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Advances in plant phenotyping for more sustainable crop production

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Contents

Part 2 Sensor types

Part 3 Carrier/delivery systems

Part 4 Data analysis

Introduction

Plant phenotyping is rapidly developing technology that involves the quantitative analysis of structural and functional plant traits. It is widely recognised that phenotyping needs to match similar advances in genetics if it is to not create a bottleneck in plant breeding.

This volume reviews the wealth of research on advances in plant phenotyping for more sustainable crop production, focusing on new technologies such as optical and thermographic sensors, as well as alternative carrier systems such as field robots and unmanned aerial vehicles (UAVs). The book is split into five parts: Part 1 examines the development of phenotyping as a research field, chapters in Part 2 describe the different sensor types that can be used and Part 3 chapters look at the use of carrier and delivery systems in crop phenotyping. Chapters in Part 4 review data analysis in crop phenotyping systems. The three final chapters of the book in Part 5 provide case studies on using phenotyping techniques in different settings.

Part 1 The development of phenotyping as a research field

The book opens with a chapter that focuses on origins and drivers of crop phenotyping. Chapter 1 begins by outlining how plant phenotyping has developed over recent decades, driven by factors such as advances in optical sensors, image analysis and automation as well as multidisciplinary cooperation in establishing facilities for high throughput plant phenotyping. The chapter also describes successful uses of plant phenotyping and stresses the importance of collaboration in further development, particularly to address emerging potential bottlenecks such as management of data to enable interoperability. It highlights the development of networks across centres and why plant phenotyping will always require multi-disciplinary and multi-site resources and expertise to improve the knowledge about plant-environment interactions.

Chapter 2 examines the evolution of trait selection in breeding. It first outlines a historical basis for plant selection and examines the areas in which sensor technology has evolved from our eyes to the application of proximal and remote sensing. The chapter also discusses the use of none-invasive methods that can aid with selection of crop characteristics critical for improving yield potential, such as photosynthesis and partitioning-related traits, as well as the detection of traits that help protect yield, related to disease and lodging resistance. It also examines how trait selection could look in the future.

Part 2 Sensor types

The first chapter of Part 2 reviews advances in optical analysis for crop phenotyping. Chapter 3 begins by focusing on optical sensors and the recently published literature in this area. The chapter also provides a comparison of advantages and disadvantages between the different sensor options. Moreover, several challenges related to the application of optical sensors are discussed. These include the distribution of stresses, compounds and colour across the canopy, but also the complexity of the canopy's 3D structure, diurnal changes in plants and the impacts of rapidly changing environmental conditions. The chapter also provides potential solutions to these challenges through the inclusion of case studies and it discusses relevant technology trends.

The subject of Chapter 4 is advances in the use of thermography in crop phenotyping. The chapter first describes the foundational theory of thermography and then goes on to highlight the principles of thermography measurements. The chapter discusses the different technologies and procedures currently in use and what their limitations are. Two case studies demonstrate the use of thermography phenotyping in different settings. The first focuses on thermography phenotyping in plant breeding and the second case study focuses on the application of thermography phenotyping in Australian environments. Sections on the main challenges and future trends of thermography in crop phenotyping conclude this chapter, highlighting the relevance of airborne imaging because this can facilitate simultaneous analyses of a high number of plots.

Chapter 5 examines advances in the use of X-ray computed tomography in crop phenotyping. The chapter addresses the physical basis and the most relevant hardware components of a computed tomography system used for crop phenotyping. The chapter reviews different system setups and ways of acquiring a computed tomography dataset. It also discusses the post processing of the generated data using specialized algorithms for segmentation. For above- and belowground phenotyping applications two examples are shown to present the utilization of X-ray computed tomography for crop phenotyping: Belowground phenotyping of potato tubers and aboveground phenotyping of wheat ears.

Part 3 Carrier/delivery systems

Part 3 begins with a chapter that focuses on the use of field robots for plant phenotyping. Chapter 6 first provides an overview of current research on different morphologies of plant-phenotyping robots and state-of-the-art sensors and technologies for automatic plant phenotyping. The chapter summarises the advantages of and problems associated with this research field and presents

prospects for future development. Platforms are developed in many variations, with some being capable of carrying high loads and allowing simultaneous measurements with multiple sensors. A section on sensors and technologies for phenotyping field robots is also provided, which is then followed by an analysis of using robotic arms for fruit phenotyping and harvesting, before providing a summary of how using field robots for phenotyping can improve the overall collection of phenotypic information from crops.

Chapter 7 examines the advances in high-throughput crop phenotyping using unmanned aerial vehicles (UAVs). The chapter first discusses approaches to UAV remote sensing and data analysis for high-throughput field phenotyping and ecophysiological research. The chapter reviews the use of UAV remote sensing to measure key plant traits through a plant's lifetime: canopy cover; leaf area index; plant emergence, density and tillering; plant height, growth and lodging; biomass; flowering, fruiting and yield; senescence; and canopy temperature. The chapter concludes with reflections on the need for plant scientists and remote sensing specialists to interact closely with each other for unlocking the full potential of their data in the interest of a more sustainable agriculture.

Part 4 Data analysis

Part 4 opens with a chapter that focuses on the advances in computer vision (CV) and crop phenotyping through data challenges. Chapter 8 begins by reviewing the key dimensions that should be considered in CV applications in plant phenotyping, focusing specifically on dimensions such as trait or scenario, plant type and organ and scale. The chapter also examines the technical issues such as hardware constraints and various data analysis needs. An analysis of the Computer Vision Problems in Plant Phenotyping (CVPPP) Workshop is also included, focusing on how the CVPPP workshop not only helped to attract experts already interested in the area, but also attracted new researchers. The chapter then goes on to discuss the various data challenges and how these can be used to accelerate progress in CV techniques, such as leaf counting and segmentation. This is followed by a review of recent agriculture-related CV challenges.

The subjects of Chapter 9 are digital phenotyping and how genotype-to-phenotype (G2P) models are implemented to predict complex traits in cereal crops. The chapter first reviews the use of digital phenotyping as a tool to support breeding programmes, then goes on to examine various G2P models and how the data collected from phenomics and envirotyping can be used in predictive breeding. The current status of statistical models to incorporate secondary traits in univariate and multivariate models, as well as how to better handle longitudinal traits - such as light interception, biomass accumulation or canopy height - is also reviewed.

Chapter 10 examines the role of crop growth models in crop improvement: integrating phenomics, envirotyping and genomic prediction. The chapter shows how crop models can be used to explore knowledge of phenotype, environment and genotype to better understand and predict their complex interrelationships. It describes how crop growth models have been used in envirotyping and in setting targets for phenotyping programmes. It also describes current limitations in modelling, how these are being addressed as well as emerging trends.

Part 5 Case studies

The final part of the book begins with a chapter that addresses using phenotyping techniques to analyse crop functionality and photosynthesis. Chapter 11 first reviews the relation of photosynthesis to crop growth and stress response. It then goes on to examine phenotyping photosynthesis in varying environmental conditions, focusing specifically on climate effects and photosynthesis. A section on using gas exchange to analyse photosynthesis is also included, which is then followed by a review of using porometry and thermal imaging of stomatal conductance and hyperspectral techniques. The chapter also provides a review of using chlorophyll fluorescence and analyses how photosynthesis can be affected by heat stress, drought stress and elevated carbon dioxide levels in the atmosphere. Four case studies analysing these effects are also provided.

Chapter 12 examines the use of phenotyping techniques to predict and model grain yield: translating phenotyping into genetic gain. The chapter describes the importance of boosting genetic gain in grain yield by focusing on phenotypic traits such as plant height, flowering time, aboveground biomass and ear density. A section on stomatal conductance is also provided, which is then followed by a review of the functional 'stay green' trait. A case study that analysed the difference between the data obtained from advanced sensors, digital cameras and UAV technology assessing grain yield in wheat in Spain is also provided.

The final chapter of the book focuses on the automated assessment of plant diseases and traits by sensors, as well as how digital technologies can support smart farming and plant breeding. Chapter 13 begins by providing an overview of digital plant disease detection, focusing on aspects such as non-invasive technologies, motivation, optical sensors and the combination with data analysis and carrier platforms at different scales. The chapter moves on to address the complexity of host-pathogen interactions as well as the complexity in a crop stand. A case study that examines the application of deep learning to foliar plant diseases is also included, which is then followed by a summary of why developing digital technologies is important in improving plant disease detection.

Chapter 1

Origins and drivers of crop phenotyping

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1 Introduction

Understanding the phenotype of plants is essential in the context of food or biomass production from crops, for efficient use of resources such as water or nutrients or in understanding plant ecological performance. All these depend on the interaction between plant genetic makeup and the prevailing environment. Understanding multidimensional plant–environment interactions has a long history in the eco-physiological sciences. The subject gained new momentum when genomics technologies became available about three decades ago. An increasing number of plant genome projects were initiated to analyse the genetic makeup of plants. Within the last few decades, about 600 genome assemblies from different plant species have been made available in public repositories (Kersey, 2019). Crop species dominated initially but a wider range of plants, including non-domesticated species, have now been analysed. In parallel with these developments, there have been advances in technologies to modify plant genetics. Recent progress in genetic engineering –specifically CRISPR/CAS9 – provides '…enormous power in this genetic tool, which affects us all. It has not only revolutionised basic science but also resulted in innovative crops and will lead to ground-breaking new medical treatments', to quote Claes Gustafsson, chair of the Nobel Committee for Chemistry. (https://www.nobelprize.org/prizes/chemistry/2020/press-release/).

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However, genetic makeup provides only one element that determines the phenotype of a plant. Genes are the toolbox with which a plant can work when the plant is exposed to challenges – especially abiotic and biotic environment – in real life. Plant phenotyping is the process of quantitatively characterising the structural and functional properties of a phenotype within a dynamic environment. It is the next step beyond genotyping towards an integrated understanding of plant–environment interactions (Fig. 1). Based on a wealth of research, in the late 1990s plant phenotyping started to integrate genomics and eco-physiological perspectives into a more holistic approach. New methods for high-throughput phenotyping were based on technologies like computers, optical sensors and automation. It is worth remembering that computers only became generally accessible in the 1990s – a fact that we often forget given their ubiquity today. High-throughput genotyping revolutionised our understanding of the genetic makeup of plants. This development then increased the need for quantitative assessment of the phenotype as a basis for understanding plant–environment interactions and for breeding applications.

The challenges posed by the complexity of plant structures and functions and their environmental plasticity continue to drive research. This research requires dynamic assessment of individual plant organs, developmental stages, entire canopies or plant processes. These components are interconnected. A change in one has an impact on the others and, in turn, the whole plant and its interactions with the environment. Continuous measurements with non-invasive technology have provided the key to connecting phenotypic dynamics, molecular properties and environmental dynamics to create models to visualise and predict these complex interrelationships and transfer them into applications such as breeding.

Phenotypic data are pivotal to understanding quantitative traits which are under polygenic control. Quantitative description of a phenotype is extremely challenging. It ranges from subcellular, cellular, tissue and organ levels up to the level of the whole organism or even the arrangement of individual plants in a stand. These different levels must be analysed in the context of dynamic

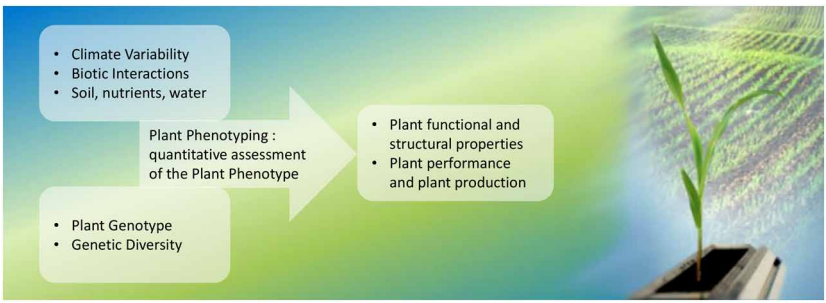

Figure 1 Multidimensional system addressed by plant phenotyping.

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environmental conditions in space and time (Pieruschka and Poorter, 2012). Houle et al. (2010) described this relation using genotype–phenotype maps to determine the phenotypic state that organisms occupy within the overall space of possible phenotypes, taking into account phenotype response to variation in environmental conditions within a complex multidimensional system (Fig. 1). This level of analysis requires robust and accurate measurement technologies (technology development), integration of technologies in phenotyping infrastructures and applications (phenotyping infrastructures), use of phenotyping systems in interaction with plant material which has been properly characterised in its genetic setup (genotype analysis) and with analysis of its often dynamic (bio-)chemical composition (chemical phenotype). Only by combining all these dimensions in adequate temporal and spatial domains, can a mechanistic understanding of interactions of genetics, structural, physiological and environmental processes be made. Since this is the basis for crop improvement, a concerted effort is essential to further advance the quantitative understanding of crops in a dynamic environment. This is particularly important with multiple grand challenges related to feed and food for a growing population in times of climate change (FAO, 2017).

Selection based on phenotypic traits has been the basis for crop improvement for thousands of years and has been fundamental for breeding from the beginning of crop domestication until genetic tools became available. Wheat is an outstanding example where phenotypic selection based on traits such as large seeds, plants with low toxicity and reduced seed dispersal was essential for adaptation of plants to crop cultivation. The success of using early, non-quantitative phenotyping was vital to the shift from hunter-gatherer to agricultural societies, and it stimulated the development of cities and modern civilisations. The increased understanding of genetics, which became available during the Green Revolution, allowed genetic improvement towards much higher-yielding varieties through the introduction of dwarfing traits with huge increases in wheat yield (Vergauwen and De Smet, 2017). The development of various DNA/gene marker technologies shifted the emphasis of breeders from selection based on phenotypes to marker-assisted methods based on access to cheap sequencing methods (Collard and Mackill, 2008). Phenotypes were linked to DNA-markers which could then be used to assess crossing material without expressing the phenotype at each step of the selection process. This has been critical to the success of modern breeding. One study has shown that, on average and across all major arable crops cultivated in Europe, quantitative phenotyping contributes approximately 74% to overall productivity growth (Noleppa, 2016).

Plant phenotyping has been recognised as one of the major limitations to further advances in breeding and pre-breeding (Watt et al., 2020). Progress in phenotyping requires improved technology and access to state-of-the-art

methods and facilities, as well as dissemination of novel technologies to phenotype an increasing diversity of traits under controlled and field conditions above and belowground. These must be combined with integrated data management techniques to allow reuse of data.

This chapter outlines how plant phenotyping has developed over recent decades, driven by factors such as advances in optical sensors, image analysis and automation as well as multidisciplinary cooperation in establishing facilities for high throughput plant phenotyping. The chapter describes successful uses of plant phenotyping and stresses the importance of collaboration in further development, particularly to address emerging potential bottlenecks such as management of data to enable interoperability.

2 Technological progress in plant phenotyping

The terms phenotype and genotype were first mentioned over a hundred years ago and quantitative plant phenotyping has been a central element of eco-physiological research ever since (Johannsen, 1903, 1911). Over the past two decades, however, there has been a dramatic increase in the accuracy and speed of phenotyping with new technologies in high-throughput based on huge volumes of genetic information from next-generation sequencing. Since then, plant phenotyping has made impressive progress, developing novel sensors and imaging techniques able to quantitatively measure a wide range of traits at many spatial scales and in many different temporal dimensions. Quantitative phenotyping to understand the interaction between plants and environmental conditions is now possible, using low and high-throughput phenotyping for quantitative screening of a high number of genotypes under well-defined conditions at cellular, organ, plant and canopy levels under a variety of environmental conditions.

This progress in phenotyping can be seen in the massive increase in the number of publications addressing plant phenotyping from around 2010 (Costa et al., 2019). This period coincides with the development of tools for quantitative assessment of plant traits that allowed the matching of phenotypic analysis with genetic information from DNA sequencing.

Non-invasive imaging sensors and computer vision technologies have significantly improved measurement of plant traits. These were based on the rapid increase in affordable and robust imaging systems in the 1990s following the development of digital imaging sensors (Blais, 2004). Non-invasive technology initially focused on recording time series at the level of single organs in single (or a few) plant(s). Schmundt et al. (1998) were able to demonstrate the temporal and localised character of growth in leaves of *Ricinus communis* and tobacco with growth restricted to the base of the leaf and to a few hours at the end of the night and the start of the day. The ability to obtain

high-resolution time-lapse videos, paired with development of algorithms in image analysis, represented a substantial advance in understanding growth processes. The progress was possible by linking image analysis expertise with plant physiology. Similar examples include characterisation of growth of primary roots (Walter et al., 2002) and functional imaging of photosynthesis (Genty and Meyer, 1995).

Newly developed imaging systems and the increasing number of image analysis programs led to the next step in the evolution of phenotyping. These phenotyping approaches were integrated into automated systems allowing increasingly high throughput. Automated systems first addressed phenotyping of small plants such as *Arabidopsis* to measure growth dynamics (Granier et al., 2006) or *Arabidopsis* and tobacco seedlings to measure growth and photosynthetic properties (Jansen et al., 2009). These marked an important step towards measuring genetic variability. The importance of imaging approaches in plant phenotyping can be seen in the growth in publications using the terms plant phenotyping with different types of imaging over the last decade (Fig. 2). This analysis shows that functional imaging (spectral, hyperspectral, thermal) also grew over the same decade. Merging these different imaging modes (sensor fusion) may become an important tool in the future, enabling simultaneous monitoring of the dynamics of structural and functional properties by combining measurement of 3D plant structure with spectral measurements (Paulus, 2019). Increased use of imaging can be expected with the development of affordable, mobile technologies providing high-quality images (Mueller-Linow et al., 2019; Reynolds et al., 2019).

Many of the ongoing developments in plant phenotyping are driven by developments in non-invasive sensors providing information from the entire electromagnetic spectrum. A wealth of information can be obtained from data from reflected, transmitted or emitted radiation. The magnetic resonance

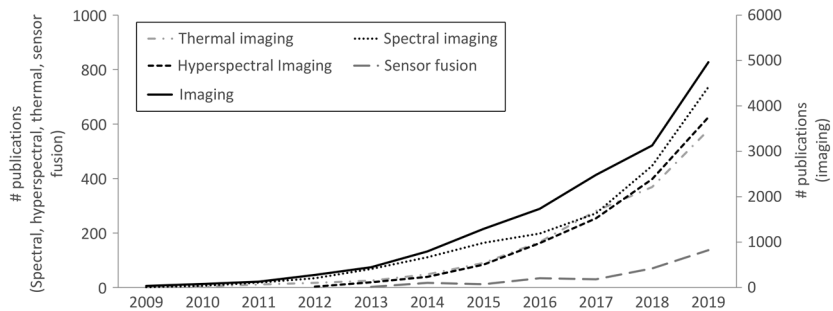

Figure 2 Number of publications using imaging technology. Web of Science (Clarivate Analytics) was used for a simple evaluation of publications within the last 10 years with search terms: phenotyping x imaging, spectral imaging, hyperspectral imaging, thermal imaging and sensor fusion.

Index