Roberto Fritsche-Neto · Aluízio Borém
Editors

Phenomics

How Next-Generation Phenotyping is Revolutionizing Plant Breeding

Springer

Phenomics

Roberto Fritsche-Neto · Aluízio Borém
Editors

Phenomics

How Next-Generation Phenotyping is Revolutionizing Plant Breeding

Springer

Editors
Roberto Fritsche-Neto
Departamento de Genética
University of São Paulo
Piracicaba, SP
Brazil

Aluízio Borém
Departamento de Fitotecnia
Federal University of Viçosa
Viçosa, MG
Brazil

Translation from the Portuguese language edition published by Suprema Editora, Visconde do Rio Branco.

ISBN 978-3-319-13676-9 ISBN 978-3-319-13677-6 (eBook)
DOI 10.1007/978-3-319-13677-6

Library of Congress Control Number: 2014956365

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

In recent years, plant breeding has experienced a revolution. Because of a reduction in genotyping costs and single-nucleotide polymorphisms, it is possible to obtain a large amount of genotypic data in a short time. This flood of genomic information has triggered the development of new strategies for the integration of molecular information in breeding programs. However, there is still a need for quality phenotypic data. This will not only foster efforts in mapping initiatives, but also in genomic selection and direct phenotypic selection. Tuberosa (2012) addressed this issue by saying that "phenotyping is now king, and has taken heritability as queen."

The objective now is phenomics—that is, phenotyping a large number of individuals for a great amount of traits throughout the development of the plants, in a nondestructive manner and with good accuracy. However, the development of high-throughput phenotyping platforms is still a bottleneck. Thus, several initiatives involving many species and several traits are underway to develop automation and robotics for the next generation of phenotyping in the field, greenhouses, and laboratories. Many of those technologies have shown promising results for practical applications in breeding programs.

This book aims to describe the new technologies for high-throughput phenotyping as applied to plant breeding. Written in an easy-to-understand style, this book can serve as a reference for students, educators, and researchers who are interested in innovative technologies in plant breeding. Enjoy it!

Roberto Fritsche-Neto
Aluízio Borém

Reference

Tuberosa R (2012) Phenotyping for drought tolerance of crops in the genomics era. Front Physiol 3:347

Contents

1 **New Technologies for Phenotyping** 1
José Luis Araus, Abdelhalim Elazab, Omar Vergara,
Llorenç Cabrera-Bosquet, Maria Dolors Serret,
Mainassara Zaman-Allah and Jill E. Cairns

2 **Experimental Designs for Next Generation Phenotyping** 15
Luiz Alexandre Peternelli and Marcos Deon Vilela de Resende

3 **Statistical Analysis of Gene Expression and Genomic Data** 33
Marcos Deon Vilela de Resende, Fabyano Fonseca e Silva,
Moysés Nascimento, Camila Ferreira Azevedo
and Luiz Alexandre Peternelli

4 **Root Phenomics** 49
Júlio César DoVale and Roberto Fritsche-Neto

5 **Seed Phenomics** 67
Jeffrey L. Gustin and A. Mark Settles

6 **Agronomic Field Trait Phenomics** 83
Dhyaneswaran Palanichamy and Joshua N. Cobb

7 **Disease Phenomics** 101
Éder A. Giglioti, Ciro H. Sumida and Marcelo G. Canteri

8 Proteomics and Metabolomics as Large-Scale Phenotyping Tools 125
Simone Guidetti-Gonzalez, Mônica T. Veneziano Labate, Janaina de Santana Borges, Ilara G. Frasson Budzinski, Felipe Garbelini Marques, Thaís Regiani, Andressa Peres Bini, Marisângela Rodrigues Santos and Carlos Alberto Labate

Index 141

Chapter 1
New Technologies for Phenotyping

José Luis Araus, Abdelhalim Elazab, Omar Vergara, Llorenç Cabrera-Bosquet, Maria Dolors Serret, Mainassara Zaman-Allah and Jill E. Cairns

Abstract Improvements in agronomical practices and crop breeding are paramount responses to the present and future challenges imposed by water stress and heat (Lobell et al. 2011a, b; Cairns et al. 2013; Hawkins et al. 2013). On what concerns breeding, constraints in field phenotyping capability currently limit our ability to dissect the genetics of quantitative traits, especially those related to yield and water stress tolerance. Progress in sensors, aeronautics and high-performance computing is paving the way. Field high throughput platforms will combine non-invasive remote-sensing methods, together with automated environmental data collection. In addition, laboratory analyses of key plant parts may complement direct phenotyping under field conditions (Araus and Cairns 2014). Moreover, these phenotyping techniques may also help to cope with spatial variability inherent to phenotyping in the field.

Water stress is the main factor limiting agricultural productivity worldwide. Global change scenarios for the coming decades suggest an increase in water stress in many regions of the world, either directly due to a lower precipitation or as a response to increases in air temperature. As a consequence, crop yields will be affected, even for crops such as maize (Lobell et al. 2011a, b; Cairns et al. 2013; Hawkins et al. 2013). Improvements in agronomical practices and crop breeding are paramount responses to the present and future challenges imposed by water stress. Constraints in field phenotyping capability currently limit our ability to dissect the genetics of

J.L. Araus (✉) · A. Elazab · O. Vergara · M.D. Serret
Unit of Plant Physiology, Department of Plant Biology, University of Barcelona, 08028 Barcelona, Spain
e-mail: jaraus@ub.edu

L. Cabrera-Bosquet
INRA, UMR759 Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, Place Viala, 34060 Montpellier, France

M. Zaman-Allah · J.E. Cairns
International Maize and Wheat Improvement Center, CIMMYT Southern Africa Regional Office, Harare, Zimbabwe

R. Fritsche-Neto and A. Borém (eds.), *Phenomics*,
DOI 10.1007/978-3-319-13677-6_1

quantitative traits, especially those related to yield and water stress tolerance. Progress in sensors, aeronautics and high-performance computing are paving the way. Field high throughput platforms will combine non-invasive remote-sensing methods, together with automated environmental data collection. In addition laboratory analyses of key plant parts may complement direct phenotyping under field conditions (Araus and Cairns 2014). Moreover these phenotyping techniques may also help to cope spatial variability inherent to phenotyping in the field.

1.1 Field Phenotyping

Crop management has benefited strongly from the adoption of techniques to monitor crop water status and growth, as well as to predict yield through the fast development of fields, such as precision agriculture or deficit irrigation schedule. These agronomical approaches are helping to reduce the gap between the actual (farmer's) yield and the yield potential. In the case of crop breeding, genetic advances in yield and stress resistance have decreased in recent decades despite the increased adoption of molecular approaches (e.g. marker-assisted selection, transformation). Increased evidence shows that phenotyping, particularly at the field level, is actually limiting the efficiency of conventional breeding as well as preventing molecular breeding from delivering all its potential (Araus et al. 2008; Cabrera-Bosquet et al. 2012; Cairns et al. 2012; Cobb et al. 2013). Constraints in field phenotyping capability limit our ability to dissect the genetics of quantitative traits, particularly those related to stress tolerance. The development of effective field-based high-throughput phenotyping platforms (HTPPs) remains a bottleneck for future breeding advances (Araus and Cairns 2014). However, progress in sensors, aeronautics, and high-performance computing are paving the way. 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 of genotypic variability is usually far smaller than that caused by changing environmental conditions, and the target is to assess a large number of genotypes.

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; therefore, they are difficult to extrapolate to the field (Fig. 1.1). 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 because declining soil moisture content is associated with increased mechanical impedance in the field, which is an effect that is hard to replicate within pots (Cairns et al. 2011).

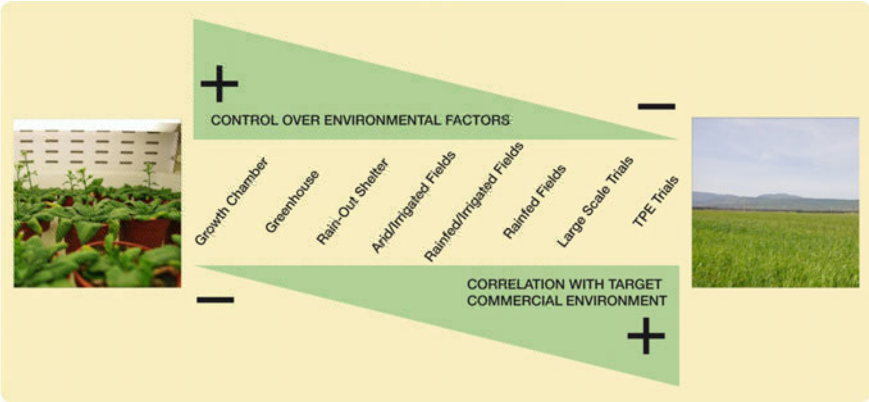

Fig. 1.1 Continuum of environments for drought resistance screening. The control over environmental factors decreases from the use of growth chambers to the target population environment (*TPE*) while the correlation of performance with the target commercial environments increases. Figure redrawn from Passioura (2006)

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, 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 (Fig. 1.2). 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). In practical terms, the second and third categories of traits may be considered within the same group of traits because NIRS may be eventually applicable to many of the traits usually analyzed in the laboratory.

1.2 Phenotypic Traits: Remote Sensing

Ground-based HTPPs allow data to be captured at the plot level, thus requiring little postprocessing. Moreover, this approach allows the implementation of closed multispectral imaging systems, which shut 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). Also, in the case of maize, its use is not very feasible, except for early stages of the crop (Montes et al. 2011).

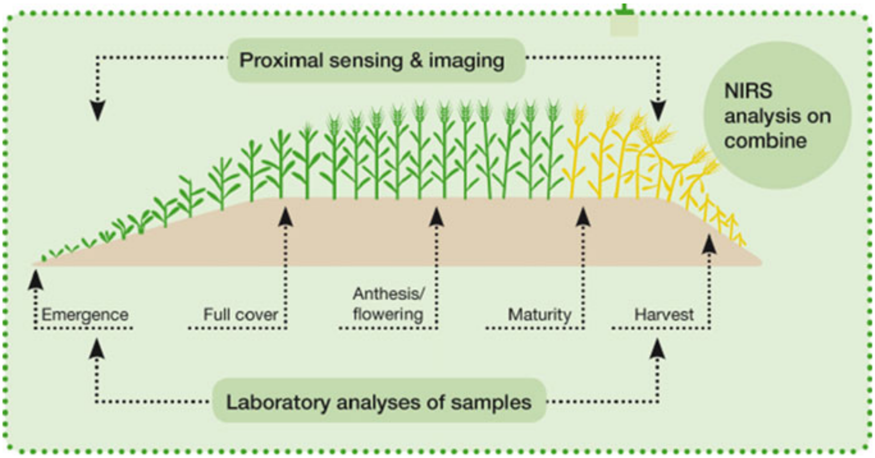

Fig. 1.2 Diagram of the main categories of phenotyping techniques deployed over the lifecycle of an annual seed crop, such as a cereal. Types of data acquisition include proximal sensing and imaging at frequent intervals, laboratory analyses of samples taken at specific intervals, and near-infrared spectroscopy (*NIRS*) on leaf matter or seeds to assess phenotypic traits potentially related with cereal performance under water stress, such as mineral content, stable carbon and oxygen isotope composition, or total nitrogen content (Cabrera-Bosquet et al. 2009a, b, 2011b). Redrawn from White et al. (2012) and Araus and Cairns (2014)

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

For almost any of the remote techniques, the use of imaging allows upscaling of the measurements—for example, from a single plot basis to dissecting an entire trial composed of different plots—provided 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 (Fig. 1.3A). It allows the estimation of green biomass, through a vegetation indices such as the normalized difference vegetation index (NDVI). Estimating the green leaf area index (GLAI, the ratio of green photosynthetic leaf area per ground area) is the proper way to assess the effect of drought (or any other stress that accelerates senescence) on potential canopy photosynthesis and thus grain yield (Lopes et al. 2011; Nguy-Robertson et al. 2012). 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 near infrared (NIR), with the latter model having a weight of 100 g. Multispectral cameras are widely used for crop monitoring via remote sensing (Fig. 1.3B). They can acquire a limited number of spectral bands at once in the visible (VIS)–NIR regions.

Fig. 1.3 Different categories of imaging systems for remote-sensing evaluation of vegetation. These include RGB/CIR **a** multispectral; **b** hyperspectral; **c** thermal; **d** conventional RGB; **e** cameras

Besides vegetation indices for evaluating green biomass, multispectral imagers can be formulated to other different 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–visible near-infrared (VNIR) imagers (Fig. 1.3C) allow the acquisition of hundreds of images at once, covering the entire electromagnetic spectrum between the VIS and the NIR regions in a continuous mode (wavelengths ranging from 400 to 900 nm). Other configurations cover the range from 1,000 to 2,500 nm. Therefore, it is possible to run empirical calibrations (like in a "NIRS-mode") against a wide and miscellaneous set of traits.

Figure 1.3C depicts the Micro-Hyperspec VNIR model (http://www.headwallphotonics.com/Portals/) which measures up to 260 bands of 5–7 nm full-width half-maximum in the 400–885 nm spectral region. This is a particularly promising approach given the possibility for multispectral information to predict complex traits, such as grain yield (Weber et al. 2012). Longwave infrared cameras or thermal imaging cameras render infrared radiation in the range of micrometers as visible light (Fig. 1.3D). The potential use of thermal imaging in phenotyping includes predicting water stress in crops. Thermal sensing has been used to assess maize response 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 × 512 resolution (http://www.flir.com/cvs/cores/view/?id=51374) and the Thermoteknix Miricle camera with a 640 × 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 thermostabilized. Conventional digital RGB cameras (Fig. 1.3E) 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 one to assess chlorophyll and nitrogen content from digital images (Rorie et al. 2011). They can eventually replace portable chlorophyll meters, which cost several thousands of dollars. Moreover, the software needed is usually freely available (Casadesús et al. 2007).

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).

In the case of maize, its height prevents (or at least makes difficult) the use of growth-based platforms, such as phenomobiles (Deery et al. 2014), except for in the early phases of the crop. In these crops, the use of aerial HTPPs becomes a need. Considering cost and versatility, the use of unmanned aerial vehicles (UAVs) is the most promising alternative, compared with the use of cranes, tethered balloons, or manned aircrafts, to install remote-sensing approaches (Fig. 1.4). On the other hand, research on affordable technologies also should be a priority if the adoption of quality field high-throughput phenotyping is pursued for small companies and national agricultural systems from developing countries. These low-cost technologies include remote-sensing approaches, such as the use of RGB imaging and the implementation of NIRS calibrations of key analytical components.

In any case, improvements in user-friendly data management, together with a more powerful interpretation of results, should increase the use of field HTPP. Overall field high-throughput precise phenotyping needs to be placed in its right context as a one of the components that integrates advanced crop breeding, together with molecular biology, quantitative genetics, and even modelling (Cabrera-Bosquet et al. 2012; Araus and Cairns 2014; Cooper et al. 2014).

Fig. 1.4 Example of an aerial platform developed by the University of Barcelona in collaboration with Airelectronics and the Instituto de Agricultura Sostenible (Spain), sponsored by the Global Maize Program of CIMMYT (International Maize and Wheat Improvement Center)

1.3 Phenotypic Traits: Laboratory Analyses

In addition to proximal sensing approaches, the analysis of plant samples may complement direct phenotyping under field conditions. This is the case, for example, with stable isotopes (Yousfi et al. 2012). When breeding for yield potential and adaption to abiotic stresses such as drought, carbon isotope composition ($\delta^{13}C$) in dry matter, frequently expressed as a discrimination ($\Delta^{13}C$) against the source (i.e. atmospheric) CO_2, is a very promising tool that frequently exhibits high heritability and genetic correlation with yield (Condon et al. 2002, 2004; Araus et al. 2013); it has already been applied to breeding programs for C_3 cereals such as wheat (Rebetzke et al. 2008). However, its use as a phenotypic trait for crops such as maize (as well as sorghum, sugar cane, pearl millet, and others) appears to be limited because the specific characteristics of their photosynthetic C_4 metabolism makes the range of response of $\delta^{13}C$ to varying water conditions far smaller (and in the case of maize, in a opposite direction) than for crops with C_3 metabolism (Farquhar 1983; Henderson et al. 1992). Even so, $\delta^{13}C$ still allows one to differentiate between growing water conditions in maize (Cabrera-Bosquet et al. 2009a), as well as between hybrids and inbred lines (Araus et al. 2010) and highly

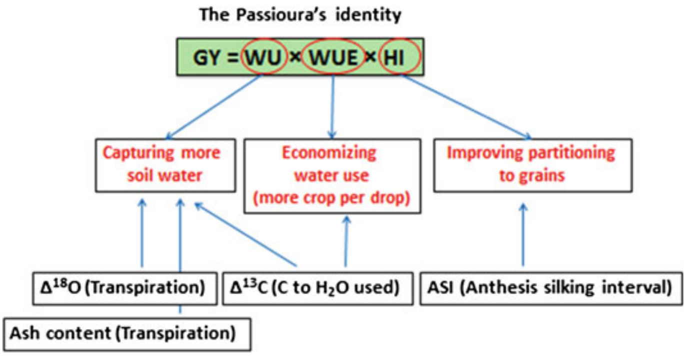

Fig. 1.5 Potential analytical traits to phenotype for crop performance under water-limited environments. The physiological meaning of traits is placed in the context of the Passioura's identity (Passioura 1977). $\delta^{13}C$, carbon isotope composition or $\Delta^{13}C$, carbon isotope discrimination; $\delta^{18}O$, oxygen isotope composition; $\Delta^{18}O$, oxygen isotope enrichment with regard to water. ASI is placed here as example of successful trait related with HI, in this case for maize. For this and other crops phenological traits such as date of flowering may be also relevant

heritable significant genetic variation for Δ13C has been detected under field and greenhouse conditions (Gresset et al. 2014).

As for C_3 species, in maize, $\delta^{13}C$ (or $\Delta^{13}C$) is an indicator of water use efficiency (Farquhar 1983; Henderson et al. 1992) but it also informs indirectly on water use (Cabrera-Bosquet et al. 2009a) (Fig. 1.5). Oxygen isotope composition ($\delta^{18}O$) on dry matter (sometimes expressed as enrichment from the source water, $\Delta^{18}O$) is an indicator of transpiration and therefore water used by the plant (Barbour et al. 2000; Farquhar et al. 2007; Cabrera et al. 2011a). Moreover, it is independent of the kind of photosynthetic metabolism that makes at first its use in feasible for maize (Cabrera-Bosquet et al. 2009b; Araus et al. 2010). However, to date, the use of $\delta^{18}O$ for breeding has been less promising than initially expected, probably due to a set of miscellaneous factors that affects ^{18}O isotopic signature, such as the plant's source (s) of water (irrigation, rainfall, water table may have different $\delta^{18}O$) or the kind of tissue analyzed (^{18}O fractionation in the assimilates moving from the photosynthetic to the reproductive tissues probably exists). A relatively low-cost trait with low technical demands to assess plant transpiration and thus water used in an integrated manner is the total amount of minerals accumulated in transpiring organs, which in its simplest approach consists of analyzing the ash content (Cabrera-Bosquet et al. 2009a).

NIRS is regularly used to analyze in (intact) seeds the protein, nitrogen, starch, and oil content, as well as grain texture and grain weight, among others (Montes et al. 2007; Hacisalihoglu et al. 2010; Mir et al. 2012; White et al. 2012). In any case, the NIR spectrum captures physical and chemical characteristics of the samples, either of vegetative plant tissues or harvested seeds. By using calibration models, several traits can be determined on the basis of a single spectrum. However, the same spectrum may be used to develop prediction models for analyzing traits of potential