EXPERT VIEW

All roads lead to growth: imaging-based and biochemical methods to measure plant growth

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Abstract

Plant growth is a highly complex biological process that involves innumerable interconnected biochemical and signalling pathways. Many different techniques have been developed to measure growth, unravel the various processes that contribute to plant growth, and understand how a complex interaction between genotype and environment determines the growth phenotype. Despite this complexity, the term ‘growth’ is often simplified by researchers; depending on the method used for quantification, growth is viewed as an increase in plant or organ size, a change in cell architecture, or an increase in structural biomass. In this review, we summarise the cellular and molecular mechanisms underlying plant growth, highlight state-of-the-art imaging and non-imaging-based techniques to quantitatively measure growth, including a discussion of their advantages and drawbacks, and suggest a terminology for growth rates depending on the type of technique used.

Keywords: Biomass, growth, imaging, kinematics, morphometrics, phenomics, phenotyping, relative expansion rate of growth (RER), relative growth rate (RGR).

Introduction

Due to the continued increase of the world's population, it is projected that annual global food production needs to increase by ~25–70% by the year 2050 (Hunter et al., 2017). Already today, the Food and Agriculture Organization of the United Nations (FAO) estimates that over 800 million people are undernourished (FAO et al., 2018). Furthermore, climate change and global warming are predicted to dramatically reduce crop yields (Zhao et al., 2017). Environmental stresses cause massive agricultural losses by restricting photosynthetic capacity and nutrient acquisition, resulting in inhibition of plant growth and development. To overcome these limitations, it is essential to decipher the complex relationship between a plant's genotype and environment that determines the plant's growth phenotype (Fig. 1A), in order to understand how plants grow throughout their life cycle under various conditions. This review will highlight the complexity of plant growth, focusing on above-ground organs (shoots) of vegetatively growing plants, and explore state-of-the-art methods for growth quantification (Fig. 1B; Box 1). It should be noted that many studies concentrate on the model plant Arabidopsis thaliana, which we will focus on in this review. However, knowledge gained from Arabidopsis has to be transferred to crops, such as wheat, maize, rice, potato, and tomato in the future (Kojima et al., 2002; Lifschitz et al., 2006; Navarro et al., 2011).

Shoot growth: processes and definitions

Plant growth begins with embryogenesis and involves passing successively through several developmental stages to establish
the architecture of the mature plant, including the shoot apical meristem (SAM) (Boscá et al., 2011). Once formed, the SAM is responsible for the overall shoot growth of the plant through the presence of stem cells. These allow plants to grow throughout their whole life span (Williams and Fletcher, 2005), and provide highly plastic developmental flexibility to adapt and reprogram growth in response to environmental perturbations (e.g. Olas et al., 2019). Many interconnected processes control shoot growth. First, cell division is followed by cell expansion and cellular differentiation (Vanhaeren et al., 2015). Second, it involves developmental programs that regulate when cells divide, in which plane they divide and whether they continue to divide or start to expand and differentiate, and what type of cell they differentiate into. Third, growth requires the synthesis of cellular components, for example nucleic acids, proteins, and lipids. Fourth, especially in the cell expansion phase, it involves ion and water uptake into the large central vacuole, that typically occupies up to 90% of a mature plant cell (Taiz, 1992). Thus, understanding growth requires information about these factors and how they interact to regulate the various processes that contribute to growth. However, the term ‘growth’ itself is used inconsistently in the plant science community, with the usage depending on the method used to measure growth. Three different definitions of growth are currently used: (i) an increase in observed plant size, typically quantified by taking images of plants, for example by phenotyping systems; (ii) dynamic changes in cell architecture (size, number, and shape) and the emergence of new organs, for example measured by microscopic imaging techniques; and (iii) gain in structural biomass, for example quantified by measuring fresh weight, structural dry weight or, more specifically, carbon (C) deposition in biomass. Although none of these definitions of growth is inherently wrong, the hierarchical organisation of plant morphology and anatomy (whole plant, organs, tissue, cell, organelle, membrane, and metabolites, proteins, and genes) limits our comprehensive understanding of plant growth when only one or a few of these are studied in isolation (Passioura, 2010). The integration of the different functional levels and large-scale biological interactions between scientists of different disciplines is needed to capture the essence of plant growth (Passioura, 2010).

Plant growth rates are best determined by sequential measurements at several time-points and can be derived as the slope of a semi-logarithmic plot (Evans, 1972; Hoffmann and Poorter, 2002). In favourable conditions, a vegetative plant typically grows at over 20% per day, which in a few days leads to a doubling in size or biomass. The growth rates of individual parts of the plant can vary greatly, with young organs showing much higher growth rates (Asl et al., 2011). At a whole-plant level, growth rate typically declines with plant age. Importantly, there is no necessary connection between plant size or biomass and the rate of growth at a given time. Larger size or biomass may reflect faster growth at that time, but can also reflect faster growth at an earlier time in the plant’s life history. For this reason, temporal analyses and determination of growth rates are an integral component of any study of
Box 1. Key developments in measuring plant growth

• Imaging system based on laser-scanning
  
  Dornbusch et al. (2012) used laser-scanning to obtain 3D image sequences of plants growing in 12–36 pots (each containing six plants) over long time periods with a temporal resolution of 40–60 min. The system was used to study leaf elongation simultaneously with hyponasty (Dornbusch et al., 2014). A laser stripe is emitted and a camera records the reflectance. The information is used to calculate the distance based on the known positions of the camera and laser.

• Imaging system based on a light-field camera
  
  This phenotyping system presented by Apelt et al. (2015) is built around a light-field camera that provides 3D information. The camera utilises an array of calibrated microlenses with various focal lengths that is placed in front of an image sensor, onto which the single lens projects the image. For each recording, the camera provides two types of images, a classical 2D focus image and a depth image that provides highly spatially-resolved information about the distance of the object from the camera. A robotic platform enables high-throughput time-series imaging of up to 48 Arabidopsis rosettes with a time resolution of five images per plant per hour. This system enables monitoring of the plant’s expansion growth as well as analyses of leaf movement and rosette architecture.

• Imaging system based on photometric stereo
  
  This low-cost and portable system described by Bernotas et al. (2019) employs a photometric stereo-imaging technique in order to obtain 3D plant growth over the diel cycle for up to nine Arabidopsis plants simultaneously. Sets of plant images are captured under controlled, varied, and directional illumination that allows surface 3D reconstruction at high spatio-temporal resolution and accuracy. Artificial neural networks are used for leaf segmentation using 3D data.

• Kinematic model of leaf development
  
  Asl et al. (2011) present a model that describes the division and expansion rates for individual epidermal cells of leaf pairs of Arabidopsis. The following kinematic growth parameters are recorded at 1-day intervals throughout leaf development: leaf area and LER, number of guard and pavement cells and their sizes, stomata index, and cell division. From these data, the authors were able to calculate the probability for a cell to divide into two guard cells or two pavement cells and the maximum size of the cell at which it can divide and its RER. The model demonstrated that RER differs between guard and pavement cells, being initially much higher in pavement cells compared to guard cells and declining afterwards. This demonstrates that pavement cells grow faster in young, proliferating leaves than in older ones.

• Quantification of morphogenesis
  
  de Reuille et al. (2015) present an open-source 3D imaging platform called MorphoGraphX that is used to quantify temporal plant morphogenesis, thus providing an opportunity to investigate size, geometry, growth, and proliferation of cells. The platform has a user-friendly interface for data visualisation and editing.

• Whole-plant C balance model
  
  In this approach, first used by Sulpice et al., (2014), measurements of photosynthesis, respiration, and changes in starch and metabolite levels between two time-points (e.g. dawn and dusk, or vice versa) are used to estimate the rate of C deposition into biomass (Mengin et al., 2017; Flis et al., 2019; Moraes et al., 2019). In the model, the amount of C in each major metabolite (starch, malate, fumarate, sucrose, glucose, fructose, amino acids) is obtained by multiplying the amount of a given metabolite by the number of C atoms in the molecule. The rate of C deposition in metabolites is then calculated as the difference in total summed C between the two time-points divided by the time interval between them. C allocation to growth is estimated in the light as the net rate of C assimilation minus the rate of C deposition. C allocation at night is estimated as the rate of C deposition minus the net rate of respiratory C loss (Sulpice et al., 2014; Mengin et al., 2017; Moraes et al., 2019). This approach can be extended to investigate plant strategies to optimise growth, and to identify metabolites that correlate with biomass gain, for example the sugar-signal trehalose 6-phosphate (Tre6P) (Sulpice et al., 2014).
growth. We recommend that growth rates in terms of biomass increase are defined as relative growth rates (RGR), whereas growth rates in terms of increase in observable size or area are defined as relative expansion rates of growth (RER).

**Imaging-based techniques to determine shoot and cellular growth**

**Shoot phenotyping: imaging platforms**

Imaging-based plant phenotyping is nowadays an essential aspect of virtually all plant-related research and aims to accurately quantify and measure anatomical and physiological plant features of increasingly large numbers of biological samples (Fiorani and Schurr, 2013; Walter et al., 2015). With increasing computational power and digitalisation, the potential of high-throughput phenotyping methods based on imaging has greatly increased, and such methods are gaining more and more attention (Jaffe et al., 1985; Spalding and Miller, 2013). This is reflected by an increasing number of publications, many national and international networks, and grant initiatives that support plant phenotyping (Box 2). The technical applicability depends on the biological question: while cameras for spectral reflectance/absorbance and thermography are quickly becoming more common as indicators for compositional features such as chlorophyll content (Malenovský et al., 2013) and transpiration/water content (Jones et al., 2009), respectively, they are usually not used to quantify growth. Instead, standard (RGB) cameras are often used for imaging-based methods to study plant expansion growth. Although phenotypic observations cannot directly uncover the molecular network underlying plant growth, they do provide initial information about key physiological features for plant science and agriculture (Passiourea, 2010).

Most imaging-based phenotyping techniques are inseparably linked with automated image analyses (Gupta and Ibaraki, 2014). Segmentation is one of the most critical steps in image processing and splits the pixels of an image into distinct areas, for example plant structure and background. Several obstacles have to be addressed regarding an automated image analysis of plant image sequences: (i) overlapping plant structures, (ii) low contrast between the plant structure and background, and (iii) varying image quality (Walter et al., 2012; Pape and Klukas, 2014).

Many (open-source) plant growth image analysis tools are available at https://www.plant-image-analysis.org/ (Lobet et al., 2013). However, many methods lack a robust validation (benchmarking) and long-term maintenance (Lobet, 2017). Shoot phenotyping is technically challenging and the methods currently used often lack high spatial or temporal resolution (Spalding and Miller, 2013). Non-invasive continuous day and night imaging requires near-infrared (NIR) light sources that are not sensed by plants, as exciting photoreceptors (Kelly and Lagarias, 1985) at night can change plant growth and shape (Nozue et al., 2007). Even if a compromise is made with regards to the time resolution, for example by avoiding the dark phase (Millenar et al., 2005), or by using non-photosynthetically active green light (Zhang et al., 2012), the crucial problem is that conventional 2D imaging camera systems (Schmidt et al., 1998; Leister et al., 1999; Granier et al., 2006; Mullen et al., 2006; Arvidsson et al., 2011; Dhont et al., 2014; Faragó et al., 2018; Fujita et al., 2018) do not distinguish whether an increase in the size of an object represents plant growth, leaf movement (hyponasty), or both. One way to avoid this pitfall is to compare pictures taken at the same time-point each day when leaf angles are supposedly the same, which results in low temporal resolution without information about dynamic changes in diel RER (Pantin et al., 2011). Another approach is to prevent leaf angle changes by physically constraining leaf movement (Schmidt et al., 1998; Walter and Schurr, 2005; Wiese et al., 2007; Poiré et al., 2010), but this means applying forces to growing tissues and potentially interfering with cellular organisation.

Thus, the third dimension is needed for accurate expansion growth quantification with high precision and time resolution. One of the most widely used approaches is (pseudo-) 3D reconstruction by imaging plants from different angles using two (or more) cameras (Biskup et al., 2007; Burgess et al., 2017) or by rotation (Pound et al., 2016). Such systems are affordable and for many biological questions they provide sufficient spatio-temporal resolution and accuracy.
Another method is the ‘depth from focus’ technique that uses the image sharpness as a parameter to estimate the 3D shape of an object, which is set on a motorised stage (Minhas et al., 2009). Other developed techniques are based on non-structured lighting, for example with time-of-flight cameras (Lange and Seitz, 2001). NIR light-emitting diodes (LEDs) are used as a light source to emit a wave, the reflection of which is sensed and measured in order to calculate the distance to the object (Gupta and Ibaraki, 2014). Imaging systems based on structured light have been developed as well. For example, with 2D structured lighting (Salvi et al., 2010) the distance of the object is determined by the deformation of the emitted 2D pattern on the object's surface (Gupta and Ibaraki, 2014). Relatively low-cost Microsoft Kinect-based systems that produce depth images from the analysis of spatially structured lighting patterns using NIR light have also been introduced to study plant growth, for example for crop monitoring in the field (Andújar et al., 2016; Hämmerle and Höflé, 2016; Jiang et al., 2016), in controlled conditions (Vazquez Arellamo et al., 2016), and at the level of individual plant resolution (Chéné et al., 2012; Azzari et al., 2013; Hu et al., 2018).

Currently, one of the most advanced 3D imaging techniques employs laser-scanning based on triangulation (Kaminuma et al., 2004; Dornbusch et al., 2012). For example, Dornbusch et al. (2012, 2014) used laser-scanning to obtain 3D image sequences of plants for several days with a relatively high temporal resolution of 40–60 min to simultaneously study leaf elongation and hyponasty. However, laser-scanning systems tend to be expensive (Paulus et al., 2014) and can be invasive depending on the laser wavelength, power, and exposure time.

An alternative is to use light-field (plenoptic) camera systems (Lippmann, 1908; Ng et al., 2005; Perwass and Wietzke, 2012), which provide a focus image and the depth information in a single exposure. Such a system forms the basis of a phenotyping system that enables high temporal resolution and accuracy in determining Arabidopsis growth behaviour, providing information about the plant's RER, the timing of leaf appearance, hyponastic movement, and the shapes of individual leaves and whole rosettes (Apelt et al., 2015, 2017). Although costly, the system has been the first that has been able to capture 3D rosette area, individual leaf growth, and hyponasty throughout the diel cycle.

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**Box 2. A selection of national and international phenotyping networks and grant initiatives supporting plant phenotyping**

- International Plant Phenotyping Network (IPPN, a global association of phenotyping centers)
- European Infrastructure for Multi-scale Plant Phenomics and Simulation (EMPHASIS)
- French Plant Phenomic Infrastructure (PHENOME)
- Italian Plant Phenotyping Network (PHEN-ITALY)
- Austrian Plant Phenotyping Network (APPN)
- European Plant Phenotyping Network (EPPN2020)
- German Plant Phenotyping Network (DPPN)
- North American Plant Phenotyping Network (NAPPN)
- Nordic Plant Phenotyping Network (NPPN)
- Australian Plant Phenomics Facility (APPF)
- Wheat Initiative

**Illustration of the constantly increasing number of publications related to phenotyping and imaging in plant science over the last 20 years.**
Recently, another alternative low-cost system has been described that uses photometric stereo imaging to obtain 3D images to capture dieI growth and hyponasty of Arabidopsis plants (Bernotas et al., 2019). This technique relies on images acquired under controlled, varied, and directional illumination using one camera with high spatio-temporal resolution and accuracy.

Organ phenotyping: cellular imaging

It is well established that organ growth is determined by cell division and subsequent cell expansion (Green, 1976). Typically, cell division occurs at an early stage during organ growth and is associated with rapid synthesis of cellular components (Shapiro et al., 2016), and is followed by cell expansion (Lockhart, 1965). Analysis of cell division and expansion is essential to understand the mechanisms regulating whole-organ growth.

Destructive methods such as 2D imaging of fixed and morphologically stained tissue sections or imaging of β-GLUCURONIDASE reporter lines are insufficient to provide detailed quantification of cellular growth (Bougourd et al., 2000; Kang and Dengler, 2002), because they are limited by the number of cells that can be imaged simultaneously. Development of fluorescence and laser-scanning confocal microscopy (LSCM) has enabled a new generation of high-sensitivity detectors to image living plant cells in 3D, providing accurate quantitative information about organ morphogenesis. LSCM generates images called z-stacks of 2D optical sections through the sample. This allows the same cells to be monitored over a time series using vital fluorescence markers (Pawley, 2010), and the images are used to reconstruct a 3D object, the parameters of which provide a measure of volume growth. A number of fluorescent proteins (Rizzuto et al., 1995; Nagai et al., 2002; Bindels et al., 2017), targeted to different cell components, can be monitored and quantified, providing information about different aspects of growth (Waters, 2009). In particular, tracking tagged nuclei over time provides detailed information about cell division rates (Dammle et al., 2004), while cell wall or membrane tags enable cell geometry to be studied (Paredez et al., 2006; Crowell et al., 2009; Bassel et al., 2014; Fujimoto et al., 2014; Vellosillo et al., 2015; Liu et al., 2016; Ivakov et al., 2017). Typical cell geometry analyses require 3D segmentation algorithms and specific geometry-based cell-type identification methods to provide quantitative information. To date, different programs have been developed to visualise 3D organ morphogenesis, topological structures, and cellular growth, including open-source applications (e.g. MARS-ALT, MorphoGraphX, and DRACO-STEM) (Fernandez et al., 2010; de Reuille et al., 2015; Cerutti et al., 2017) and costly commercial software (e.g. IMARIS; https://imaris.oxinst.com/). With ongoing improvements of 3D confocal imaging resolution, as well as engineering of novel bright fluorescent proteins with reduced phototoxicity and increased tissue accessibility, those programs can provide better and comprehensive information about cellular growth (Bindels et al., 2017). Moreover, the 3D visualisation of plant structures can be performed using fluorescent staining with vital dyes such as propidium iodide or via macro-optical projection tomography (Lee et al., 2016).

Shoot and cellular phenotyping: morphometrics and kinematics

Basic or local quantitative information about organ or shoot growth and changes in shape can be obtained by measuring morphometric parameters from a time-series of images, for example by using midline-finding techniques (Spalding and Miller, 2013). Time-lapse imaging or replica methods to analyse cell RER can be used to measure the total strain rate of the cell walls (Dumais and Kwiatkowska, 2002; Kwiatkowska, 2005; Kierzkowski et al., 2012). Information about the biological structure can also be obtained by measuring the distribution of local curvature (Kwiatkowska and Dumais, 2003). Using time-lapse images, the plant's surface area can be extracted using open-source software, such as ImageJ (Abramoff et al., 2004), for example to determine total rosette (leaf) surface area (Schnaubelt et al., 2013).

 Whereas morphometric analyses extract geometric features, kinematic approaches have been used to determine the spatial distribution of organ or plant growth over time. Based on the laws of fluid flow dynamics, kinematics provide a powerful mathematical framework to quantify the rates of cell division and cell expansion. For example, to describe organ growth using kinematic parameters, a batch of various features, such as leaf elongation rate (LER), cell length profile, mature cell size (S), the number of cells (N), stomatal density and index, need to be extracted from the microscopic images of cleared leaves, or epidermal peels or imprints, and analysed by mathematical equations (Nelissen et al., 2013). Next, cell production (P) can be calculated as LER/S, and the average division rate equals P/N (Beemster and Baskin, 1998). Kinematics analysis has been widely applied to determine the growth of different plant organs such as roots, stems, shoot apices, and leaves in diverse species (Erickson and Sax, 1956; Silk and Erickson, 1979; Silk, 1984; Sacks et al., 1997; Beemster and Baskin, 1998; Ma et al., 2003; Nelissen et al., 2013). Although kinematic analyses provide accurate results and some specific image-analysis programs have been created, many measurements are performed manually (Lièvre et al., 2013).

Non-imaging-based techniques to measure plant growth

Biochemical methods to estimate RGR based on structural biomass production

Imaging techniques have dramatically improved over recent years and can now monitor changes at the cellular, organ, and rosette level with considerable precision. However, they monitor mainly expansion growth, which is driven by water uptake and vacuole expansion (Gonzalez et al., 2012). Such measurements do not provide information about the timing of synthesis of structural biomass, a fundamental aspect of plant growth. Here, we summarise the commonly used techniques that use the synthesis of structural biomass as a proxy for RGR.

Plants cannot expand in the long term without an increase in structural biomass and proteins. Sulpice et al. (2014) therefore used combined measurements of photosynthesis, respiration,
starch, and other metabolite levels at dawn and dusk to estimate RGR by calculating the rate of C deposition into structural biomass, and numerous studies have also used this technique (Mengin et al., 2017; Flis et al., 2019; Moraes et al., 2019). Such studies, using this so-called whole-plant C balance model, have shown that growth is slower at night than in the daytime, especially in short photoperiods (Stitt and Zeeman, 2012).

The rate of protein synthesis is regulated by changes in total ribosome abundance and the loading of ribosomes into polysomes (Bailey-Serres et al., 2009). Polysome loading is measured by a non-equilibrium density gradient to separate free ribosomes from polysomes. The distribution of ribosome abundance in the gradient is measured by determining their absorbance at 280 nm. Cytosolic polysome loading correlates with sucrose levels during the day, showing that polysome loading tracks the availability of C (Pal et al., 2013; Ishihara et al., 2015) and thus can be used as a proxy for sucrose/C availability and protein synthesis, an important aspect of growth. However, changes in RGR that are estimated based on polysome loading have been found to be smaller than changes in RGR based on the C balance model (Sulpice et al., 2014). Furthermore, polysome loading underestimates the extent to which protein synthesis is inhibited at night (Pal et al., 2013) and it is difficult and time-consuming to use this method (Piques et al., 2009).

Therefore, it is advantageous to measure protein synthesis directly, which can be done using stable isotopes to label proteins and their amino acid precursors. In contrast to single-cell organisms where labelled amino acids, nutrients, or water can easily be supplied via the medium, this is quite difficult in multicellular organisms such as plants. A method to quantify protein synthesis was developed by Ishihara et al. (2015) based on the introduction of a label via $^{13}$CO$_2$ in the atmosphere (Box 1). A key feature is the use of $^{13}$C enrichment in free amino acids to correct for isotope dilution as it moves through the metabolite pools in the central metabolism. The time resolution is limited by the need to obtain enough $^{13}$C incorporation in protein and the need for a large enough period of time between consecutive sampling points to obtain a precise measure of the increase in labelling in a given time interval. Nevertheless, labelling for a few hours, in either the light or the dark, is sufficient and allows growth to be measured at different times in the day. There is a good quantitative agreement between estimated RGRs based on the C balance model and the RGRs estimated by protein synthesis measurements (Sulpice et al., 2014; Ishihara et al., 2015, 2017; Moraes et al., 2019). However, it should be noted that the rate of protein synthesis is not necessarily equivalent to the net rate of growth, because of the protein degradation that happens at the same time (Ishihara et al., 2017).

The cell wall accounts for up to 55% of the rosette dry weight in Arabidopsis (Williams et al., 2010). Furthermore, most cell wall material is not subject to turnover. Thus, measuring the rate of cell wall synthesis provides another way of estimating RGR based on the gain in structural biomass (reviewed in Verbanič et al., 2018). Cell walls are a mixture of cellulose, hemicellulose, pectins, and proteins. About 20–30% of the cell wall consists of cellulose (Somerville et al., 2004; Lodish et al., 2013), and both it and the backbone of hemicellulose consist of glucose moieties. Therefore, incorporation of labelled glucose into the cell wall provides a relatively fast and simple proxy for cell wall synthesis (Ishihara et al., 2015, 2017). This approach has led to estimated average RGRs that are similar to estimates based on plant size measurements (Ishihara et al., 2015).

However, all biochemical methods to estimate RGR based on structural biomass production share common disadvantages: they are time-consuming and intrusive, and, in the case of protein synthesis measurements, only applicable for time-scales that allow a large enough increase in enrichment to be accurately detected.

Conclusions

Plant growth is a highly complex biological process that involves countless interconnected pathways. Several definitions of plant growth are currently used by the plant science community (i.e. increase in the observed size of the whole-plant or parts of the plants, increases in cell number and cell size, and gain in biomass). These definitions largely reflect different ways of measuring growth and they capture various aspects of the interacting processes that underlie plant growth. We recommend using RGR for biochemical-based approaches, and RER for imaging-based techniques to avoid ambiguous usage of the term growth rate itself.

While whole-shoot phenotyping provides accurate information about expansion growth over longer periods with high spatio-temporal resolution, the results can vary depending on the method used (Box 3). In addition, imaging-based shoot phenotyping does not provide information about cell division and biomass accumulation. This information, however, seems to be essential to determine how variable cells form reproducible organs. Cell growth displays high heterogeneity, for example RER measured by increase in cell area varies from 0–2% per hour in Arabidopsis leaves (Elsner et al., 2012) and from 0–5% per hour in flowers (Tauriello et al., 2015), whereas the growth of organs formed from heterogeneous cells and that of the overall plant shoot is highly homogeneous (Hong et al., 2016). This robustness allows developmental reproducibility to occur despite environmental perturbations.

In addition, growth rates over a whole day–night cycle estimated from synthesis of structural biomass (RGR) and relative expansion rates (RER) are fairly similar. However, RER can substantially deviate from both cell division and the synthesis of structural biomass, for example as seen for different photoperiods when looking at the timing of growth within a 24-h cycle (Box 3). These observations suggest an uncoupling of the two processes needed for growth, namely synthesis of structural components associated with C deposition during the day and cell expansion associated with water uptake during the night. Thus, it is necessary to use more comprehensive studies that functionally integrate different aspects of plant growth simultaneously to understand the processes underlying plant development (Fig. 1B).

Phenomics is one of the fastest developing research areas in plant biology and the availability of new high-throughput phenotyping techniques has increased the quality and speed of data production. We expect highly accurate and large-scale field-based phenotyping of crops to be a major research focus in the

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**Box 1**

Phenomics is one of the fastest developing research areas in plant biology and the availability of new high-throughput phenotyping techniques has increased the quality and speed of data production. We expect highly accurate and large-scale field-based phenotyping of crops to be a major research focus in the...
coming years. This will not be a trivial undertaking, as the complex shoot structure of crops ideally requires imaging from several perspectives to obtain accurate estimations of area, and the use of automatic sensors to allow field phenotyping at a large scale, for example by using drones (Pederi and Cheporniuk, 2015).

In addition, the vast majority of experiments are performed under highly controlled conditions, which may not always be relevant for growth in natural environments that are more variable and less predictable (Passioura, 2006; Rebetzke et al., 2014). Metabolism and growth strongly respond to fluctuating conditions in ways that research in constant environmental conditions, which may not always be relevant for growth in natural environments that are more variable and less predictable (Passioura, 2006; Rebetzke et al., 2014). In short-day conditions, the RER per hour is nearly twice as high during the day than during the night based on 4D shoot imaging (Apelt et al., 2017). This appears to conflict with measurements of deposition of C in structural biomass, which is faster in the light period (Sulpice et al., 2014), and results from $^{13}$CO$_2$ labelling of cell wall components, which indicates that RGR h$^{-1}$ is three times higher in the light than in the dark (Ishihara et al., 2015; Ivakov et al., 2017). Interestingly, over a complete 24-h cycle, similar rates of growth are measured with regards to the daily increase in rosette area (Apelt et al., 2017) and glucose deposition in cell walls (Ishihara et al., 2015).

**Box 3. Examples of differences in growth estimations depending on the system used**

- **Growth differences reported within shoot phenotyping imaging platforms (RER versus RER)**
  - Apelt et al. (2015) reported that the maximum RER of Arabidopsis Col-0 plants was at the beginning of the night, while Wiese et al. (2007) and Dornbusch et al. (2014) reported the highest RER at the beginning of the light period. Although all three systems use whole-shoot imaging techniques, they varied by using different read-outs: while Apelt et al. (2015) used the whole shoot surface area to calculate RER, Dornbusch et al. (2014) used average leaf RER and Wiese et al. (2007) measured the growth of individual leaves that were fixed to prevent leaf movements, which potentially interferes with expansion growth. Thus, while these results seem contradictory at first, contrasting results can co-occur depending on the method used for scoring growth. A comparison of the methods can often reveal the reason for the contrasting results.

- **Growth differences reported between imaging-based and non-imaging-based techniques (RER versus RGR)**
  - Cell division is accompanied by rapid synthesis of structural components but little expansion, whilst the reverse is true for expansion growth (see also Czedik-Eysenberg et al., 2016). Similarly, leaf thickness increases from a 12-h to a 16-h or 22-h photoperiod, thus decreasing the amount of new leaf area per unit fresh weight (Sulpice et al., 2014). In short-day conditions, the RER per hour is nearly twice as high during the day than during the night based on 4D shoot imaging (Apelt et al., 2017). This appears to conflict with measurements of deposition of C in structural biomass, which is faster in the light period (Sulpice et al., 2014), and results from $^{13}$CO$_2$ labelling of cell wall components, which indicates that RGR h$^{-1}$ is three times higher in the light than in the dark (Ishihara et al., 2015; Ivakov et al., 2017). Interestingly, over a complete 24-h cycle, similar rates of growth are measured with regards to the daily increase in rosette area (Apelt et al., 2017) and glucose deposition in cell walls (Ishihara et al., 2015).

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