High Throughput and Precision Phenotyping for Improving Abiotic Stress Resilience in Maize

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Phenotyping – a key component in the maize adaptation to future challenges

Crop production must double by 2050 to meet future consumption demands (Tilman et al. 2011). However, climate change scenarios for the coming decades imply a penalty in the productivity of maize due to increases in air temperature, decreases in precipitation or both factors together. As a consequence, yields will be affected, even in crops like maize which possesses a C4 photosynthetic metabolism that exhibits higher yield potential and water use efficiency as well as photorespiration which is less sensitive to increases in temperature than most other crops with C3 metabolism (Lobell et al. 2011a,b; Cairns et al. 2013; Hawkins et al. 2013). In fact, climate change has already restrained genetic advances in maize during recent decades, primarily related to heat and secondarily to water stress (Lobell et al. 2011a). In addition, the increasing cost of fertilizers, together with concerns about their environmental impact, will limit their indiscriminate use in the future. Improving agronomical practices and crop breeding are paramount if we are to respond to the present and future challenges imposed by global change. Crop management has benefited strongly from the adoption of techniques to monitor crop physiological status and growth and to predict yield through the rapid development of technologies such as precision agriculture (Rodrigues Junior et al. 2014). These agronomical approaches are helping to reduce the gap between actual (farmer's) yield and the yield potential.

In the case of crop breeding, genetic advances on yield improvement and stress resistance during the past decades despite the increased adoption of molecular approaches (e.g. marker assisted selection, transformation, etc.). Increased evidence exists that phenotyping, particularly at the field level, is actually limiting the efficiency of conventional breeding as well as preventing the delivery of molecular breeding at its full potential (Araus et al. 2008: Cabrera-Bosquet et al. 2012; Cairns et al. 2012; Cobb et al. 2013). Constraints in field phenotyping limit our ability to dissect the genetics of quantitative traits, particularly those related to abiotic stress tolerance. The development of effective field-based high-throughput phenotyping platforms (HTPPs) remains a bottleneck for future breeding advances (Prasanna et al. 2013; Araus and Cairns, 2014). However, progress in sensors, aeronautics, and high-performance computing are paving the way. In fact, some of these technologies have been successfully implemented in precision agriculture but their use for breeding requires more accuracy and high-throughput because the range genotypic variability is usually far lower than that caused by changing environmental conditions, and the target is to assess a large number of genotypes.

The need for field phenotyping

Field conditions are notoriously heterogeneous and the inability to control environmental factors makes results difficult to interpret. However, results from controlled environments are far removed from the situation plants will experience in the field and, therefore, are difficult to extrapolate to the field (Figure 1). For example, the volume of soil available to roots within a pot is considerably smaller than in the field, thereby reducing the amount of water and nutrients available to plants (Passioura, 2006; Porter, 2012). The soil environment plays a crucial role in plant growth and development and is difficult to simulate under controlled conditions (Whitmore and Whalley, 2009). Drought stress phenotyping is particularly challenging due to declining soil moisture content which is associated with increased mechanical impedance in the field, which is an effect that is hard to replicate within pots (Cairns et al. 2011).

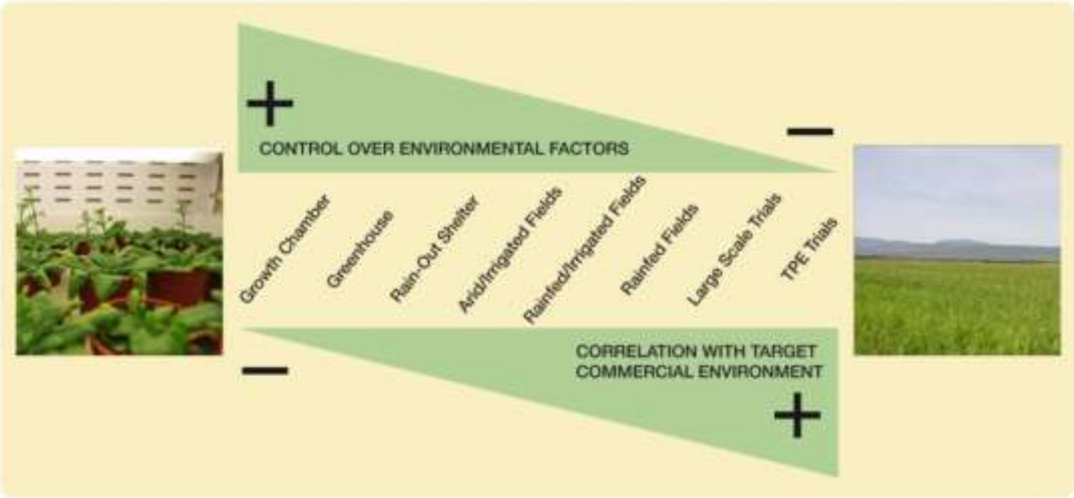

Figure 1. Continuum of environments for drought resistance screening. The control over environmental factors decreases from the use of growth chambers to the target population environment (TPE) while the correlation of performance with the target commercial environments increases. Figure redrawn from Passioura (2006).

Traits and tools for phenotyping

The most successful traits for field phenotyping integrate in time (throughout the crop cycle) and space (at the canopy level) the performance of the crop in terms of capturing resources (e.g. radiation, water and nutrients) and how efficiently these resources are used (Araus et al. 2002, 2008). Different methodological approaches have been proposed to evaluate these traits in the field (Figure 2). They can be summarized into three categories: (i) proximal (remote) sensing and imaging; (ii) laboratory analyses of samples; and (iii) near-infrared reflectance spectroscopy (NIRS) analysis in the harvestable part of the crop (White et al. 2012).

Ground-based HTPPs allow data to be captured at the plot level requiring little post-processing. Moreover, this allows the implementation of closed multispectral imaging systems, which shuts out wind and sunlight to ensure the highest possible precision and accuracy (Svensgaard et al. 2014). However, this also limits the scale at which ground-based HTTPs can be used. Furthermore, ground-based platforms do not allow simultaneous measurements of all plots within a trial (Busemeyer et al. 2013) and in the case of maize its use is not really feasible except for early stages of the crop (Montes et al. 2011).

Field HTPPs should combine a high capacity for data recording or scoring and processing and non-invasive remote-sensing methods, together with automated environmental data collection and all at an affordable cost. Laboratory analyses of key plant parts may complement direct phenotyping under field conditions.

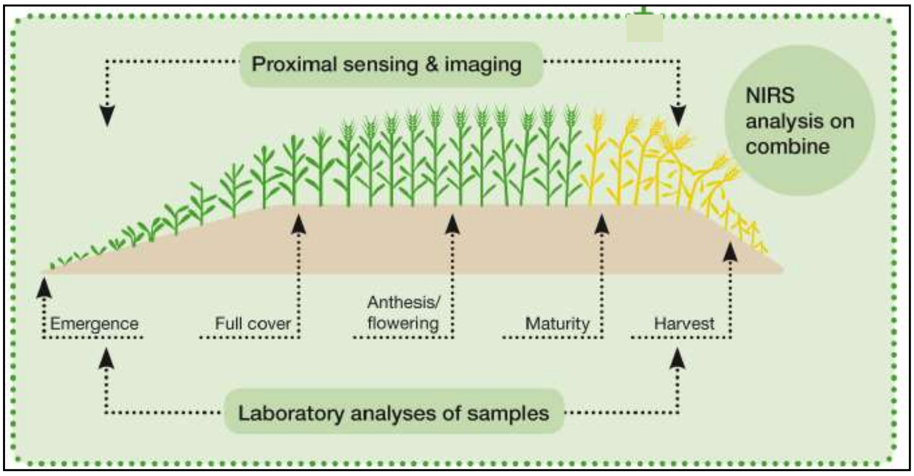

Figure 2. Diagram of the main categories of phenotyping techniques deployed over the life-cycle of an annual seed crop like maize. Types of data acquisition include: proximal sensing and imaging at frequent intervals, laboratory analyses of samples taken at specific intervals, and near-infrared spectroscopy (NIRS) on leaf matter or seeds to assess phenotypic traits potentially related to maize performance under stress (Cabrera-Bosquet et al. 2009a,b, 2011). Redrawn from White et al. (2012) and Araus and Cairns (2014).

For almost all of the above remote techniques, the use of imaging allows up-scaling of the measurements, for example, from a single plot basis to dissecting an entire trial composed of different plots, providing that the image has enough resolution (pixels). There are different categories of sensors. RGB/CIR cameras combine color infrared (CIR) and red, green and blue light (called visible or RGB) imagery (Figure 3A). This allows the estimation of green biomass (vegetation index type of information). For example, the ADC Lite (http://www.tetracam.com/adc_lite.html) and the ADC Micro (http://fieldofviewllc.com/tetracam-adc-micro) have spectral range bands in red, green and NIR, with the latter model having a weight of 100 g. Multispectral cameras are widely used for crop monitoring via remote sensing (Figure 3B). They can acquire a limited number of spectral bands at once in the VIS-NIR regions.

Besides vegetation indices for evaluating green biomass, multispectral imagers can be formulated to other spectral indices targeting senescence evaluation, nutrient status, pigment degradation, photosynthetic efficiency, or water content (Gutierrez et al. 2010). An example of a widely used camera is the Tetracam MCA (http://www.tetracam.com/Products-Mini_MCA. htm). Hyperspectral VIS-VNIR imagers (Figure 3C) allow acquisition of hundreds of images at once, covering the entire electromagnetic spectrum between the visible and the near infrared regions in a continuous mode (wavelengths ranging from 400-900 nm). Other configurations cover the range from 1000 to 2500 nm. Therefore, it is possible to run empirical calibrations (like in a “NIRS-mode”) against a wide and miscellaneous set of traits. The image in 3C depicts the Micro-Hyperspec VNIR model (http://www.headwallphotonics.com/ Portals/), which measures up to 260 bands – 5-7 nm full-width half-maximum (FWHM) in the 400–885 nm spectral region. This is a particularly promising approach given the possibility that multispectral information can predict complex traits such as grain yield (Weber et al. 2013).

Longwave infrared cameras or thermal imaging cameras render infrared radiation in the range of µm as visible light (Figure 3D). Potential use of thermal imaging in phenotyping, includes predicting water stress in crops. Thermal sensing has been used to assess maize responses to drought (Romano et al. 2011, Winterhalter et al. 2011; Zhia et al. 2013). Low resolution may represent a limitation to the use of such cameras from aerial platforms. Examples of light thermal cameras are the FLIR Tau 640 LWIR with a 640 x 512 resolution (http://www.flir.com/cvs/cores/view/?id=51374) and the Thermoteknix Miricle camera with a 640 x 480 resolution (http://www.thermoteknix.com/products/oem-thermal-imaging/miricle-thermal-imaging-modules/). Due to their small size and weight, these cameras are not thermo-stabilized. Conventional digital RGB cameras (Figure 3E) are very low-cost instruments that allow estimating plant cover (green biomass), senescence and yield (Casadesús et al. 2014). At the leaf level, it allows chlorophyll and nitrogen content to be assessed from digital images (Rorie et al. 2011) and eventually, replace the portable chlorophyll meters that cost several thousands of dollars. Moreover, the software needed is usually freely available (Casadesús et al. 2007).

Figure 3. Different categories of imaging systems for remote-sensing evaluation of vegetation.

Other remote sensing techniques are starting to be adopted for field phenotyping, such as the use of laser imaging detection and ranging (Lidar). This is an active, remote sensing technique that uses Lidar sensors to directly measure the three-dimensional distribution of plant canopies as well as subcanopy topography, thus providing high-resolution topographic maps and highly accurate estimates of vegetation height, cover, and canopy structure (Weiss and Biber, 2011; Comar et al. 2012; Deery et al. 2014).

The height of maize makes it difficult to use ground-based platforms such as phenomobiles (Deery et al. 2014), except for early phases of the crop. Therefore, the use of aerial HTPPs becomes necessary. Due to cost and versatility, unmanned aerial platforms (UAPs) offer the most promising alternative for carrying remote sensors at height when compared to cranes, tethered balloons, or manned aircraft (Figure 4). Moreover, unmanned planes may be more adaptable than polycopters due to the greater flight autonomy of the former, which makes them suitable for additional tasks such as exploring spatial variability in a breeding trial or even supporting precision agriculture.

Low-cost phenotyping approaches: a case study

Research on affordable technologies is also a priority if the adoption of quality field high-throughput phenotyping is to be pursued by small companies or national agricultural systems in developing countries. These low-cost technologies include remote sensing approaches like the use of RGB imaging or the implementation of NIRS calibrations of key analytical components. The use of RGB images is a very plastic approach since it may be implemented at different levels of organization, from the entire trial (Elazab et al. submitted) to a single leaf (Figure 5). Moreover, the vegetation indices derived from RGB images can be formulated using freely available software (Casadesús et al 2007; Casadesus and Villegas, 2014).

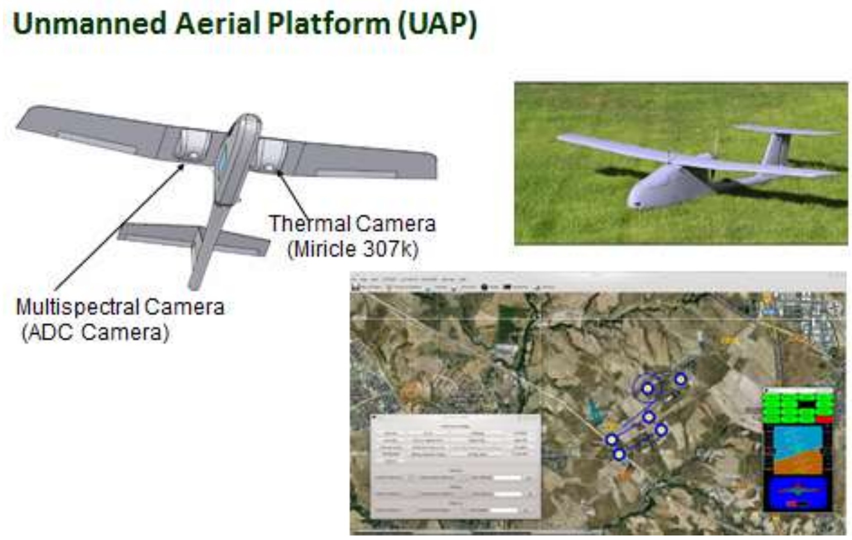

Figure 4. Example of an unmanned aerial platform sponsored by the Global Maize Program of CIMMYT through the project "Affordable HTPP." The UAP carries a multispectral and a thermal camera. The autopilot allows the flight to be scheduled in advance.

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Figure 5. Different approaches to measure vegetation indices at the whole trial, plot and single leaf levels. Left: Measurement of an entire trial derived from an image taken with an RGB/CIR camera from an unmanned aerial platform. Middle: measurements at the single plot level using a portable spectroradiometer or images taken with a conventional RGB camera or of individual leaves in situ using a leaf chlorophyll meter. Right: evaluation of detached leaf segments using a leaf chlorophyll meter, a conventional RGB camera or a desk scanner.

Indeed, when measured at the same level (e.g. individual plot level) the use of a classical vegetation index derived from spectroradiometrical data on the visible/near infrared spectrum, such as the normalized difference vegetation index (NDVI), performs less efficiently than vegetation indices formulated from RGB digital images predicting grain yield (Figure 6).

Vegetation indices from RGB images also perform reasonably well when assessing individual leaf traits such as nitrogen concentration. This is the case, for example, of indices measured on leaf segments and assessed with a conventional digital camera, or even better, a desk scanner in comparison to a leaf chlorophyll meter (Figure 7). This is relevant considering the several thousands of US dollars needed to buy a leaf chlorophyll meter compared with the few hundreds of dollars for a camera or scanner.

RGB images taken under natural field conditions may be used in other areas, such as developing an image-based method to automatically detect and monitor the severity and progression of foliar diseases. This would enable a significant improvement in the throughput and precision of disease screening in maize. In the case of wheat, a vegetation index (VI) such as the green area (GA), formulated using RGB images have proven to perform better in predicting the impact of yellow rust on grain yield than, for example, the NDVI measured with a portable spectroradiometer or the chlorophyll content of individual leaves recorded with a leaf chlorophyll meter (Vergara 2014). In that context, UAPs of low cost, even polycopters carrying only a light high-resolution RGB camera (e.g. http://panasonic.net/avc/lumix/) with image analysis done with free access software (and therefore costing just few thousands of dollars overall) which may represent a viable alternative. Improvements in user-friendly data management, together with a more powerful interpretation of results, will spread the use of field HTPP.

Future perspective

Field high-throughput precision phenotyping needs to be placed in context as a one of a number of components alongside molecular biology, quantitative genetics and even modelling that can assist advanced crop breeding (Cabrera-Bosquet et al. 2012; Prasanna et al. 2013; Araus and Cairns, 2014; Cooper et al. 2014). For the near future, what will pave the way for adoption of field HTPP is the efficient integration of all the components of the system. This includes more user-friendly data management combined with data gathering and processing. Moreover, technology is reaching a stage of maturity where affordable, low-cost approaches for high throughput phenotyping are becoming more and more available. This will further spread the adoption of these technologies for maize breeding.

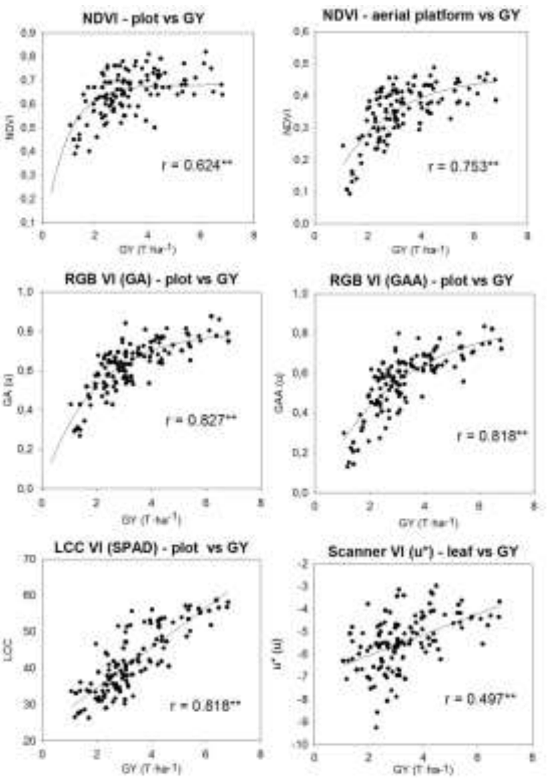

Figure 6. Relationship of different vegetation indices measured at the whole trial, plot- and leaf- levels with grain yield. The normalized difference vegetation index (NDVI) measured at the plot- level with a portable field spectroradiometer (GreenSeeker, Trimble) (upper left) and from the entire trial, with an ADC Lite camera placed in an unmanned aerial platform described in Figure 5 (upper right), Green Area (GA, middle left) and Greener Area (GAA, middle right) derived from images taken at the plot-level with a conventional RGB camera and the chlorophyll content of individual leaves measured with a leaf chlorophyll meter (SPAD, lower left) and from the index u* calculated using RGB images taken with a desk scanner (lower right). Measurements were performed around flowering at the CIMMYT Southern Africa Regional Office, Harare, Zimbabwe. The trial was composed of 10 hybrids, six levels of nitrogen fertilization (0, 10, 20, 40, 80 and 160 Kg ha^{-1}) and two replications per genotype and N level. The VI derived from RGB images is calculated as described elsewhere (Casadesus et al. 2007; Casadesus and Villegas, 2014).

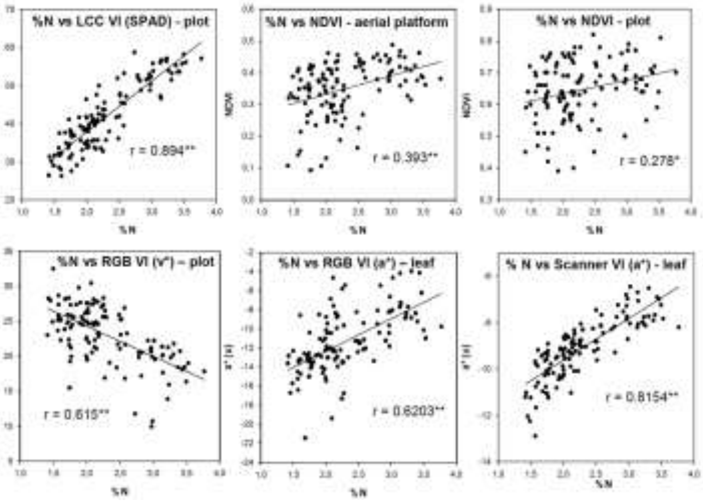

Figure 7. Relationship of different vegetation indices measured at the whole trial, plot and leaf levels with nitrogen content of individual leaves. Measurements taken with a leaf chlorophyll meter (upper left) are taken as a reference. NDVI measured at the plot level (upper center) and from an aerial platform (upper right), the v* index derived from images of the plots taken with an RGB camera (lower left), the a* index derived from images of the leaf segments taken with an RGB camera (lower center) and with a desk scanner (lower right). Leaf chlorophyll content and the RGB measurements on individual leaves were performed on leaf segments sampled in the field and further assessed in the laboratory. Trial and growing conditions are as in the previous Figure.

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References
Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant Breeding and Water Stress in C3 Cereals: what to Breed for? *Annals of Botany* 89: 925-940.

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Science* 27: 1–36.

Araus JL, Cairns J (2014) Field high-throughput phenotyping – the new crop breeding frontier. *Trends in Plant Science* 19: 52-61.

Busemeyer L, Mentrup D, Möller K, Wunder E, Alheit K, Hahn V, Maurer HP, Reif JC, Würschum T, Müller J, Rahe F, Ruckelshausen A (2013) BreedVision – A multi-sensor platform for non-destructive field-based phenotyping in plant breeding. *Sensors* 13: 2830–2847.

Cabrera-Bosquet L, Sánchez C, Araus JL (2009a) How yield relates to ash content, Δ^{13}C and Δ^{18}O in maize grown under different water regimes. *Annals of Botany* 104: 1207–1216.

Cabrera-Bosquet L, Sanchez C, Araus JL (2009b) Oxygen isotope enrichment (Δ^{18}O) reflects yield potential and drought resistance in maize. *Plant Cell & Environment* 32: 1487–1499.

Cabrera-Bosquet L, Sánchez C, Rosales A., Palacios-Rojas N, Araus J. (2011) NIRS-assessment of δ^{18}O, nitrogen and ash content for improved yield potential and drought adaptation in maize. *Journal of Agricultural and Food Chemistry* 59: 467–474

Cabrera-Bosquet L, Crossa J, von Zitzewitz J, Serret MD, Araus JL (2012) High-throughput phenotyping and genomic selection: the frontiers of crop breeding converge. *Journal of Integrative Plant Biology* 54: 312–320.

Cairns JE, Impa SM, O'Toole JC, Jagadish SVK, Price AH (2011) Influence of the soil physical environment on drought stress and its implications for drought research. *Field Crop Research* 121: 303-310

Cairns JE, Sanchez C, Vargas M, Ordoñez RA, Araus JL (2012) Dissecting Maize Productivity: Ideotypes Associated with Grain Yield under Drought Stress and Well-watered Conditions. *Journal of Integrative Plant Biology* 54: 1007–1020**.**

Cairns J, Hellin J, Sonder K, Araus JL, MacRobert JF, Thierfelder C, Prasanna BP (2013) Adapting maize to climate change in sub-Saharan Africa. *Food Security* 5: 345-360.

Casadesús J, Kaya Y, Bort J, Nachit MM, Araus JL, Amor S, Ferrazzano G, Maalouf F, Maccaferri M, Martos V, Ouabbou H, Villegas D (2007) Using vegetation indices derived from conventional digital cameras as selection criteria for wheat breeding in water-limited environments. *Annals of Applied Biology* 150: 227-236.

Casadesús J, Villegas D (2014) Conventional digital cameras as a tool for assessing leaf area index and biomass for cereal breeding. *Journal of Integrative Plant Biology* 56: 7-14.

Cobb JN, DeClerck G, Greenberg A, Clark R, McCouch S (2013). Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype–phenotype relationships and its relevance to crop improvement. *Theoretical and Applied Genetics* 126: 867-887.

Comar A, Burger P, Benoit de Solan C, Baret F, Daumard F, Hanocq J-F (2012) A semi-automatic system for high throughput phenotyping wheat cultivars in-field conditions: description and first results. *Functional Plant Biology* 39: 914–924

Cooper M, Messina CD, Podlich D, Totir LR, Baumgarten A, Hausmann NJ, Wright D, Graham G. (2014) Predicting the future of plant breeding: complementing empirical evaluation with genetic prediction. *Crop & Pasture Science* 65: 311–336

Deery D, Jimenez-Berni J, Jones H, Sirault X, Furbank R. (2014) Proximal Remote Sensing Buggies and Potential Applications for Field-Based Phenotyping. *Agronomy* 5: 349-379.

Elazab A, Ordoñez RA, Savin E, Slafer GA, Araus JL. Detecting terminal heat stress effects on maize biomass and grain yield by remote sensing techniques (submitted)

Gutierrez M, Reynolds MP, Klatt AR (2010) Association of water spectral indices with plant and soil water relations in contrasting wheat genotypes. *Journal of Experimental Botany* 12: 3291–3303.

Hawkins E, Fricker TE, Challinor AJ, Ferro CAT, Ho CK, Osborne TM (2013) Increasing influence of heat stress on French maize yields from the 1960s to the 2030s. *Global Change Biology* 19: 937-947

Lobell DB, Schlenker W, Costa-Roberts J (2011a) Climate trends and global crop production since 1980. *Science* 333: 616–620.

Lobell DB, Bänziger M, Magorokosho C, Vivek B (2011b) Nonlinear heat effects on African maize as evidenced by historical yield trials. *Nature Global Change* 1: 42-45.

Montes JM, Technow F, Dhillon B, Mauch F, Melchinger A (2011) High-throughput non-destructive biomass determination during early plant development in maize under field conditions. *Field Crops Research* 121: 268–273.

Passioura JB (2006) The perils of pot experiments. *Functional Plant Biology* 33: 1075–1079.

Prasanna BM, Araus JL, Crossa J, Cairns JE, Palacios N, Mahuku G, Das B, Magorokosho C (2013) High-throughput and precision phenotyping in cereal breeding programs. In: Cereal Genomics, 3rd Edition, In: Gupta, P.K. and Varshney R.K. ed. Cereal Genomics II, Springer Netherlands, pp. 341-374.

Poorter H, Bühler J, van Dusschoten D, Climen J, Postma J.A. (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology* 39: 839-850.

Rodrigues Junior A, Ortiz-Monasterio I, Zarco-Tejada PJ, Gérard AB (2014) Using precision agriculture and remote sensing techniques to improve genotype selection in a breeding program. CIMMYT report.

Romano G, Zia S, Spreer W, Cairns J, Araus JL, Müller J. (2011) Use of thermography for high throughput phenotyping of tropical maize adaptation in water stress. *Computers and Electronics in Agriculture* 79: 67–74.

Rorie RL, Purcell LC, Karcher DE, King CA (2011) The assessment of leaf nitrogen in corn from digital images. *Crop Science* 51: 2174–2180.

Svensgaard J, Roitsch Y, Christensen S (2014) Development of a Mobile Multispectral imaging platform for precise field phenotyping. *Agronomy* 4: 322-336.

Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of. Sciences, USA* 108: 20260–20264.

Vergara O (2014) Avaluació de la sensibilitat del blat al rovell groc (*Puccinia striiformis* f.sp. *tritici*) mitjançant diferents tècniques de teledetecció. Master Thesis. University of Barcelona.

Weber VS, Araus JL, Cairns JE, Sanchez C, Melchinger AE, Orsini E (2012) Prediction of grain yield using reflectance spectra of canopy and leaves in maize plants grown under different water regimes. *Field Crop Research* 128: 82-90.

Weiss U, Biber P (2011) Plant detection and mapping for agricultural robots using a 3D LIDAR sensor. *Robotics and Autonomous Systems* 59: 266-273.

White JW, Andrade-Sanchez P, Gore MA, Bronson KF, Coffelt TA, Conley MM, Feldmann, KA, French AN, Heun JT, Hunsaker DJ, Jenks MA, Kimball BA, Roth RL, Strand RJ, Thorp KR, Wall GW, Wang G (2012) Field-based phenomics for plant genetics Research. *Field Crops Research* 133: 101-112.

Whitmore AP, Whalley WR (2009) Physical effects of soil drying on roots and crop growth. *Journal Experimental Botany* 60: 2845-2857.

Winterhalter L, Mistele B, Jampatong S, Schmidhalter U (2011) High throughput phenotyping of canopy water mass and canopy temperature in well-watered and drought stressed tropical maize hybrids in the vegetative stage. *European Journal of Agronomy* 35: 22–32.

Zia S, Romano G, Spreer W, Sanchez C, Cairns J, Araus JL, Müller J (2013) Infrared thermal imaging as a rapid tool for identifying water stress tolerant maize genotypes of different phenology. *Journal of Agronomy and Crop Science* 199: 75-84.