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Measurement of plant growth in view of an integrative analysis of regulatory networks

Nathalie Wuyts[1,2], Stijn Dhondt[1,2] and Dirk Inzé[1,2]

As the regulatory networks of growth at the cellular level are elucidated at a fast pace, their complexity is not reduced; on the contrary, the tissue, organ and even whole-plant level affect cell proliferation and expansion by means of development-induced and environment-induced signaling events in growth regulatory processes. Measurement of growth across different levels aids in gaining a mechanistic understanding of growth, and in defining the spatial and temporal resolution of sampling strategies for molecular analyses in the model *Arabidopsis thaliana* and increasingly also in crop species. The latter claim their place at the forefront of plant research, since global issues and future needs drive the translation from laboratory model-acquired knowledge of growth processes to improvements in crop productivity in field conditions.

Addresses
[1] Department of Plant Systems Biology, VIB, Technologiepark 927, 9052 Gent, Belgium
[2] Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 927, 9052 Gent, Belgium

Corresponding author: Inzé, Dirk (dirk.inze@psb.vib-ugent.be)

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Introduction

Cell growth and division constitute the most elementary processes of growth that bring about final organ and whole-plant size and shape, and plant reproduction. Although the molecular understanding of growth regulatory processes at the cellular level is already quite impressive [1•,2], it is steadily becoming clear that they need to be regarded as situated within a spatial and temporal framework, defined by the tissue, organ and whole-plant level, on the one hand, and the influences of the plant's environment, on the other hand. This was pointed out many years ago by mechanistic insights into growth and development, based on descriptive measurements at multiple levels, but the molecular evidence has only been catching up more recently in the model plant *Arabidopsis thaliana*, and also in crop species. Next to breeders interested in crop improvement, and researchers involved in quantitative genetics approaches in crops under field and laboratory conditions, increasing numbers of scientific institutes are engaging in bridging the (molecular-level) knowledge gap between model plants and crop species, and in the translation of controlled environment findings to actual improved crop traits under field conditions [3]. This is brought about by an awareness of global issues such as climate change, improved wealth in newly developed countries and increasing population pressure, while the economic fallback of recent years influences policy makers in prioritizing application-oriented research.

This review has no intention of providing a comprehensive overview of the state of the art in growth measurement or the current knowledge of growth regulation, as the scope would simply be too large; rather, current issues in growth measurement and considerations in regard to plant growth conditions and sampling strategies for molecular analyses of growth regulatory networks are discussed, and where appropriate, crop species are included.

Growth measurement, an issue of levels and scalability

Plant growth can be regarded as a multi-level process, operating from the cellular to the whole-plant and plant community level. The choice of the level at which growth is measured depends heavily on the reason for measuring. Research on the mechanistic understanding of growth, based on measured phenotypic traits, their correlation or causal relation, and their variability in response to the atmospheric and belowground environment, continues to deliver data for both modeling purposes and quantitative genetics approaches ([4–7] and references therein). The latter assist in breeding and crop improvement in line with next-generation sequencing and the development of mapping populations and diversity panels. The vast agricultural area required to grow the corresponding number of plants, raises scalability issues in the measurement of growth and other physiology-related traits. Areal modes of imaging provide low resolution and canopy level growth measurements related mostly to ground cover, while vehicles for proximal sensing of individual plants for height and architecture are under development [8,9]. In controlled conditions, as opposed to field conditions, levels range from the plant (shoot or root system), organ and down to the cellular level in both *Arabidopsis* and crop

model species. The same range applies to research on the insights into the molecular networks governing cell growth and division, and to a lesser extent cell expansion, which become more and more comprehensive [1•,2]. Controlled environments offer scalable growth monitoring systems, *in vitro* [10–12], or in soil, in phenotyping platforms with automated weighing, irrigation and imaging [13–15]. However, image-based, nondestructive measurement of growth is currently restricted to the plant level (shoots) aboveground, and down to the organ level (individual root) belowground (for an overview, see [16] and Supplementary Table 1). Destructive sampling and/or visualization is still required for organ-level and cellular-level growth measurements, even in *Arabidopsis*, although progress is being made in the measurement of individual leaf growth parameters from rosette images [17•]. In the case of crop species, an evolution toward three-dimensional reconstruction and quantitative analysis, including the number and size in length and area of individual leaves, promises to deliver automated, nondestructive measurement of growth at the organ level [18,19]. Compared to the analysis of growth in the *Arabidopsis* rosette, crop species pose a number of additional challenges in automated morphological phenotyping, including stem growth and internode elongation, stem branching, tillering and leaflet development, to name just a few. At present, crop biomass accumulation over time, as an expression of the crop growth rate, is modeled based on the correlation between variables extracted from two-dimensional images and measured samples [20,21]. However, caution is warranted, since model parameters are expected to differ between genotypes, growth conditions and even developmental stages. Belowground, crop species show a higher complexity in root system architecture, especially in monocots where branching is achieved through adventitious roots [22,23]. Even so, the number of available and advanced tools for the measurement of root growth and root system architecture in crop species is impressive (Supplementary Table 1). *In situ* root system assessment, however, remains problematic, despite its importance in crops in particular, but image analysis is now applied in an upgraded version of 'shovelomics' [24,25] and is under development for X-ray computed tomography of plants grown in soil cores [26]. At the cellular level, crop roots pose challenges because of their thickness compared to *Arabidopsis* roots. More elaborate clearing and microscopy techniques are required for the visualization and quantification of their cellular organization [27].

Methods and tools for growth measurement across different levels have been reviewed in [28] and are being collected by [16]; the most recent additions have been integrated in Supplementary Table 1. The extent of Supplementary Table 1 is a clear demonstration of the continued dynamics in the field, with crop species gaining in importance.

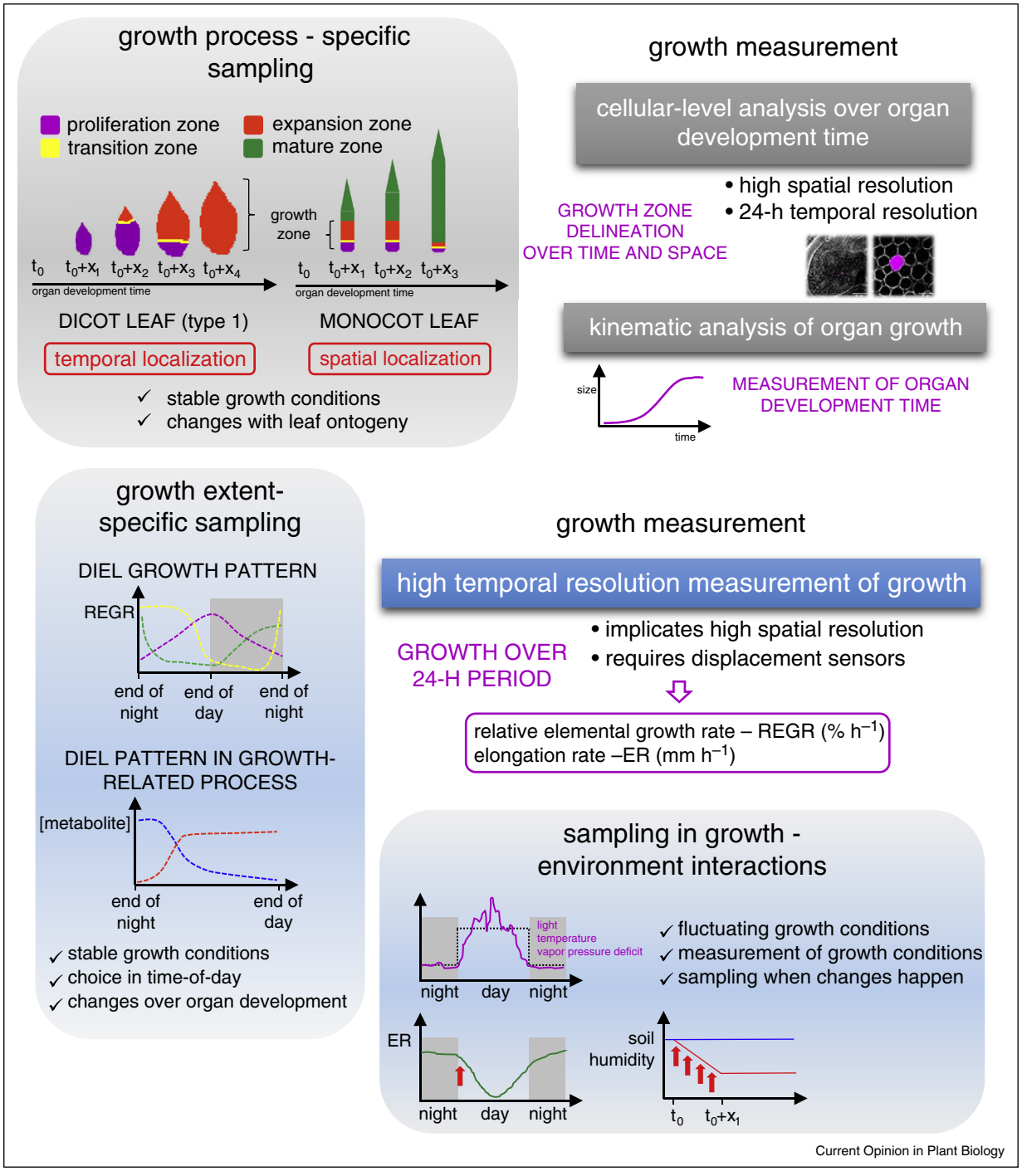
Know how plants grow — growth conditions and sampling strategies

The description of growth across different levels constitutes an important aspect of research into growth regulatory processes. When the focus lies on a particular process at the molecular level, stable conditions with predictable plant, organ and cellular growth and development assist in the definition of the temporal and spatial resolution of sampling (sample where, when and how frequent?) (Figure 1). A transcriptome analysis in either proliferating or expanding cells, for example, necessitates a precise delineation of the growth zone as it consists of spatially distinct sections of cell proliferation and cell expansion at the tip of roots and at the base of monocot leaves [29,30]. Dicot leaves do not have determinate zones of proliferation, expansion or maturation; rather, any zone within the leaf passes through all three developmental phases [29]. Walter *et al.* [31] have distinguished two types of dicot leaves based on the spatial localization of relative elemental growth rates (REGR) and diel leaf growth cycles. The Type 1 pattern of growth occurs in leaves of *Arabidopsis*, and is characterized by a tip-to-base gradient in REGR and the transition between developmental phases. In this case, a detailed characterization over time of the spatial localization of cell division activity and cell size can deliver time points in which the entire leaf is within one developmental phase [32]. In leaves with Type 2 growth patterns, such as in *Populus deltoides* and *Glycine max*, proliferation, expansion and maturation occur throughout the leaf and throughout leaf development which makes sampling for molecular analyses of specific growth processes extremely difficult [31].

Furthermore, if the pattern of the diel growth cycle of specific organs in stable environmental conditions is known, the choice of time points, for sampling of plant growth zones, within a 24-h period can be tuned to maximum growth states. The determination of the pattern in diel growth cycles requires specific methods capable of measuring displacement (growth) at high spatial and temporal resolution ([31,33,34], and references therein). Here as well, the progression through organ developmental stages needs to be considered as diel growth cycles may shift phases, as shown for post-emergence *Arabidopsis* leaves [35]. Alternatively, instead of focusing on growth itself, daily patterns in processes directly related to growth may serve as a basis for the definition of sampling strategies, an example of which is found in the distribution of carbon resources toward either structural or storage components in sink and source leaves [36].

Lastly, clever sampling strategies can be devised by taking the timing of plant developmental stages into account, such as in the recent work on the involvement of shoot photosynthesis-derived glucose in target-of-rapamycin (TOR) signaling, where sampling was targeted

Figure 1



Major decisive factors in the design of sampling strategies for molecular analyses of growth regulatory networks and accompanying growth measurements in plant leaves. Sampling for specific growth processes, cell proliferation and expansion, requires knowledge about the developmental program of leaves and a cellular analysis of the growth zone. In the case of dicot leaves (Type 1, [31]) the timing of growth process transition enables sampling for specific growth processes [32]. Monocots show linear growth related to a determinate growth zone at the leaf base. The spatial localization of proliferation, transition and expansion zones allows for growth-process-specific sampling. The time t_0 represents the zero starting point in the chosen reference frame for growth measurements (time after sowing, germination, leaf initiation, leaf emergence, among others). Sampling related to the extent of growth (increase in size per unit time) and underlying molecular and metabolite-level processes is facilitated by a detailed knowledge of growth patterns over a 24-h period. Diel growth cycles for different species (dicot and monocot) have been determined in ([31,34] and reference therein). Measurement of absolute and relative organ expansion at high-temporal resolution requires dedicated equipment such as high-spatial resolution displacement transducers [31,33,34]. An alternative sampling strategy may be based on daily

specifically at the transition between heterotrophic and photoautotrophic growth in *Arabidopsis* root meristems [37••]. Once potential components of regulatory nodes have been identified, their tissue, cellular and sub-cellular localization may be determined by means of marker lines, such as the recently established collection in maize [38], or in the case of hormones, by means of synthetically engineered fluorescent surrogates [39].

Stable growth conditions may not be suitable when the focus lies on the effect of environmental factors. Important changes in growth and associated molecular processes rather occur at boundary conditions [40]. The 'time of day' effect is controlled by the circadian clock which adjusts growth according to day-night rhythms imposed by the plant's environment with a species-specific pattern in the diel growth cycle [34,41,42••], but allows for attenuation of the amplitude of daily gene expression under influence of temperature, solar radiation [43] and conditions that provoke drought, as recently shown [42••]. In most phenotyping platforms that provide automated weighing and irrigation of soil-grown plants [13,14], pots are watered once a day with the time of day differing between plants. Samples for molecular analyses taken before or after watering may already differ in cases where plants experience drying soil conditions within the 24-h period between watering. Moreover, the extent of the effect of drying soil on growth and corresponding regulatory processes may differ significantly between times of the day, especially when working under naturally fluctuating conditions of temperature, relative humidity and light [40].

The timing of responses to disturbances, measurable at the organ and cellular level, and analyzed at the molecular level, warrants careful consideration. Effects on cell expansion can be measured at a temporal resolution of minutes and appear quickly ([40,44] and references therein). Effects on cell proliferation, however, can only be measured over time periods corresponding to the cell division rate, despite the fact that they may have been triggered very early in cell cycle regulation. Molecular responses may indeed occur rapidly, within the hour [40,45,46•], or even within 10 min in the case of the maize phosphoproteome upon rewatering after a mild drought stress [47]. Due to the plethora of possible mechanisms for transcriptional, translational and post-translational modification and control, and the variability in response times caused by their actions, a multi-tool molecular approach to unravel growth regulatory processes, combining genomic and transcriptome data with analyses of the proteome and post-translational modification mechanisms, is imposing itself. Novel insights obtained by an in-depth molecular network evaluation may give incentives for the measurement of particular phenotypic traits related to plant growth or physiology for confirmation or novel discoveries. The small size of *Arabidopsis* plants, organs and growth zones may be limiting this approach because of the required amount of sampling material, while crops may lend themselves better in this respect.

The spatial-temporal context of growth and development at the organ and whole-plant level

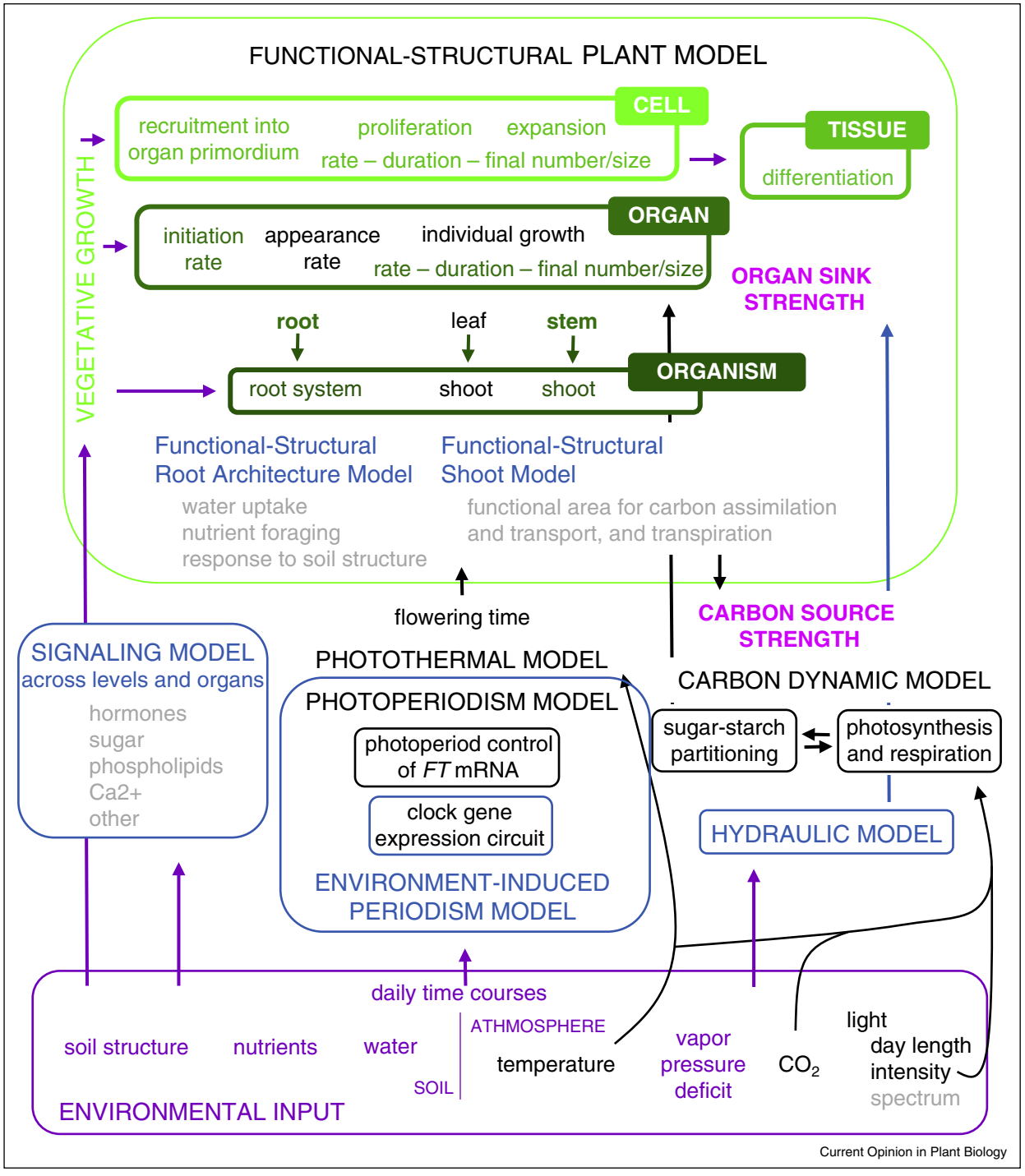
In addition to the multiple modes of growth regulation at the molecular level, and the accompanying need for multiple tools, it is no longer possible to ignore the effect of the whole-plant level on the spatial and temporal regulation of growth at the cellular, tissue and organ level. Sugar, hormones, and other signaling mechanisms such as phospholipids and waves of calcium ion (Ca^{2+})-gradients [1•,37••,48,49,50•,51], have a central role in affecting growth processes in developing organs under influence of existing organs (defining the plant nutrient and developmental status) [37••,44,48,49], the environment sensed in distant organs [52–54] or according to the developmental program of the plant, which in itself is influenced by the plant's environment [55]. Similarly, increasingly important molecular-level data are accumulating for intercellular communication in the coordination of growth in tissues constituting an organ [56–58], and intracellular, cell-autonomous effects on cell growth [59]. An awareness of the spatial and temporal context provided by processes at a higher organizational level (plant and organ) for growth processes characterized at a lower level, may give incentives to further include these aspects in growth measurements and sampling strategies. A possible, but not unlikely, consequence may be a future decrease in *in vitro* growth experiments on artificial media, often supplemented with sugar, under conditions that do not favor photosynthesis and transpiration, or trigger natural environment interaction responses.

Modeling aids in getting a grip on the complexity of growth regulation

Modeling constitutes an important tool in making complex, interconnected processes tangible and in providing simulations of disturbances with predictions of their outcome. Modeling may even be ultimately required to enable the translation of knowledge of growth regulatory processes into biotechnology-driven crop improvement. Crop research is certainly further ahead in the development and application of functional-structural [60,61] and

(Figure 1 Legend Continued) patterns of processes related to growth, such as those for carbon partitioning between structural and storage components [36]. Finally, under non-stable conditions, both aboveground and belowground, and in the frame of strategies aimed at characterizing gene-environment interactions, the most interesting time points for sampling most likely occur at boundary conditions [40]. The time t_0 in the curve describing soil humidity is the starting point for the drying of soil to new stable conditions at a lower soil humidity at $t_0 + x_1$. The arrows indicate boundary conditions.

Figure 2



Hypothetical extension of the framework model for *Arabidopsis thaliana* rosette growth developed and compiled by Chew and co-workers [63••]. The framework model links genetic regulation (circadian clock component) and biochemical dynamics (photosynthesis-derived sucrose and starch for growth) to growth at the organ and organism level with input from the environment at both the genetic and biochemical level of regulation. The original model parts are indicated in black. Extra modules and input parameters, in colors other than black, have been added with the focus remaining on the vegetative phase of plant growth. The functional-structural plant model here comprises cellular growth processes, and was split into a functional-structural shoot model and a functional-structural root system architecture model inspired by [69]. The effect of environmental input arguments, such as temperature, vapor pressure deficit, water and nutrient availability, on growth and thus sink strength, have been included according to recent literature on growth modeling, which questions the emphasis on growth as a consequence of the amount of assimilated carbon [44,70]. The environment-induced periodism model, extending the existing photoperiodism model [63••], suggests the influence of environmental conditions other than light on the circadian clock, and was inspired by [42••]. It also encompasses the shift from metabolic to

environment-interaction models with genetic inputs [7,62]. These models are, however, mainly based on a mechanistic understanding of growth processes. The framework model for *Arabidopsis* rosette growth, developed and compiled by Chew and co-workers [63••] provides a clear path, and possibly the required incentive, for further developments in the incorporation of growth regulatory networks, besides the photoperiodism model that has already been integrated. A hypothetical extension of the current framework model of Chew and co-workers [63••] is proposed in Figure 2. An important, but difficult, issue remains in including crop species, both dicot and monocot, in models incorporating growth regulatory networks determined primarily in *Arabidopsis*. Is the definition of a 'gene space' [3] sufficient to use the same model in crop species, potentially with a distinction between C_3 and C_4 carbon fixation model components [64]? Or will a whole new model be required? Likewise, as growth regulatory networks seem to be shared among organs [65–67], will a 'gene space' suffice, or will the network need to be defined per organ and integrated into a developmental framework?

Concluding remarks

Research into growth regulatory processes, under influence of plant development and in interaction with a more or less extreme environment belowground and aboveground, is highly dynamic and boosted by developments in techniques on the one hand, and specific requirements toward crop improvement on the other hand. Methods and tools for growth measurement have evolved progressively toward visualization at a higher spatial resolution and (semi-)automated quantitative analyses, at both cellular and organism levels, and rapidly toward noninvasive techniques, thereby adding a temporal resolution to growth measurements at the individual plant level. The concurrent development of molecular tools and insights into molecular-level processes at the transcriptional, translational and post-translational level, and performant metabolome characterization, continues to reveal potential control mechanisms in regulatory nodes, which calls for integrative approaches. These require, however, larger amounts of sampling material, which may become problematic in *Arabidopsis*, in contrast to crop species with larger organ sizes and growth zones. Moreover, both growth measurements and molecular analyses of regulatory processes will increasingly need to consider the spatial and temporal context of growth and development at multiple levels. The multi-level approach is shared by research in root growth [68] and stress response [51,54]. Ideally, one would be able to measure whole-plant growth (*i.e.* both shoot and root systems) at the organism down to the cellular level, and at the same time, the expression of the plant's mechanism to sense its aboveground and belowground environment. Likewise, molecular networks would be incorporated into whole-plant level models enabling the simulation of environmental and genetic perturbations. Finally, a potentially underexplored line of research lies in consideration of the sharing of regulatory networks by different types of organs [65–67]. Knowledge of the extent and the organ specification may prove to be important in model development, in the organ-specific targeting of biotech-driven crop improvement measures, and in the translation of growth regulatory networks from models to crop species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pbi.2015.05.002.

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