorophyll, Anthocyanins and Carotenoids values and calculating $PSRI_{500}$ (Plant senescence reflectance index). Fruits with similar condition were collected to determine tissue water potential (Ψ) and content of proline, chlorophylls, anthocyanins and carotenoids by spectrophotometry.

Significant differences among sampling dates and initial condition of the fruit were found for six of nine response variables. $PSRI_{500}$ differed only for initial fruit condition and anthocyanin reflectance index and carotenoids, only differed for evaluation date.

The correlation between proline and PSRI500, and proline and Ψ were higher than correlation between pigment concentrations values obtained by extraction and those calculated by spectroradiometry indices (rPro_PSRI = -0.41 r_Pro Ψ = -0.65). The determination coefficient for the linear regression model for Proline versus Ψ was 0.4, allowing an early and rapid assessment of fruit by non-destructive methods.

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Organic systems in Goiânia, GO

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Keywords: Zeas mays, cultivars, rizotrons, root mass, shoot mass.

Popcorn corn has a reduced participation in the volume of maize crop yield, but it is of nutritional and sociocultural significance. Phenotyping technique can be applied to select creole varieties suitable for organic family farming system. The present work aimed to evaluate the phenotypic characteristics of creole popcorn maize for agro-ecological systems in Goiânia, Goiás. Three seeds of popcorn corn were sown in standard rhizotrons, German Research Centre of Jülich, Jülich, Germany [1]. The cultivars of popcorn maize (MP) were four creole popcorn (CRV-red, CRA-yellow, CRB-white and CRP-black) and two conventional ones (both yellow, CO1 and CO2). Two plants per rhizotron were grown. Samples were taken at four sampling times of 14, 21, 28 and 35 days after planting. The stem diameter and the plant height were measured with a digital caliper and anthropometric measurement, respectively. Later, the plants were cut and evaluated for green shoot and root mass (fresh weight). After drying at constant weight under 65°C the dry mass of the shoot and root were weighted. These data (Rhizotrons) were compared with the field experiments of an organic farming area of the Agronomy of UFG, which is certified by IBD. Data were submitted to analysis of variance by SAS software (GLM and univariate procedures). Experiments conducted of phenotyping in rhizotrons indicated high potential discrimination of phytomass production with strong correlation with the field experiments. The cultivars MP-CRB-white, MP-CRP-black (Creoles) presented the best results even better than MP-CO1 (conventional). The cultivars MP-CRA-yellow and MP-CRV-red (Creoles) showed intermediate performance. MP-CO2 (conventional) presented the worst performance and was eliminated from the selection program. The phenotypic variables in the rhizotrons evaluation of plant growth in the field more quickly and economically.

Acknowledgments:
CNPq, CAPES, Biomass and Biogas Laboratory/UFG-IFG

Reference:
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Novel Non-Invasive Technologies

Keynote

Prof. Dr. Ulrich Schurr
Forschungszentrum Juelich / Diretor do IBG-2: Plant Science, Juelich, NRW, Alemanha

Title:
State of the art phenotyping: New windows to crops as the basis for better understanding and improvement of crops

Keynote

Prof. Dr. Jörg-Peter Schnitzler
Helmholtz Zentrum München, Institute of Biochemical Plant Pathology,Research Unit Environmental Simulation (EUS), D-85764, Neuherberg, Alemanha

Title:
Volatile organic compounds as non-invasive markers for plant phentoyping

Plants emit a great variety of volatile organic compounds (VOCs) that play an important role in atmospheric chemistry. Furthermore, these compounds actively participate in the plant growth and their protection against biotic and abiotic stresses. VOC emissions are extremely dependent on environmental conditions; a biggest ambiguity is whether the change in climate will influence plant-pest interactions that are mediated by VOCs or not. The constitutive and induced emission patterns between genotypes and species are highly variable and can be used as pheno(chemo)typic markers to distinguish between different origins and proveniences. In recent years significant progress has been made in molecular and genetic plant breeding. However, we actually have a lack of knowledge in understanding about genes and phenotypes, particularly in analyses of plant-environment interactions. Plant phenotyping, the assessment of complex plant traits such as growth, development, tolerance, resistance, etc., has become a major bottleneck and quantitative information on genotype-environment relations is the key to address major future challenges. With increasing demand to support and accelerate progress in breeding for novel traits, the plant research community faces the need to accurately measure increasingly large numbers of plants and plant parameters. This presentation we will focus on the technological challenge in developing a VOC phenotyping and demonstrates the usefulness of VOC phenotyping as fast and non-invasive measure of phenotypic dynamics.

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Invited Speakers

Prof. Dr. Gustavo A. Lobos
Plant Breeding and Phenomic Center, Universidad de Talca, Talca, Chile.

Title:

Assessing wheat traits by spectral reflectance: do we really need to focus on predicted trait-values or directly identify the elite genotypes group?

Phenotyping, via remote and proximal sensing techniques, of the agronomic and physiological traits associated with yield potential and drought adaptation could contribute to improvements in breeding programs. In the present study, 384 genotypes of wheat (Triticum aestivum L.) were tested under fully irrigated (FI) and water stress (WS) conditions. The following traits were evaluated and assessed via spectral reflectance: grain yield (GY), spikes per square meter (SM2), kernels per spike (KPS), thousand-kernel weight (TKW), chlorophyll content (SPAD), stem water soluble carbohydrate concentration and content (WSC and WSCC, respectively), carbon isotope discrimination (Δ13C), and leaf area index (LAI). The performances of spectral reflectance indices (SRIs), four regression algorithms (PCR, PLSR, ridge regression RR, and SVR), and three classification methods (PCA-LDA, PLS-DA, and kNN) were evaluated for the prediction of each trait. For the classification approaches, two classes were established for each trait: the lower 80% of the trait variability range (Class 1) and the remaining 20% (Class 2 or elite genotypes). Both the SRIs and regression methods performed better when data from FI and WS were combined. The traits that were best estimated by SRIs and regression methods were GY and Δ13C. For most traits and conditions, the estimations provided by RR and SVR were the same, or better than, those provided by the SRIs. PLS-DA showed the best performance among the categorical methods and, unlike the SRI and regression models, most traits were relatively well classified within a specific hydric condition (FI or WS), proving that classification approach is an effective tool to be explored in future studies related to genotype selection.

Invited Speakers

Prof. Dr. Odemir Martinez Bruno
IFSC / USP – São Carlos (SP), BRAZIL

Title:
Chaos Theory and Nonlinear Science: A New Approach to Data Analysis Applied in Plant Phenotyping

Exploring soybean traits through multivariate analysis in contrasting rust environments

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Keywords: Glycine max, P. pachyrhizi, mixed models, high-throughput phenotyping.

Soybean has a key role in the agricultural economy worldwide. Its currently most devastating disease is Asian rust that is already widespread in almost every producing country around the globe. This research aimed to verify changes in associations between soybean traits in contrasting rust environments. Four similar experiments were carried out using a randomized block design with three replications of 19 genotypes in two contrasting sites (soil types). In each site, we executed two (protected and unprotected) side by side experiments contrasting only for the fungicides used to manage the disease. This strategy allowed us to estimate the rust effect of each genotype on eleven key traits per plot (see Fig.1). We applied the SmartGrain software for high-throughput phenotyping of seed shape using digital images [1]. The collected data for each experiment was first analyzed fitting a linear mixed model using the lme4 R package, assuming block as random effect and genotype as fixed. Them, we computed the blue best linear unbiased estimators for each genotype. The multivariate analysis was performed in R. In general, soybean seed traits were positively associated with protected experiments in both sites. We noticed a statistical evidence suggesting that high grain yield (GY) is associated with longer seeds than spherical ones (negative correlation with circularity). Finally, agronomic value appears to be effective in ranking the genotypes in terms of GY.

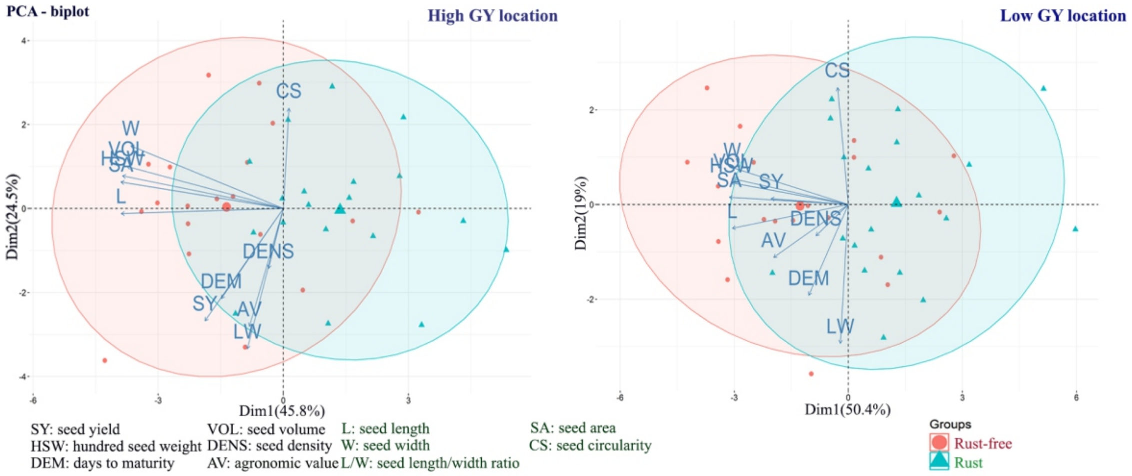

Fig.1 - Biplots of the principal component analysis.

Acknowledgments:
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Monitoring device of the health conditions of the plants using the photoacoustic detection by RGB LED lighting

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Keywords: photoacoustic, photosynthesis.

The photoacoustic (PA) effect occurs when the modulated light impinges on the sample that absorbs it and converts in heat and generates a pressure wave or sound detected by a microphone or by a piezoelectric element. The advantages of this technique are: to study since optically opaque to highly absorbing samples; to provide thermic information related to sample; the variation in temperature on the surface of the sample is about 10^{-4} K. The PA signal is directly proportional the absorption coefficient of the samples. The PA has been applied several in scientific research fields. In the vegetable study [1], PA has been used as tools to understand the photosynthesis systems [2], loss of energy, photosynthetic saturation effect, diseases, stress conditions and others. This work presents a small PA cell, which function is to detect the health conditions of plants due to the loss or excess nutrients and water or pest attacks. The sample is a leaf that someone wants to examine it. The modulated frequency of the light is defined between 5 to 10 Hz. The PA spectra show intense red band due to chlorophyll, low intensity in green region and increase in the blue region. Comparatively to spectral intensity in green region, the band amplitudes in three wavelengths must to provide information about the health conditions of the plants.

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Low-cost thermal imager for plant phenotyping

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Keywords: Thermal Images, Lepton, Raspberry Pi, Low-Cost.

This work uses a Flir Lepton 3 longwave infrared imager integrated with a Raspberry Pi computer to produce thermal images of crops. The longwave infrared sensor can detect the thermal radiation emitted by objects near room temperature and then determine its temperature. The data acquired is sent through Serial Peripheral Interface (SPI) communication to the computer and with this data a false-color image of 160x120 pixels is created. With the generated thermal images the status of the plants can be evaluated and it may be possible to predict drought or salt stress, for example. This technique has great advantages because it is a non-invasive, non-contact and non-destructive way of determine the quality of the crops. Another advantage is its low-cost and its small size, the hardware of the project costs less than $ 400,00 and it is composed by a Flir Lepton 3 that measures 10.5 x 11.7 x 6.4 mm, a Raspberry Pi model 2B with 85.60 x 56 x 21 mm and a Flir Lepton Breakout Board to integrate them.

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Collecting physiological parameters in field conditions with a dedicated multispectral camera

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Plant phenotyping in field conditions is often limited by the low throughput of the invasive measurements, traditional methods are also labor-intensive. Commercial availability of unmanned aerial vehicle (UAV) have opened new opportunities to accelerate breeding efficiency. However, there is still a need of research to improve the accuracy of the evaluated phenotypical traits. The AIRPHEN multispectral camera was specifically designed for accessing canopy biophysical variables from UAV observations within field phenotyping experiments. The camera weigh about 200 g, records the GPS and IMU information and allows triggering companion devices including a thermal infrared or a RGB high resolution camera. Six narrow wavebands selected to sample the chlorophyll specific absorption coefficient are recording the reflected radiation with a 1280x960 pixels resolution. The 8mm focal length provides resolutions at the ground level from few millimeters to few centimeters depending on flight altitude. One of the 6 cameras can be equipped with a 4.2 mm focal length lens providing an increased throughput allowed by the larger swath of the camera in this band.

An associated pipeline called 'phenoscript', was developed to process the raw images of the AIRPHEN camera and extract the microplots from the original images. The extracts can be later used to compute vegetation indices, and derive few biophysical variables.

The exploitation of the AIRPHEN camera with two different approaches were benchmarked: an empirical approaches and a radiative transfer model inversion. The results are presented over several experiments conducted on a range of species including wheat, maize and sugar beet. The accuracy of estimates of some biophysical variables including the leaf area index (LAI), the green fraction (GF), the Green Area Index (GAI) and the fraction of intercepted radiation (FIPAR) content is discussed. The capacity to combine the multispectral camera with thermal infrared imagery is also illustrated for the characterization of water stress.

Monitoring of evapotranspirated plant water in the SITIS Platform of Plant Phenotyping for Drought Tolerance

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Keywords: phenotyping, tolerance to water deficiency, greenhouse.

In the development of new cultivars that are more tolerant to water deficiency, it has become important to identify plants that consume less water during their life cycle and are able to uptake water from deeper soil layers. For phenotyping of populations and germplasm in water use, a greenhouse system was developed [1] where each plant is grown in a stationary soil column with up to approximately 0.049 m^3 of soil (up to 5 PVC rings, 25 cm in diameter and 20 cm high each). Each soil column is placed on a digital scale with a capacity of 100 kg and a precision of 10 g. In turn, each scale is connected to an electronic module capable of requesting and storing the mass at any desired moment. Thus, by the difference in mass of the soil column at two different instants of time, it is possible to determine the amount of water evapotranspirated. If it is desired to reduce the soil evaporation and obtain the plant transpiration value, it is possible to cover the soil with impermeable material, so that the evaporated water condenses and returns to the soil column. The system allows scheduling of monitoring either at predefined times or at regular intervals, or the combination between them. With this, it is possible to construct curves of plant water use daily, monthly or according to their phenological stages. Currently, the automated system is in the development and the process using the scale and manual irrigation was validated for rice, beans, soybean and cotton crops. In rice, for example, each unit of increase in water stress, from 100% to 20% of evapotranspiration replacement, reduced cumulative evapotranspiration of 0.57 L in 'Douradão' (more tolerant) and of 0.67 L in 'BRS Soberana' (more susceptible).

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Use of a capacitive sensor in the SITIS Phenotyping Platform for the automated determination of the effective area of the root system to uptake water

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Keywords: volumetric moisture, soil column, greenhouse.

The best performance of upland rice in front of the water deficiency may be associated with several parameters [1]. Among them, those associated with the plant's greater ability to uptake water in the deeper soil layers seem to be the most important when the drought is short and there is good availability of water stored in the deeper soil layers. Deeper and more branched root systems contribute to better utilization of soil water. In addition, primary roots with larger diameter have thicker xylem vessels, which offer less resistance to water flow. All these characteristics are associated with the effectiveness of the root system in uptaking water, which is better the bigger it is in the deeper soil layers. In this direction, the total use of water by the root system was determined using capacitive sensors conditioned in soil columns made in PVC pipes 25 cm in diameter and 100 cm in height. The following equation was used to it:

$$\sum_{i=1}^{n} \Delta\,(\theta \mathrm{v}_j - \theta \mathrm{v}_{j-1}),$$

Where, i = soil layer, j = time and θv = volumetric soil moisture. The percentage of individual water use of 20 cm layers, from the surface to 100 cm depth, is determined by the use of each layer, θvj-θvj-1, in relation to the total use. For the monitoring of soil volumetric moisture, a basic version of the SITIS Platform of Plant Phenotyping for Drought Tolerance at Embrapa Rice and Beans is used, with FDR sensors [2] to measure the soil moisture.

Acknowledgments:
To the DREBCROPS, SecaCereal and Dryce Projects for the resources made available.

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SITIS – Plant Phenotyping Platform

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Keywords: drought stress, soil moisture, monitoring, irrigation, climatic variables.

Plant phenotyping in greenhouse requires high physical effort for management and data collection [1, 2]. To minimize this effort, SITIS Platform of Plant Phenotyping for Drought Tolerance was projected and built by Embrapa Rice and Beans to automate the management and data collections in greenhouse and consists of 384 columns of soil (diameter: 25 cm; height: 100 cm) placed on an digital scale with individualized monitoring of the water supply. SITIS is composed by two integrated modules: 1) SITIS Web – responsible by the planning and supervision of the environment variables, irrigation during the experiment and recording the results. The monitored variables are: soil moisture until it reached five depth layers (20, 40, 60, 80 and 100 cm), evapotranspiration and climatic conditions: temperature, relative air humidity and solar radiation; 2) SITIS Embedded – responsible for the monitoring and actions of water supply, it uses an embedded system connected to an digital scale with capacity of up 100 kg and accuracy of 10 g, capacitive sensors of soil moisture, solenoid valve connected to an hydraulic system and sensors of air temperature, air relative humidity and solar radiation. This automated SITIS platform will allow reducing the human effort and will improve the quality of the data collected.

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3-Demeter: an affordable 3-D phenotyping software

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Keywords: 3-D phenotyping, affordable phenotyping, software, stereo vision.

3-D models are an useful representation for plant phenotyping. Different traits can be extracted from 3-D models of shoots and roots, and such models can be stored for further analysis and for the computation of new or unplanned traits. Occlusions and volumetric information are better handled by the 3-D approach. However, the construction of 3-D models can involve expensive sensors and/or precise automation. An affordable alternative is to employ multiple-view stereo vision for building 3-D point clouds from RGB images captured by an ordinary camera, and exchanging expensive precision positioning systems by visual odometry (camera pose tracking) [1]. This work introduces 3-Demeter Capture, a software application that combines visual odometry and multiple-view stereo components to produce dense 3-D points clouds from images captured by an ordinary high-definition webcam. Using a printed plane board with markers of known geometry, the application is able to transform the 3-D model to standard scale and reference frame, allowing millimeter precision measurements. The application uses a robust SLAM system for visual odometry [2] that allows free movement of the camera and automatic re-localization in case of tracking failure. Such flexibility allows the 3-D modeling of plant shoots for different species in different developmental stages. The software was employed to produce point clouds for potted specimens of maize, sunflower, wheat and dendê (*Elaeis guineensis*) in greenhouses and growing chambers and for vines in field.

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Color correction method applied to plant phenotyping

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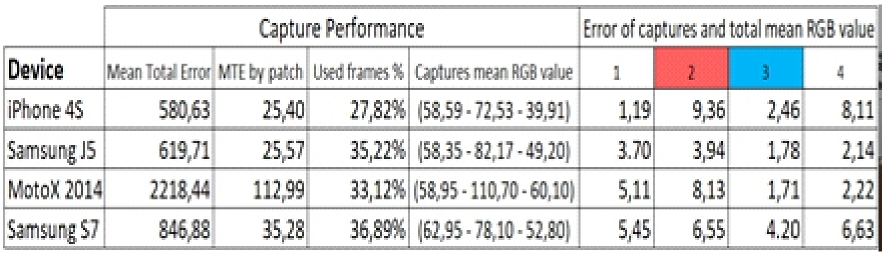
Keywords: plant phenotyping, camera calibration, color correction.

Large agricultural producers have become more interested in big data analytics and the development of decision aid systems for production planning. Thus, the importance of algorithms and automatic capture methods for collecting crop data has grown. Most systems designed to this day resort to different types of cameras and sensors in an effort to capture distinct types of data for the phenotyping process. Also, one important tool for plant phenotyping is the analysis of the crop's color characteristics [1]. This paper presents a color correction method that improves color constancy of any consumer grade camera in several different capture conditions, lowering the costs of designing such systems.

The method uses a color target with known values in the image and estimates a transform function between the captured samples and the target values using linear regression. Although the method is largely based on [2], approximately 300 frames are captured and sampled instead of a single image, in order to estimate statistical parameters and improve performance by removing outliers. This has greatly improved performance in environments or devices with artificial lights due to their more unstable nature.



	Capture Performance				Error of captures and total mean RGB value			
Device	Mean Total Error	MTE by patch	Used frames %	Captures mean RGB value	1	2	3	4
iPhone 4S	580,63	25,40	27,82%	(58,59 - 72,53 - 39,91)	1,19	9,36	2,46	8,11
Samsung J5	619,71	25,57	35,22%	(58,35 - 82,17 - 49,20)	3.70	3,94	1,78	2,14
MotoX 2014	2218,44	112,99	33,12%	(58,95 - 110,70 - 60,10)	5,11	8,13	1,71	2,22
Samsung S7	846,88	35,28	36,89%	(62,95 - 78,10 - 52,80)	5,45	6,55	4.20	6,63

Fig. 1 - Capture performance, error measures and capture setup

Validation was performed by capturing and correcting images of a plant in 4 different devices and 4 different conditions (1 - indoor without flash, 2 - indoor with flash, 3 - outdoor at noon and 4 - outdoor late afternoon), averaging RGB values in different regions of interest of the plant and measuring their distance to target values and the average RGB values of all captures. Results shown in Figure 1 show that the values obtained after correction are similar enough between the different capture conditions, ensuring more consistency of the data captured for analysis.

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An open source tool for automated phenotyping of seed germination through image analysis

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Keywords: Seed, germination, image analysis, open source.

The objective of this work was to develop a system that allows the quantification of the germination of seeds of different species by means of image analysis. Unlike existing closed-source tools (e.g., [1]), we developed a system based on open source technologies, which allows not only its free use but also its modification for adaptation to other species or experimental systems, as well as its improvement or expansion. We also aimed at detecting germination through changes in the geometry of the seed, rather than color changes, so as not to be limited to dark-colored seeds only. The software source code and hardware design are available at [2].

The experimental device consists of a light box in which a Petri dish is placed with the seeds to be evaluated, sown on blue germinating paper. Two versions of this box were built: i) one with a fixed digital camera, driven automatically by an Arduino board, which is useful for time-lapse imaging of a single sample, and ii) one in which samples can be easily swapped, and images are captured with a smartphone, with an optional app for automatic image tagging. The images obtained are then processed automatically using a protocol written for the image analysis program ImageJ, through which the germinated seeds are identified based on geometric parameters.

This system was tested by acquiring serial images of wheat, rice, and agropyron seeds, being able to satisfactorily quantify germination parameters such as rate and duration of phases, uniformity and final percentage of germinated seeds.

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Open tools for plant phenotyping

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Keywords: Open source, software, hardware.

Technology is not neutral. This also applies to scientific tools, which play an essential role in research and can even be one of the main drivers in some scientific fields (with Genomics and Phenomics as clear examples). But what values are embedded in the tools used by scientists? High-tech, complex, high-quality, prestigious instruments are what scientists and science institutions usually seek.

Science is a community endeavor, which relies on a set of social norms that define a scientist's (ideal) behavior, which Robert Merton structured into four principles: communality (common ownership of knowledge), universalism (everyone capable of contributing), disinterestedness (not for personal gain), and organized skepticism (subject to critical scrutiny and testing). What if we could have scientific tools that represented those principles?

The number of open source tools being developed is rapidly increasing in most scientific fields, and plant and crop phenotyping is no exception. There are many good examples, including sensors (e.g., the MultispeQ multispectral sensor [1]), platforms (e.g. aerial vehicles for phenotyping based on open-source hardware and software [2]), and image analysis software (e.g. for automatic measurement of germination parameters [3]).

Besides being able to freely download them or build them, these tools allow scientists to make modifications and adaptations to specific use cases, instead of being only recipients of the tools delivered by the market. Also, open source tools are not “black boxes”, and the specific protocols, alogorithms and processes in which they are based are known and subject to evaluation and peer review. Future work should include not only the development of new tools, but also their evaluation and benchmarking against well-established commercial counterparts.

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Non-invasive measurement of soil water, using Time Domain Reflectometry (TDR) to study roots growth

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Keywords: Non-invasive technique, TDR, soil-water-root, rhizobox

New tools and approaches are needed to evaluate soil-water-plant interactions for crop phenotyping. Plants can affect soil moisture distribution and the soil hydraulic properties, both directly by root water uptake and indirectly by modifying the soil structure [1]. A variety of instruments may be used to measure soil moisture; including microwave transmittance and microstrip patch antennas, as a non-invasive method [2], and invasive devices as for example Time Domain Reflectometry (TDR) or tensiometers, based on gypsum blocks or ceramic plates. However, these instruments must be inserted as measuring devices, and thus may interfere with soil conditions and plant growth reducing the analytical power of the experimental results. This study evaluated the performance of a probe consisting of two planar electrodes, developed for TDR measurements, made with circuit board. A rhizobox was built from Plexiglas, to investigated root growing and soil moisture. The rhizobox was filled with a *Typic Haplorthox (Rhodic Ferralsol)* soil with bulk density in the rhizobox varying from 1.09 to 1.20 g.cm^{-3} and two maize (*Zea mays* L.) seeds. TDR measurements were carried out using cable tester Tektronics 1502 C. The experiment was conducted during 42 days in laboratory conditions and the dielectric constant (εr) of the environment (soil-water-root), room temperature (°C) and air relative humidity (%) were recorded. Preliminary conclusions of this experiment indicate the possibility to observe the effect on the hydraulic properties, with time, during roots growing, using the soil as a baseline. Results presented in this study are promising and shows that TDR can be used as a non-invasive technique to assess root growth. Further measurements will be conducted to evaluate the 2-D distribution of the water content in the rhizobox with and without roots, the effect of bulk soil density, as well a development new probes configurations.

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A selective VOC biosensor based on microcantilever functionalized with enzyme. Potential application on plant investigations

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Keywords: Biosensor, Microcantilever, VOC,

In nature, the detection of volatile organic compound (VOCs) can signal plant immunity, with potential application in plant phenotyping [1]. In this context, this work describes the development of a biosensor based on the immobilization of alcohol dehydrogenase enzyme, employing self-assembled monolayer (SAM) arrays in a microcantilever of Si_3N_4 for detection of volatile short-chain alcohols. The frequency response of the microcantilever during excitation was measured using a Veeco V Dimension equipment. Silicon nitride microcantilevers were acquired from NT-MDT. All chemicals and buffer components were used as packaged received. Alcohol dehydrogenase from *Saccharomyces cerevisiae* A7011, lyophilized powder ≥ 300 units/mg protein, MW 141-151 kDa, was used as active biomolecule. The activation process was characterized by X-ray Photoelectron Spectroscopy (XPS). The calculation of the functional layer thickness was performed through AFM. To verify the selectivity of the biosensors, tests were carried out with controlled concentrations of different volatile short-chain alcohols, analyzing the resonance frequency response. The device can operate in air or, allowing the estimate of short-chain alcohols concentration. Repeatability tests revealed similar responses after 15 repetitions, with a shelf life around 432 hours. This biosensor can also operate in almost any conditions of relative humidity with optimum temperature around 25 ± 3° C and pH 8.6. This technique opens a new potentiality to exploit measurements about VOC´s in plants, using nanosensors. Further studies will be conducted with the aim of developing a biosensor for the detection of VOCs in conditions of plant disease and drought stress indicators.

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Non-destructive Fluorescence Spectroscopy as a phenotyping technique in soybeans

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Keywords: soybean, non-destructive tool, laser spectroscopy, fluorescence, phenotyping.

This work describes the results obtained with Laser-Induced Fluorescence Spectroscopy (LIFS) and Laser-Induced Fluorescence Images (LIFI) associated with chemometrics methods, as an efficient tool for soybean cultivars characterization. Each cultivar has a specific concentration of multiple fluorophores, which alters the characteristic fluorescence spectra of the leaves in a way that can be successfully identified by this optical technique Leaves of three different soybean cultivars, TMG1188RR, TMG4182 and M9144RR, from Parnaiba, Xingu I and Xingu II, respectively, located in Maranhão state, Northeast region of Brazil were tested in this work. Soil management, irrigation, fertilization, temperature were adequate for a correct plant growing. All leaves were collected from a region with low infestation of diseases and pests and their spectral profile are specific for the plant. Only fresh leaves were used. The portable LIFS system used in this work has an excitation wavelength at 405 nm and a high sensitivity mini-spectrometer (USB4000) with spectral range of 194–894 nm. The LIFI system is composed by a laser with excitation wavelength at 375nm and the fluorescence emission is detected by a Sony alfa 55 camera. From each image, 38 attribute per pixel were extracted. The discrimination of the soybean varieties was performed by a classifier based on the combination of classification via regression and partial least square regression using cross validation, employing the fluorescence emission spectra obtained with LIFS system and the attribute extracted from the image system for LIFS and LIFI, respectively. As result, two confusion matrices were obtained with an average accuracy for correct classification of 96.26% and 85% of instances, respectively.

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Laser Induced Breakdown Spectroscopy as Analytical Tool for soybeans cultivars discrimination

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Keywords: soybean, non-destructive tool, laser spectroscopy, ablation, phenotyping.

The aim of this work was to introduce laser-induced breakdown spectroscopy (LIBS) as a new tool for soybean variety identification measuring the variations of elemental concentration in its leaves, since different cultivars has different elements uptake from soil, resulting in different elemental concentration in the leaves. LIBS performs elemental analysis by measuring the spectral emissions of the analyte in the plasma, with no or very limited sample preparation, avoiding the use of chemical reagents, in a rapidly way, allowing large-scale and real-time monitoring providing data featuring high sensitivity and reliability. The main objective of this work was to show that LIBS associated with chemometric methods could represent a new promising potential economically viable tool for the identification and discrimination of soybean variety It was used three different soybean cultivars leaves, TMG1188RR, TMG4182 and M9144RR from Parnaiba, Xingu I and Xingu II, respectively, located in Maranhão state, Northeast region of Brazil. Soil management, irrigation, fertilization, temperature were adequate for a correct plant growing. All leaves were collected from a region with low infestation of diseases and pests and their spectral profile are specific for the plant. Only fresh leaves were used. The discrimination of the soybean varieties was performed by a classifier based on the combination of classification via regression and partial least square regression models using cross validation, employing the elemental concentrations measured by LIBS as input data. As result, a confusion matrices was obtained with an average accuracy for correct classification of 99,091% was obtained.

Acknowledgments:
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Fast Detection of Tan Spot and Powdery Mildew on Wheat using Deep Learning

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Keywords: GPU Computing, Image Processing, Wheat Leaf Diseases, Transfer Learning.

Several diseases affect the potential for wheat production, usually related to leaf disease area what limit the whole plant development. Our work makes use of deep learning procedures to train a neural network to predict the occurrence of leaf diseases in the field with a fast and good accuracy. Digital images of tan spot and powdery mildew symptomatic and non-symptomatic wheat leaves were collected at Embrapa Wheat using a standard RBG digital camera. All processing and classification are done using a workstation with GPU support, TensorFlow, and Python. For the neural network architecture, we choose a convolutional neural network (CNN), but this procedure is time and computational consuming what could restrict the use of this novel technologies in areas other than applied computation. To cut off this, we adopt the transfer learning (TL) methodology which utilizes a pre-trained neural network, and allows the detachment of outer the classification layer and uses the remains structure to retraining and get new weights for the new image set and classes of interest. We use the output from [1] as a pre-trained neural network. During the training were used 25,884 arranged into four categories: healthy, dry, tan spot and powdery mildew. We could share our positive experience in using the transfer learning for an entirely new set of images and obtain a good overall accuracy. The validation set presents a total of 4.4% of misclassification. The most common error occurs between tan spot and powdery mildew with an index of 1.4% and 1.3%, respectively. In conclusion, the use of transfer learning for deep neural network cut down the overall time, effort and resources necessary to utilize this cutting-edge technology from artificial intelligence applied to fast non-invasive diagnosis of wheat leaf diseases with an overall accuracy of 95.6%.

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Image analysis for measuring pod dimensions in vegetable soybean

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Keywords: Glycine max, ImageJ, Plant phenotyping

Food-type soybean is used for human consumption as a vegetable, and additionally to other traits, it must have large pods and seeds. Furthermore, large seeded genotypes are associated with a better flavor for fresh consumption. In order to obtain high quality soybean, a breeding program needs to implement an efficient procedure to analyze pod and seed size to select the most prominent genotypes. Pod dimensions usually are measured using a caliper or in a visual evaluation, which are time consuming and prone to error. Here, we employed ImageJ [1] a software for image analysis to measure the pod length (PLR6) and width (PWR6) in the R6 stage. We also study the correlation between these two traits with one hundred seeds weight (HSW) which is an evaluation to select large seeded genotypes. An F3 population consisting of 21 progenies was evaluated in a randomized complete block design with four replicates. We performed an analysis of variance, a Scott-Knott test and a correlation study of the traits. The ANOVA showed that genotypes had a significant difference in all the traits evaluated and the Scott-Knott test formed two groups for PLR6, three for PWR6 and two for HSW. The traits exhibited a high correlation, confirming that the use of image analysis in the R6 stage may optimize the process of selection of large seeded soybean.

Table 1- Phenotypic (above diagonal) and genetic (below diagonal) correlation coefficients among three traits in soybean F3 progenies.

Trait	PLR6	PWR6	HSW
PLR6	-	.8694	.8809
PWR6	.8916	-	.9507
HSW	.9389	.9786	-

Acknowledgments:
CNPq for the financial support and Dr. G. Polder for developing the plugin used for our analysis.

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Portable NMR equipment applied to a non-invasive study of the water within the soil matrix

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Keywords: Portable NMR; CPMG signal; adsorption; soil – water matrix

The development of non-invasive instruments, and sensors to measure soil moisture distribution would open new approaches to investigate plant strategies to deal with low moisture and especially the most relevant heterogeneity of water availability in soils. A portable Nuclear Magnetic Resonance (NMR) Metter was used to determine and monitor the evolution of water in sand soil and glass-beads substrate after the equilibrium state. A home-built C-shaped magnet with a 13-turn solenoidal r.f and a field strength 0.26 T. coil, with an inner diameter of 15 mm, was used for excitation and detection [1]. The temperature was set to 25°C. For each data point a Carr-Purcell-Meiboom-Gill sequence (CPMG) type measurement was run, with a repetition time of 8.0 μs, 2000 echoes, an inter-echo time of 100μs, eight complex points per echo, 32 averages. The Larmor frequency was 10.227 MHz and a spectral width of 100 kHz. The total scan time for each point was 2 min. To this experiment the kaldenkirchen – magnetic sandy soil was used as a soil sample, with a bulk density 0.96 (g/ml) (73% sand, 23% silt, 3.6% clay and 0.25% iron) and a single batch soda lime glass beads 500 – 750 μm in diameter, with bulk density 1.51 (g/ml). The CPMG signal of the sandy soil and glass beads with water is linearly correlated with degree of moisture. In this investigation was possible to observe changes in the CPMG signal with the time and the amount of water. In the sandy soil, with volumetric soil moisture of 6.75%, the CPMG signal decrease after 12 min. This was compared with glass beads, in the same experimental condition. We conclude that there is a reduction of the signal that could be related to adsorption of water in the soil matrix. Further experiments will be conducted to evaluate the behavior in other kinds of substrates and how is the effect of the roots in the matrix.

Acknowledgments:
FZJ / IBG-2: Plant Science and Embrapa Labex Europa to financial support of project of common interest # 10200.13/0076-2

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Chlorophyll content measured directly and indirectly in high yield potential rice genotypes

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Keywords: SPAD, chlorophyll, Pearson correlation.

Chlorophyll (Chl) content in rice genotypes is one of the most important traits affecting photosynthetic rate. Several studies demonstrated that high yielding rice possessed high leaf Chl content. Methods used for Chl determination are mainly based on destructive methodology. The Soil Plant Analysis Development (SPAD-502, Minolta Camera Co., Osaka, Japan) provides a fast and non-destructive Chl measure content under field conditions. The aim of this work was to correlate the Chl content measured directly using DMSO and indirectly using SPAD in five high yield potential irrigated rice genotypes. The assay was conducted on 2016/2017 season at the experimental station of the Rio Grande do Sul Rice Research Institute (IRGA), Cachoeirinha/RS. Twelve flag leaves completely expanded were selected to each rice genotyping at the beginning of the reproductive stage for SPAD reading. Soon after the reading all leaves were collected for direct Chl determination using Hiscox [1] protocol. The dataset was analyzed using Tukey and Pearson correlations were carried out between direct and indirect Chl measurements. Statistical analyses were performed on Sisvar and Sigmaplot. SPAD values ranged from 31.5 to 34.2 and presented no statistical differences among genotypes. Chl a and carotenoids did not differ among the genotypes, while Chl b, Chl a/b ratio and total Chl differed significantly. Pearson correlation showed a value of 0.97 ($p<0.00524$) between SPAD and the total content of Chl. Followed by Chl b, which presented a correlation of 0.93 ($p>0.0195$) with SPAD. Based on these results, SPAD values were no able to discriminates statistically the genotyping. Although to suggest to be a good indicator of the total Chl content at the reproductive rice stage in Brazilian rice flooded genotypes.

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Phenotyping for Crop Improvement

Keynote

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Title:
Phenotyping for crop improvement in a diversity of climatic scenarios

The plant science community needs to design new genotypes able to cope with the diversity of 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. We have adopted a multi-scale multi-environment approach. In the field, it consists of (i) clustering time courses of environmental variables simulated by a crop model in 60 sites x 30 years in current and future conditions into six scenarios of temperature and water deficit; (ii) performing field experiments in contrasting environmental conditions across Europe with a panel of maize hybrids; (iii) assigning individual experiments to scenarios, based on environmental conditions measured in each field; (iv) analysing 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 have estimated (v) intercepted light and radiation use efficiency of each hybrid of the same panel via a functional-structural model using 3D reconstructions of each plant, (vi) stomatal conductance, estimated from transpiration via inversion of the Penman Monteith equation via joint analysis of 4 experiments with contrasting light, evaporative demand and soil water potential. As a whole, the combination of field and platform steps results in a dataset allowing one to identify genomic regions associated with tolerance in specific scenarios of heat and drought, and with traits associated to these genomic regions. Finally, models allow identifying geographical regions in which a given combination of alleles is likely to have comparative advantages.

Keynote

Dr Michael Schaefer and Dr. Xavier Sirault
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Australian Plant Phenomics Facility, The High Resolution Plant Phenomics Centre, CSIRO email: michael.schaefer@csiro.au

Title:
Automated Phenotyping and Analytics

Plant Phenotyping is key to understanding the physiological and genetic bases of plant growth and performance and their application for crop improvement. In Australia within the Australian Plant Phenomics Facility (APPF), to increase our capacity at measuring plant traits, a range of high-throughput phenotyping platforms have been engineered. These phenotyping platforms range from automated systems in controlled environments to ground-based or aerial vehicles (manned or unmanned) in the field. The commonality in all these platforms is the range of sensors that they integrate, ranging from visible imaging sensors (RGB) to imaging spectroscopy sensors or from thermal infrared (IR) sensors to Light Detection and Ranging (LiDAR) sensors. The availability of and access to these platforms is today transforming phenotyping at scales ranging from individual plots for breeding and physiological studies, to entire farms to characterize agro-eco-systems.

For analysis of the vast array of high-throughput phenotyping data, the Australian Plant Phenomics Facility's Collaborative e-infrastructure platform phenoSMART□ is utilised. This platform allows the user to easily extract real information and value from the data collected using phenotyping tools. The platform also serves as a base to allow computational tools developed by other research groups across Australia to become available to others and/or the Agro-business sector.

This keynote presentation will provide a background into some of the plant phenotyping work currently being performed in Australia utilising the services of the APPF's national infrastructure as well as an insight into the technology and techniques being used.

Invited Speakers

Prof. Dr. G. Habermann
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Title:
Aluminum stress in plants: an alternative view

Keywords: Al^{3+}, Al-accumulating species, acidic soil, biomimetism.

Although almost neglected by scientific community, we live in the age of aluminum (Al). It is the third most abundant element in the Earth's crust. Approximately 30-45% of soils from the world's ice-free land are acidic ($pH < 5.0$) and, under this condition Al^{3+} from the soil is toxic to most plant species. The reduction in root growth is the most conspicuous symptom in sensitive plants, including many herbaceous and woody crop plants. The Al-induced decrease in CO_2 assimilation and low aboveground plant growth are also reported as indirect symptoms. There are few crop plants tolerant to Al that exude organic acid to quelate Al in the rhizosphere and avoid its uptake. In addition, lime is used to neutralize acidity and reduce Al, but it costs US$350 ha^{-1} year^{-1}. On the other hand, there are Al-accumulating species that are able to concentrate more than 15000 mg Al per kg dry leaves, with no damage to their metabolism and growth. In the Cerrado vegetation in Brazil, Al-accumulating and non-accumulating species co-exist, growing on soils that are acidic ($pH < 4.0$) and extremely rich in Al. Unfortunately, there has been almost no effort in studying Al-accumulating (and non-accumulating) plants. Understanding their genome and metabolism could lead to different strategies for Al resistance in crop species.

Acknowledgments:
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Use of image phenotyping for screening Pyrenophora tritici-repentis resistance in wheat

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Keywords: Triticum aestivum L.; Tan spot disease; flag leaves.

Tan spot disease caused by *Pyrenophora tritici-repentis* is an important threat to wheat crops, causing significant economic losses in many areas where wheat is cultivated, especially in the southern Brazil, where nearly 90% of the national production is located. Genetic resistance is one of the best strategies to control the disease. Modern approaches to identify resistant genotypes would save time and resources for breeding new varieties. Image phenotyping based on percentage of leaf area covered with symptoms can be used as a simple tool for disease screening. The major objective of this study was to image-phenotype 30 wheat varieties representing more than 90% of the wheat area cultivated in Brazil. Image phenotyping was conducted in Passo Fundo, RS, in the experimental area of Embrapa Wheat, as a complete randomized design, with five replicates. The plants were artificially inoculated with one isolate of the pathogen, besides one mock inoculation as control. Disease was evaluated during booting stages, using flag leaves and flag leaves (-1). Images from each genotype were recorded and submitted to computational analysis. Diseased leaf area was calculated by AF Software, based on artificial neural network, developed by Embrapa Instrumentação Agropecuária, São Carlos, SP. The software was efficiently able to identify the different classes of symptoms, which were characterized by the presence of green (healthy), chlorotic and necrotic areas. From this segmentation, it was possible to classify two contrasting groups for resistance to Tan spot. Genotypes with the lowest area reduction due to the disease were: BRS Marcante, BRS Parrudo, Estrela Átria, LG Oro, TBIO Pioneiro, TEC Frontale and Topázio. Genotypes with the largest leaf area reduction due to the disease were: Esporão, LG Prisma, Mirante and TBIO Toruk. The approach reported here shows the potential application of Image-Phenotyping to screen disease resistant genotypes for Tan spot in a more precise way.

High Resolution Magic Angle Spinning and Solid-State NMR spectroscopy methods to explore the metabolome of soybean genotypes upon abiotic stress

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Direct analysis by NMR is ideally suited to high-throughput metabolite profiling applications and has the advantage of detecting a wide range of metabolites in an inherently quantitative and unbiased manner. In this work, we explored the utility of using the High Resolution Magic Angle Spinning (HR-MAS) and Solid-State NMR (SSNMR) techniques to identify metabolic changes in soybean tissues subjected to water-deficient conditions. Control and water-deficient soybean leaves were analysed using 1D HR-MAS and SSNMR and the NMR data were submitted to Partial Least Square Discriminant Analysis (PLS-DA). Total RNA was extracted from the leaves for the transcriptomic analysis. The ^{1}H HR-MAS and CP-MAS ^{13}C{^{1}H} spectra of soybean leaves grown with and without water deficiency stress revealed striking differences in metabolites. A total of thirty metabolites were identified, and the impact of water deficiency on the metabolite profile of soybean leaves was to induce amino acid synthesis. High expression levels of genes required for amino acid biosynthesis were highly correlated with the compounds identified by ^{1}H HR-MAS. The integration of the 1H HR-MAS and SSNMR spectrum with the transcriptomic data provided a complete picture of the major changes in the metabolic profile of soybeans in response to water deficiency.

High-throughput phenotyping as a tool for cotton breeding for drought tolerance

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Keywords: Gossypium hirsutum, water stress, genetic parameters, cotton improvement, breeding indicators

In most countries, cotton production relies on rainfall. The availability of water during development impacts on productivity but also on fiber quality, and the development of plant ideotypes capable of maintaining yield potential and fiber quality under limited water supply is highly desirable. Tolerance to water stress depends on the complex and specific interaction of a number of mechanisms whose morphological, physiological and genetic bases are still not well understood. Traits related to tolerance to water stress in cotton could be used as breeding indicators, and the knowledge of their genetic bases constitutes valuable resources for cotton breeders.

Cotton (*G. hirsutum* L.) accessions were evaluated under contrasting water regimes (80% and 30% of the soil capacity) on the PhenoArch phenotyping platform in Montpellier, France. The experimental design was an alpha lattice, with water regimes as sub-plots. Plants were grown on the platform during the vegetative phase of plant development for a total of 30 days, including a 17-days period of dry-down and a 7-days period of differential water regime. Morphological, physiological, and growth parameters were recorded.

For all measured parameters, significant differences ($p\text{-value} < 0.05$) were observed between genotypes and between water regimes, demonstrating that genetic variability for response to water stress exists within the panel. Genotype x Water Regime interactions were important for some of the parameters (ex. accumulated biomass, plant height, transpiration, WUE), pointing out to a differential response of plants to water stress. Parameters that were recorded daily (ex. biomass, leaf area, transpiration) allowed to adjust local regressions, and to identify those genotypes that were able to maintain their potential under water stress.

The PhenoArch platform proved to be an efficient tool to identify potential parental accessions in cotton breeding programs aimed at developing water stress tolerant varieties.

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Evaluating the effects of microorganism biostimulants in maize to improve plant growth

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Keywords: plant growth promoters, nutrient solution, humic substance, aminoacids

Biostimulants can be defined as substances, including microorganisms, that are applied to plant, seed, soil or other growing media that may enhance the plant's ability to assimilate applied nutrients or provide benefits to plant development. This study aimed to evaluate the effect of six microorganisms strains from the collection of multifunctional and phytopathogenic microorganisms of Embrapa Milho e Sorgo on maize growth. Maize seeds were surfaced sterilized and germinated for four days and then grown in a floating system for seven days with ½ Hoagland´s nutrient solution (pH 5.65). After the acclimatization period, the maize roots were incubated with the microorganism culture (10^7 colony-forming units (cfu)/ml in 0.85% saline solution) for six hours and then transferred to the nutrient solution for ten more days. The maize roots were photographed and the root traits were quantified with RootReader2D and WhinRhizo softwares. The root and shoot dry weight was obtained at 65 °C until constant weight. Five microorganisms increased surface area of roots with diameter between 0-1 mm. Two microorganisms increased total root surface area, total root length and total dry weight. Our results showed that this methodology allowed the identification of microorganism effects on maize growth and root morphology. Moreover indicated potential microorganisms to be further used as inoculants.

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Morphological characterization of two sorghum diversity panels grown in nutrient solution with low phosphorus

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Keywords: root, P-acquisition, paper pouch.

Phosphorus (P) is an essential macronutrient for plants and its acquisition relies on plant root system. In arable land, P is one of the most unavailable macronutrients and frequently limits plant growth, limiting the productivity of crop plants. Therefore, a better understanding of how plants respond to P starvation is necessary to breed crop varieties with enhanced P use efficiency. This study aimed to analyze root traits related to P acquisition efficiency in two sorghum panels, Sorghum Association Panel Subset (SAP), composed of both tropical convert and breeding accessions and the CIRAD Panel, totaling 479 genotypes. Sorghum seeds were germinated for four days on germination rolls and then placed in a paper pouch system with modified Magnavaca nutrient solution (P - 2.5 mM) in controlled conditions. After 13 days, root images were captured using a digital photography setup and analyzed using both RootReader2D program and WinRHIZO software. Root and shoot were dried separately at 65 °C in a forced-air oven until constant weight was obtained. Total root length was highly correlated with all traits, except with root diameter and root:shoot. These suggests that greater root area combined with smaller root diameter contributed to enhanced biomass accumulation. Low coefficient of variation (6.0 - 25.2%) and medium to high heritability (31.6 - 82.1%) was found for all root traits analyzed (total root length, average root diameter, total root volume and volume of fine roots) in both panels. Principal Component Analysis (PCA) was able to differentiate contrasting sorghum lines based in the selected root traits (total root length, average root diameter, total root volume and volume of fine roots) and dry weight (root, shoot, total and root:shoot). The first principal component (PC1) explained 61% while the second principal component (PC2) explained 32% of the phenotypic variation. These data will give support to association analysis in order to identify candidate genes related to root morphology and P acquisition efficiency in sorghum.

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Correlations between reflectance indices and seed yield in contrasting Asian soybean rust managements

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Keywords: Glycine max, NDVI, NIR, Phakopsora pachyrhizi.

The vegetation indices are useful for predicting yield and monitoring diseases [1], being applicable to evaluate productivity and tolerance in soybean. In this research, 12 soybean inbred lines were sowed in field and evaluated in two rust managements: (1) with, and (2) without control by spraying fungicides. The design in each management was random complete blocks with four replicates. The variables measured were: seed yield (SY), the red-amber (590 nm, R) and near-infrared raw reads (880 nm, NIR), and NDVI (Normalized Difference Vegetation Index) using the Crop Circle ACS-210, all of them at 27th and 64th days after sowing. We analyzed Pearson correlations between the traits, as well as the joint analysis of variance (ANOVA).

Table 1- Correlations between SY and reflectance indices in each management (*significant in t-test at 5%)

	NDVI27	NDVI64	R27	R64	NIR27	NIR64
Mean	0.236*	0.328*	-0.183	0.532*	0.018	0.584*
Manag. 1	0.297*	0.267	-0.273	0.559*	0.034	0.606*
Manag. 2	0.038	0.305*	0.065	0.286*	0.104	0.357*

The trait SY was more correlated with the indices at the 64th day, moderately with R and NIR and weakly with NDVI. The correlation between SY and R64 or NIR64 in management 2 was lower than in management 1. The ANOVA revealed that genotype and management effects were significant for R64 and NIR64. For NDVI64, only the genotype effect was significant, and for SY, genotype, management and their interaction presented significance. In conclusion, the reflectance indices at 64th day were useful to identify high yielding genotypes, as well as to distinguish the presence of rust.

Acknowledgments:
To CAPES and CNPq.

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Heat priming effects on alleviating later damages of heat stress in different wheat cultivars

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Keywords: abiotic stress, gas exchange, heat priming, wheat, chlorophyll fluorescence

The increasing of extreme climate events occurrence, in particular high temperatures has become a major restrain in food production. Over the past years, evidence has shown plants subject to early stress (primed) are more resistant to future stress exposure [1]. Studies investigating priming effect in plant ability to response to stress episodes are still limited [2]. We aimed to test if repeated early abiotic stresses could improve heat tolerance during anthesis heat stress in wheat cultivars. Two wheat cultivars, Gladius and Paragon, were subjected to a pre-anthesis high temperature priming process at three and five complete developed leaves stages. Primed and control plants were subjected to either a high temperature stress or non-heat stress temperature for 7 days during anthesis. Gas exchange and chlorophyll fluorescence were used to investigate the physiological performance of plants. No difference in assimilation rate f was observed between treatments for Gladius. Heat stressed Paragon parameters were not measurable due to the premature senescence of plants. No strong evidence was observed to prove the initial assumption of early heat stress being accountable for improving heat tolerance. However; a great difference between cultivars in response to heat stress was observed. Yield parameters of Gladius primed plants did not differ from their respectively control treatment. A distinct result was observed for Paragon cultivar assumed based in this experiment to be heat sensitive, suggesting a cumulative deleterious effects caused by the repeated heat stress.

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Plant irrigation automated control in the SITIS Platform

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Keywords: water pressure, solenoid valve, greenhouse, soil columns.

Plant irrigation in greenhouse experiments executed by persons results in problems like: exposition to severe climatic conditions (high air temperature and relative humidity), low accuracy in the applied water amount and in recorded data, irrigations being executed after normal period and at the weekend [1]. The technical team of Embrapa Rice and Beans developed a system named SITIS Platform of Plant Phenotyping for Drought Tolerance for automating the planning and execution of plant irrigations in the greenhouse. The SITIS has two integrated modules: 1) SitisWeb - responsible by the planning of the irrigations in all soil columns with an human-machine interface, based on Java EE technology and PostgreSQL; 2) SitisEmbbeded - responsible by irrigation in each soil column from the rules planned and by returning with the results to the SitisWeb, based on embedded technology, Java SE, SQLite, connected to a digital scale with capacity of up 100 kg and accuracy of 10 g and with a solenoid valve connected to an hydraulic system. The valve doesn´t have flow control of water and has two states: open and close. The irrigation algorithm was projected to perform many partial water applications with a cycle of opening and closing of the valve associated to monitoring of the weight of the soil columns because there isn´t uniformity water pressure in each column. The tests showed that irrigations are executed accurately regardless of water pressure. Thus, the solution of irrigation control developed fully meets the identified problems.

Acknowledgments:
To the DREBCROPS, SecaCereal and Dryce Projects for the resources made available.

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Aerial imagery to select maize hybrids to nitrogen use efficiency

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Keywords: indirect selection, abiotic stress, RGB.

The large-scale phenotyping may increase the efficiency of the breeding process with a substantial reduction of costs and time. Therefore, the objective was to examine the genetic relationship of aerial imagery (AI) with nitrogen use efficiency (NUE) in maize. Thus, 739 maize hybrids and two checks were evaluated under two N conditions: ideal N (IN) and low N (LN), in an augment block design (22°50'51"S, 48°01'06"W, 466 m), during the second growing season in 2016. The trait evaluated was the NUE. The AI was taken at the flowering time (VT), using an RGB camera with 12Mpx attached to a drone. From these images and their positions (GPS), the mosaic and the cloud points were built by Agisoft Photo Scan software. Then, using the HTP Geoprocessor plugin on QGIS, the green index (GI) of each plot was estimated. Genotypic values of each hybrid for NUE and GI and variance components were estimated through mixed model equations by the ASReml-R® package. It was found significant differences between hybrids and all other factors for GI and NUE. The heritability of the former was 0.48 and of the latter 0.31. Based on the GI, it was possible to identify the checks and the stress level. However, the correlation between the NUE of hybrids and their GI was weak (0.03). Furthermore, it was not possible to use AI to indirectly select hybrids to NUE. On the other hand, considering the time consuming, it is a promising tool, given the relative efficiency of AI phenotyping over the traditional method, which was 1953%. We believe that using other indices such as NDVI or GNDVI we might obtain better results.

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Traits associated with seed yield in contrasting environments for soybean rust

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Keywords: Glycine max, Phakopsora pachyrhizi.

A few studies have associate shape of the leaves and leaflet area, plant architecture and canopy as mechanisms of tolerance to fungal diseases [1]. The Asian rust has caused serious damage in soybean, and the development of tolerant cultivars may lead to an alternative way to control, as it is not expected selection pressure for resistant pathogen. This research aimed to estimate the multi-traits associations to productivity and tolerance in contrasting rust managements. We tested 40 crosses (F_2 generation) derived from a partial diallel (4x10), using a RCB design with 4 replicates in two fungicide managements (with and without rust control). It was evaluated seed yield (SY) and the following traits through visual notes: leaflet shape (1- narrow, 2-medium, 3- broad leaflet, LS), plant architecture (1-less favorable to 5-ideal plant architecture, PA), leaflet area estimation (1- low to 5- hight amount of leaflet area, LA) and agronomic value (1- less adequate to 5- agronomically suitable plants, AV). The Pearson correlation revealed that AV was the most correlated trait with SY in both managements (0.46, 0.40). In the presence of the disease, LS (0.18) and LA (0.18) influenced positively SY. The Principal Component Analysis (PCA) revealed homogeneity in distribution of the genotypes, showing some tolerant crosses. In conclusion, the AV was an effective method to assist the selection of plants for productivity in both managements and there was evidence that using the other traits could help the assessment of the tolerant crosses.

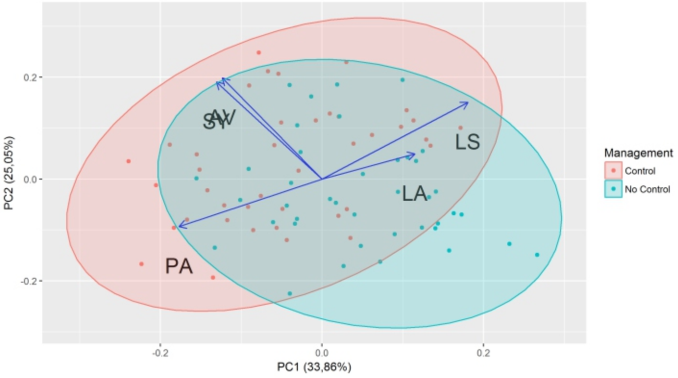

Fig. 1- PCA for 40 crosses in contrasting environment for rust.

Acknowledgments:
CNPq for financial support.

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Phenotyping transpiration dynamics in real time: the key to mine the banana biodiversity for better water use efficiency?

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Keywords: Transpiration Phenotyping, Biodiversity evaluation.

Water deficit is one of world's major constraints in agriculture and will aggravate in the future. An important crop that needs vast amounts of water for optimal production is banana (Musa spp.). Millions of people depend on banana as staple food, while it is mostly grown in rain fed systems and hence very prone to fluctuating water availability. Worldwide a huge (uncharacterized) biodiversity is available, and we believe optimal exploration and exploitation of this diversity will alleviate issues with drought and also with pests and diseases. The International Transit Center of Bioversity International holds the world's biggest collection of banana biodiversity (>1500 accessions) which is hosted by the Laboratory for Tropical Crop Improvement (KU Leuven).

Our aim is to evaluate the potential within this biodiversity collection for climate smart agricultural usage. In a controlled climate chamber, we monitored the transpiration in 10s intervals with a gravimetric, high precision phenotyping installation. This provides insight in the day to day, and within day, variations of the transpiration rate of different banana genotypes. In a constant environment, without any sign of stress, the transpiration rate changes during the day. At a genotype specific moment in time, without external signaling, a clear decline in transpiration rate is observed. This minimizes transpiration water loss up to 30%, compared to a continuous maximal transpiration rate. Among genotypes up to two hour time lags have been recorded, the most promising drought tolerant genotypes minimizing transpiration soonest. We hypothesize that bananas keep their stomata open for carbon exchange and transport, but once sufficient carbon is sequestered stomatal conductance is reduced to save water. Transport of metabolites is then maintained by root pressure. This water saving mechanism is crucial to understand differences in water use efficiency among genotypes and is currently tested in greenhouse conditions (Phenospex gravimetric installation) by combining plant growth phenotyping with the transpiration phenotyping.

Global network for precision wheat phenotyping

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Keywords: networking, wheat diseases, abiotic stress, phenotyping.

Investments in high quality phenotyping are needed to fully exploit the potential of genomic data, and consequently, to contribute for more efficient plant selections, shorter breeding cycles, and rapid advances in breeding. Based on a global network of wheat partners, several precision field-based wheat phenotyping platforms are being developed with the support of the CGIAR Research Program on Wheat and co-investing national agricultural research institutes. The objective is to generate higher precision multi-location phenotypic data on prioritized traits, under defined good practices, short- and longer-term training and sharing of the generated knowledge, as well as fostering global germplasm exchange among participating NARS and scientists. The selected locations represent key environments, as for example, hotspots for specific diseases and future climate analogue sites. Within this network, phenotyping activities are being conducted for wheat blast (*Magnaporthe oryzae*) in Bolivia, Septoria tritici blotch (STB) in durum wheat in Tunisia, and for multiple diseases (leaf rust, Fusarium head blight, and STB) in bread wheats in Uruguay. Subject to further funding, additional platforms to be developed include the ones for evaluation of heat and drought tolerance (Morocco - already funded - India, Pakistan, Bangladesh, Nepal, Turkey), yield potential (China, India, Pakistan, Egypt, Zimbabwe), and wheat diseases (China, Turkey, Bangladesh-Nepal). The generated information will contribute to a faster development of broad genetic based resistant, high yielding wheat varieties, complementing evaluations currently performed for diseases (Kenya, Ethiopia, Turkey, Mexico) and heat/drought/yield potential (Mexico).

Phenotypic screening of Toropi x IAC13 population under hydroponic solution

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Keywords: Toropi, hydroponic, aluminium, tolerance, recombinant inbred lines.

Soil aluminum (Al) toxicity is one of the major yield constraints to wheat in South Brazil. The Brazilian cultivar Toropi has been proved its contribution to Al^{3+} tolerance. Our main objective was to screen a F_{14} population of 76 recombinant inbreed lines (RILs) derived from Toropi x IAC13 in hydroponic assays conducted in controlled growth chamber. An adaptation of the protocol reported by Nava [1] was used to assess the aluminum response of each RIL and the parental lines plus Anahuac 75, a highly susceptible pattern to Al. Pre-germinated seeds were mounted on nylon-mesh floats on buckets with aerated solution pH 4.0 (free of Al) for 24h. Soon after, they were transferred to an Al solution 370 µM of $AlCl^{3+}$ for 48h and transferred back to the solution free of Al for 72h. The evaluation consisted in measuring the regrowth of the main root from point of callosity. Based on parental responses, RILs were classified as susceptible, moderately susceptible (MS), moderately tolerant (MT) and tolerant. ANOVA of data were calculated in GenStat software. ANOVA test indicated differences among the sets of experiments ($P \leq 0.005$). Toropi, IAC13 and Anahuac showed general mean primary regrowth of 6.67, 2.45 and 1.66 cm, respectively. Among the RILs analysed seven exhibited regrowth larger than Toropi, 14 RILs were lower than IAC13 and just one lower than Anahuac. This shows evidence of distortion of segregation. The distribution of RILs into the classes above resulted in 10 lines classified as susceptible, 18 as moderately susceptible, 25 moderately tolerant, 19 tolerant and four lines presented incompatible response among assays. These results confirm that Toropi is a cultivar highly tolerant to Al^{3+} and suggests that IAC13 may possesses some loci to tolerance.

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Imagery to study morphological responses of maize root traits under low nitrogen condition

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Keywords: *Zea mays*, abiotic stress, BLUP.

Root traits play an essential role in nitrogen (N) acquisition. Under abiotic stress, they tend to modify for better performance. Sixty-four tropical inbred maize lines contrasting for N-use efficiency were used to verify root changes under low N supply. Field experiments were carried out in two locations over 2015 season with an 8 x 8 lattice design with two replications. Two separated trials were conducted at each location, (a) with ideal N fertilization (IN) and (b) with low N fertilization (LN). The traits evaluated were total root length (TR), fine root length (FR), and lateral root area (LA). The root imagery measurements were performed using WinRHIZO™ Arabidopsis software coupled to an EPSON LA2400 scanner (2400 dpi definition). Genotypic values and variance components were estimated through Restricted Maximum Likelihood/Best Linear Unbiased Predictor (REML/BLUP) method, by the ASReml-R® package. In LN condition, TR, FR, and LA increased 6.90%, 6.12%, and 7.36%, respectively. Lines differed significantly as well as N level for all traits, indicating the existence of genetic variability and N doses properly considered for this kind of study. Heritability estimates were moderate to all traits, ranging from 0.49 (TR in IN) to 0.66 (FR in IN). Accordantly, the highest values of accuracy were associated with the lowest values of genetic coefficient of variation. Pair-wise Pearson's correlation showed the root traits analyzed were strongly positively correlated with each other. Under N stress, plants usually increase root length and alter root architecture [1]. Therefore, our results are consistent and the root imagery is useful and reliable for conducting phenotypic-genetic studies.

Acknowledgments:
CNPq and FAPESP (Process 2013/24135-2).

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Imagery to study the effects of Azospirillum brasiliense inoculation in maize root morphological traits

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Keywords: low nitrogen stress, diazotrophic bacteria, endophyte, Plant Growth-Promoting Rhizobacteria.

Azospirillum brasiliense is a Plant Growth-Promoting Rhizobacteria (PGPR) used as inoculant in grasses, with biological nitrogen (N) fixation and production of phytohormones mechanisms [1]. Thus, we investigated the changes in maize root morphological traits in response to *A. brasiliense* inoculation by imagery. A set of 119 single-crosses of maize were evaluated under inoculated and non-inoculated conditions in greenhouse, without an input of N fertilizer. The experimental design was a randomized block, with six replicates. A serial of root morphological traits was measured using scanner-based image and the WinRHIZO™ software. Statistical analysis was performed using mixed model equations (REML/BLUP). Genetic variability was observed for all traits, but only for root diameter (RD) and root volume (RV) the inoculation effect was significant. For these traits, the average increase was 1.92% and 2.94%, respectively. Despite these small values, positive and negative effects on hybrids were observed with the inoculation, ranging from -7.56% to +11.14% for RD and -26.39% to +25.30% for RV. The increase in the RD indicates increment of axial roots concerning the lateral roots, which are related to better soil exploration when associated with a larger RV [2]. The negative effect observed in some hybrids is because PGPR can become pathogenic depending on the host genotype and the environment conditions. Hybrids with the highest values of RD or RV in control treatment were not the same that had the highest increase with the inoculation, evidencing that the variation induced by *A. brasiliense* is not linked to intrinsic root growth rates. Therefore, there is genetic variation in maize to responsiveness to *A. brasiliense* and root traits could be used as an indirect measure of association degree between genotype-PGPR in plant breeding to improve this trait.

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Biomass phenotyping in high yield potential rice genotypes

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Keywords: NDVI, non-destructive tools, indirect measurements.

Rice is a major staple food in the world. Rio Grande do Sul is responsible for about 70% of the rice production in Brazil. Non-invasive and non-destructive tools are important approaches to characterize rice genotypes of high yield potential. The main goal of this study was to compare five rice genotypes for biomass production along different stages of development in the 2016/2017 season. The assay was conducted in the experimental station of the Rio Grande do Sul Rice Research Institute (IRGA), located at Cachoeirinha, RS. Biomass was evaluated both directly (via dry matter production in an area of 0,25m²) and indirectly (via NDVI - Normalized Difference Vegetation Index, obtained through the use of Greenseeker®). Dataset was composed of six points of data collected along the season, from V7 to R9. Pearson correlations among the direct and indirect measurements and rice grain yield was performed on Sigmaplot v. 11 software. The higher correlation was observed in the V7 stage. Good correlations were also observed in V9, R1 and R2 stages. R4 presented a high negative correlation between biomass and NDVI values while R9 presented a low negative correlation between them. In R4 this is due to panicle emission that lowers the reflectance of the plants and in R9 the senescence contributes to this negative correlation between the biomass and NDVI values. These results demonstrate that NDVI evaluation via Greenseeker® can be a good estimator of biomass production from V7 to R2, but from flowering on its relationship with biomass production lowers.

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UAV- based imagery for phenotyping in breeding and physiological pre-breeding of wheat at CIMMYT

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Keywords: wheat, phenotyping, UAV, canopy temperature, NDVI, physiological pre-breeding

Phenotyping is the cornerstone of plant breeding. Over decades, conventional phenotyping has been used to select lines based mainly in highly heritable traits such as phenology, height or kernel size. The recent incorporation of remote sensing (RS) tools in breeding programs provide an excellent opportunity to evaluate more complex traits while increasing our capacity of screening genetic material. RS allows complex integrative traits to be evaluated on scale that overcomes some of 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.

The use of unmanned aerial vehicles (UAVs) for phenotyping has been implemented in the Global Wheat Program at CIMMYT for more than five years. These platforms demonstrated to be suitable and effective to remotely measure traits such as NDVI and canopy temperature in thousands of wheat lines. Our results have support that idea that by migrating from ground-based to aerial phenotyping platforms we can improve the precision and the traits' heritability, and thus obtain a greater predictive power. In this communication we present our latest advances in the analysis of UAV based imaging for its use in the screening of genetic resources within the context of breeding and physiological pre-breeding in wheat. We also discuss possible upcoming innovations that are currently in the pipeline including the use of image analysis to estimate agronomic traits, phenology and the application of vegetative indices to correct for spatial variation in large trials.

Opportunities and Challenges of Phenotyping in the Genome-Assisted Breeding Era: Experiences from the International Potato Center

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Keywords: Genome-assisted breeding, Phenotyping, Potato, Sweetpotato

The world is faced with an ever-increasing population that requires increased production and productivity of major global food crops. This challenge is further complexed by changing environments due to climate change, reduced land sizes, extension of crop production into marginal lands, among others. The time taken to develop and release new plant varieties is quite long as it requires several generations of progeny testing in these varied environments. The rapidly changing environments on the other hand require rapid variety turn-over to cushion the farmers from this environmental plasticity. To achieve this, the generation interval between breeding and variety release needs to be shortened significantly. Genome-assisted breeding has the potential of expediting the breeding process as it can allow selection at early stages and selection on single plants. Consequently, genotyping services have undergone rapid development with high throughput next generation sequencing costs falling significantly. Despite this opportunity, genome-assisted breeding methods rely heavily on the quality of phenotypic data used to train its methods, such as tagging QTL or developing genomic selection models. Availability of high throughput phenotypic data is therefore currently the main challenge to increasing genetic gains in crop improvement. The International Potato Center (CIP) has a global mandate to improve potato and sweetpotato among other roots and tuber crops. The approaches, opportunities and challenges faced by CIP's crop improvement teams regarding phenotyping will be discussed and areas of possible collaborations explored.

Acknowledgments:
Genetics, Genomics and Crop Improvement Division, International potato Centre

Developing plant and crop phenomics in Argentina. A project in progress

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Keywords: crops diversity, phenotyping tools, phenotyping traits.

Due to its vast size and range of latitudes and altitudes, Argentina possesses a wide variety of climatic regions, ranging from the hot subtropical region in the north to the cold subantarctic in the far south. Thus, due to these contrasting environments, the country has a high potential from a Bioecomy point of view. For instance, it has a wide potential for production of food and bioenergenetical and new farming products raw materials (crops, grasses, forest…). Currently, extensive agriculture crops are widely sown around the country but several crops are grown only in some regions (i.e. sugarcane, rice) including in some cases productions of local consumption (yerba mate, Andean maize and potatos…).

Phenotyping is currently the bottleneck for crop improvement and development of efficient inputs for agriculture, in a context of increasing demands for food, feed and energy and a greater sustainability of agriculture. Also, Phenomics is useful for basic studies in Plant Science. The great diversity of agricultural productions in Argentina generates a great diversity of needs of phenotyping tools. Most high troughput and non-invasive phenotyping tools are not affordable because of their high cost or high complexity. It would therefore not be feasible to cover most of the phenotyping needs at national level with methods and technologies developed in other countries. The project presented here proposes a model potentially applicable in other Latin American countries for the development of these key technologies face to the global challenges of Bioeconomics.

The proposed solution to cover the phenotyping needs in Argentina is the creation of a National Center that will develop, transfer and use affordable methods and tools for phenotyping, specifically oriented to the improvement of the different Argentinean agricultural productions. This National Center will train human resources to support the creation of a National Phenotyping System that will include nodes located in different regions dedicated to phenotype specific agricultural productions. The project propose taking advantage of the experience, equipment and capabilities yet available at the Unidad Integrada Balcarce. These capabilities will be increased through the extension and improvement of platforms and the expansion of existing human resources. The National Center at Balcarce will be helpful to demonstrate the importance of the application of Phenomics for genetic and agronomic improvement in a sustainable context. The Ministry of Science of Argentina (MINCyT) has already granted 25% of the budget for the creation of the Center (mainly focused on the acquisition of most of the equipment).

Evaluation of rice genotypes for resistance to *Diatraea saccharalis* (Fabricius) using phenotyping methods

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Keywords: Rice, Sugarcane borer, Morphological traits, Insect host resistance.

The sugar cane borer, *Diatraea saccharalis* (Fabricius) is a pest of rice in Brazil and several American countries. An increase in *D. saccharalis* frequency of occurrence in recent years has emphasized the necessity to develop more effective stem-borer management strategies for the Brazilian rice growers. Control borers with insecticides after larvae penetrate into the rice stalk is difficult. The search for resistance to *D. saccharalis* is very important to identify source of resistance for breeding programs. Since 2010, we have conducted a series of experiments in greenhouse conditions, to identify genotypes with resistance to borers in the Brazilian rice genebank. The ultimate goal of our study is to use selected accessions as donor parents with the aim to produce rice cultivars with enhanced resistance to sugarcane borer via conventional methods of plant breeding, and in the future, through the use of molecular markers using genetic engineering methods. The experimental design was randomized blocks with five replications. Infestations were made when rice plants were 50 days old placing neonate larvae on the leaf sheaths of rice plants (2 larvae/tiller). At 30 days after infestation the rice plants were cut at ground level and taken to the laboratory where the signs of borer attack, external and internal diameter of the stem and weight of surviving larvae were determined. The morphological traits of the rice plant, response of the plant to insect attack and development of the sugarcane borer larvae indicated a genotypic variation. Our studies were successful in separating accessions into groups in relation to resistance to *D. saccharalis*. Although different groups of accessions with similar morphological traits could be discriminated, we observed that there is a considerable variability within accessions. Development of new tools to combine with traditional methods is determinant of a successful screening for insect host resistance in the future.

Phenotyping is essential to enhance phosphorus efficiency in maize and sorghum

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Keywords: paper pouch, nutrient solution, phosphorus acquisition, root traits

Phosphorus (P) is an essential nutrient to plants and is acquired as inorganic phosphate from the rhizosphere solution. P is one of the least available nutrients particularly in highly weathered, tropical soils, limiting substantially plant growth. An interesting approach to circumvent P deficiency in tropical areas is to explore the genetic diversity available in plants to breed cultivars more efficient in P acquisition. Root traits, such as root length and surface are key to determine P-efficiency. Our work aimed to study root traits involved with P acquisition efficiency and to identify putative maize and sorghum homologs to *Phosphorus Starvation Tolerance 1 (PSTOL1)*, a gene responsible for enhanced early root growth, P uptake and grain yield in rice. A combined approach of a paper pouch system in nutrient solution with field phenotyping under low-P was used to generate phenotypic data in order to investigate the role of *OsPSTOL1* homologs. Association mapping was undertaken in two sorghum association panels phenotyped for P uptake, root system morphology and architecture in hydroponics and grain yield and biomass accumulation under low-P conditions. Root length and root surface area were positively correlated with grain yield under low P in the soil, emphasizing the importance of P acquisition efficiency in sorghum adaptation to low-P availability. *SbPSTOL1* alleles reducing root diameter were associated with enhanced P uptake under low P in hydroponics, whereas *Sb03g006765* and *Sb03g0031680* alleles increasing root surface area also increase grain yield in low-P soil. SbPSTOL1 genes colocalized with QTLs for traits underlying root morphology and dry weight accumulation under low P-soil. For maize, two multiple interval models were used to map QTLs related to root traits, biomass accumulation and P content in a maize RIL population cultivated in nutrient solution. Multiple interval mapping models for single and multiple traits were combined and revealed 13 genomic regions significantly associated with the target traits in a complementary way. Some of these quantitative trait loci (QTLs) were coincident with QTLs for root morphology traits and grain yield previously mapped, whereas others harbored ZmPSTOL1 candidate genes. Maize *PSTOL1* candidate genes co-localized with QTLs for root morphology, biomass accumulation and/or P content and were preferentially expressed in roots of the parental lines that contributed the alleles enhancing the respective phenotypes. Our work indicate multiple maize and sorghum *PSTOL1* genes that have a role in the modulation of root morphology, which leads to higher P acquisition and yield.

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Attendees

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Carlos Berdugo Agudelo	Belgium	BayerCropScience N.V.
Carlos Sousa	Brazil	Embrapa Agroenergia
Carolina Saint Pierre	Mexico	CIMMYT
Carolina Trentin	Brazil	UFRGS
Celeste Molina Favero	Argentina	INTA
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Debora Milori	Brazil	Embrapa Instrumentation
Dos Santos Soares	Brazil	Universidade de Brasília
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Eduardo Kawakami	Brazil	TMG
Elesandro Bornhofen	Brazil	ESALQ/USP
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Francisco Pinto Espinosa	Mexico	International Maize and Wheat Improvement Center - CIMMYT
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