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TEACHING TOOLS IN PLANT BIOLOGY™: LECTURE NOTES

Molecular Control of Plant Shoot Architecture: How do Plants Achieve Their Form and Height?

INTRODUCTION: WHY IS THE STUDY OF PLANT FORM AND HEIGHT RELEVANT?

Like in animals, the growth rate and pattern of plants is controlled by both genetic and environmental factors. Manipulation of plant form and height has been instrumental in the domestication and improvement of crops. The balance between growth of vegetative (stems, branches, leaves) and reproductive (flowers, fruits, seeds) organs in the shoot is of chief importance for agriculture. Perhaps the most dramatic example of how human selection has modified plant growth patterns is the suite of derivatives of the wild cabbage (*Brassica oleracea*), that includes morphs (forms) as strikingly different as kohlrabi, broccoli, Brussels sprouts, kale, cauliflower and cultivated cabbage, that all belong to the same species. To a different extent, all existing crops are the result of unconscious or deliberate human selection that led to modification of the form and height of wild species. Of course, shoot architecture traits are relatively easy to select for, but selection has also affected root architecture; however, in this lesson we focus on shoot architecture in vascular plants.

The genetic mechanisms controlling shoot architecture have been the subject of intense research efforts and have revealed, in many cases, a relatively simple basis for very complex phenotypes. In this Teaching Tool we present an overview of recent advances in understanding the molecular basis of shoot architecture. We introduce basic concepts of plant growth and shape generation and provide an overview of their control. Both chemical and physical forces are involved in pattern generation. Plant hormones, transcription factors and small RNAs are key among the former, tension and pressure among the latter. We discuss how physical forces interact with chemical signals to produce specific developmental outcomes. Plants can concentrate their growth in the vertical direction, or laterally, through profuse side branching and branch outgrowth. We present a brief outline of the molecular and physiological mechanisms involved in vertical and lateral growth. Finally, we outline the potential for manipulation of plant architecture using state-of-the-art genome editing technology and how it could bring about agricultural benefits through an accelerated optimization of crop shape and size.

PLANT DEVELOPMENT: CHANCE AND NECESSITY

The optimal phenotype for life in the open sea, where conditions are relatively homogeneous, is a single planktonic cell. In other environments, there can be fitness benefits to growing larger and distributing function, so multicellularity arose. One of the great conceptual problems in biology is how a complex, patterned organism can be produced starting from a single cell, the zygote. As in man-made buildings, the bodies of plants and some animals are assembled by the repetition of small units. This is called *modular construction*. However, unlike artificial buildings where bricks are laid by a mason, living organisms are self-assembling: they contain the instructions for their own construction in each individual cell. Such instructions involve chemical and physical processes, although their combined outcome is neither chemical nor physical, but *geometrical*: an organism's form. Form and patterns are properties of cell collectives: tissues, organs and organ systems. Therefore, *development* is not only the process of producing a multicellular body from a single cell (which would simply lead to the formation of an amorphous cell agglomerate or a tumor): it is rather the sequence of events that leads to the formation and patterning of organized tissues conferring a specific form to the adult organism.

One striking difference between plant and animal development is that in animals, most organs are already present by the time the embryo is fully formed, creating a rough outline of the final form of the adult animal, the *body plan*. Put in other terms, animal *organogenesis* is a part of *embryogenesis*. This, however, is generally not the case in plants, where the mature seed contains an embryo with very few, simple organs: an axis (the hypocotyl) with cotyledons (embryo leaves) on one end, and the radicle (embryonic root) on the other. With few exceptions, adult organs are formed after seed germination, thus it is said that organogenesis in plants is almost entirely post-embryonic. This is made possible by tissues formed in the embryo that retain their embryonic nature during the whole plant's life cycle: the *meristems*. The continuous activity of meristems, which form new organs through cell division, expansion and differentiation, provides plants with a powerful tool to survive by controlling organ size and number. Whereas animals can move away from danger or explore the environment to graze or hunt, plants can form more or fewer organs and adjust their size and growth direction according to environmental conditions, while remaining anchored in a fixed physical location. Paraphrasing the title of Jacques Monod's (1910-1976) classic book 'Chance and Necessity', plant form is the result of chance (germination in a random spot with unpredictable environmental conditions) and necessity (differential survival and reproduction through post-embryonic development conditioned by natural selection).

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GROWTH AND THE ORIGIN OF FORM

Aristotle defined *form* as 'that what makes a thing what it is'. Plant form is usually referred to in a more technical manner as plant *architecture*, a description of the three-dimensional arrangement of the plant body. The main parameters that determine the architecture of a plant are *height*, *branching* and *growth habit* (characteristic shape, e.g., vine, shrub, tree). We will analyze each separately, but ultimately all three are determined by the coordinated operation of individual cells, which can engage in three basic activities: *division*, *expansion* and *differentiation*. Cell ensembles form *tissues*, which can in turn coalesce into functional units called *organs*. The size and relative arrangement of organs is what bestows plants their characteristic architectural pattern. Thus, the generation of form, or *morphogenesis*, can only be understood with a clear picture of the events that control growth at the cellular level.

Growth occurs through an increase in cell size (driven by *expansion*) and number (driven by *division*). The production of different and specialized cell types, *differentiation*, occurs concomitantly throughout plant development. The combination of cell division, expansion and differentiation leads to pattern formation from the zygote. Coordination between processes depends on cell lineage (what cell type a given cell is derived from) and position (the cellular and environmental context in which a given cell finds itself). Unlike animal cells, plant cells cannot migrate, due to the presence of a rigid cell wall, so position is considered a stronger determinant of plant cell fate rather than lineage. The regularity of form leads to patterned structures: the various parts of the plant bear predictable, repeated relations to one another.

Models for pattern formation

Plant development and pattern formation are highly dynamic processes occurring in a temporal sequence. Until recently, the analysis of plant development was restricted to snapshots taken at arbitrary points in time. Modern phenotyping technology is allowing a more realistic, dynamic picture to emerge. Non-destructive sampling using time lapse confocal microscopy and image analysis provides insight into the time dimension of plant development.

Four parameters determine plant growth and pattern formation in two dimensions. These are: 1- *growth rate*, the rate at which a given region changes in size; 2- *anisotropy*, or differential growth between two regions; 3- *direction*, the angles at which growth is concentrated and 4- *rotation*, the angle at which a region turns relative to other regions over time. For a 3D structure, nine parameters are needed (one for volume, two for anisotropy, three for direction and three for rotation). How is each of these controlled at the cellular level? In short, both chemical and physical signals contribute to direct growth and patterning.

Chemical signals controlling morphogenesis

Diffusible chemical signals underpin morphogenesis. The outstanding polymath Alan Turing (1912-1954) was one of the first to propose a chemical basis for biological pattern formation, through diffusible substances he called *morphogens*. Using ingenious mathematical modeling he suggested that morphogens may provide spatial information by forming concentration gradients, which inform cells about their relative positions. A chemical compound ought to meet three conditions to be considered a morphogen: i) it must function in a concentration-dependent manner, ii) it must be generated at one location and move from that site, and iii) it must function directly on target cells.

The local concentration of morphogen is sensed by cells, triggering a signaling cascade that activates a specific subset of transcription factors (TFs). TFs are proteins that bind to DNA and control the transcriptional activity of genes. Differential gene expression patterns arise, leading to specific outcomes in each cell: expansion, division or differentiation. Apoptosis, or programmed cell death, which can be considered an extreme case of cell differentiation, is another possible outcome, although in the context of morphogenesis it is far more relevant in animals than in plants.

Most of Turing's extraordinary insights were borne out decades later by the discovery and analysis of morphogens in *Drosophila melanogaster* (the fruit fly). Functional equivalent of morphogens in plants can be found in many forms, such as small RNAs, signaling peptides, transcription factors, and molecules with strong morphogenetic effect called plant hormones. Auxin is one such hormone and its effects on shoot elongation, side branching, root formation and leaf lamina shape determination have been well described. At the cellular level, auxin can induce cell expansion, division or differentiation. The specific outcome depends on a combination of factors, among which mechanical forces play a significant role.

Physical and mechanical signals also control morphogenesis

Plants are generally rigid structures and therefore subject to physical forces such as wind, gravity and soil impedance. Rigidity is conferred by the cell wall, a series of compounds, such as cellulose, hemicellulose and pectin, arranged in layers, which surround the protoplast. The hydrostatic pressure exerted by the protoplast onto the cell walls maintains turgor. Cell walls are highly dynamic structures, as cell expansion and division require the loosening and rearrangement of their components. Their extraordinary mechanical properties allow plants to cope with strong physical forces. High pressures develop in germinating seeds or in roots through passive water influx at night-time in the absence of transpiration. Water influx driving the increased pressure is due to higher concentration of osmolytes (small molecules such as ions, sugars or amino acids) in the protoplast.

Cell walls form a continuum called the *apoplast*, which can thus transmit physical forces between cells and tissues. These forces, in combination with chemical signals, can provide positional information and contribute to organ initiation and shape determination. For instance, chemically induced cell wall loosening can trigger regional differences in cell wall stiffness, providing positional information to direct the development of a new organ.

Chemical and mechanical forces can be integrated. As an example, auxin can switch on proton pumps that acidify the apoplast and the resulting low pH can activate proteins called

expansins. These proteins break the hydrogen bonds that tie cellulose microfibrils together, loosening the cell wall and, consequently, relieving turgor pressure. The low turgor pressure translates into reduced water potential, causing water influx from the apoplast into the cell. The influx of water builds up cell turgor pressure, leading to cell expansion. Changes in pressure in one cell are perceived and responded to by neighboring cells.

The perception, signal transduction pathways and effectors of mechanical signals remain uncertain. The cell cytoskeleton, more specifically, the highly dynamic network of microtubules, serve as 'tracks' along which cellulose deposition occurs. Microtubule orientation appears to be regulated by mechanical stress, thus providing the potential link between physical signals and morphogenetic effects. Mechanosensitive ion channels can also sense and transduce mechanical signals. New insights in this exciting research field should produce a more complete picture of plant morphogenesis in the near future.

Plant hormones are key signaling molecules

Plants and animals lineages diverged before the beginning of multicellular life; therefore, the developmental mechanisms that integrate their bodies evolved independently. In animals, large-scale coordination of the organism's function is achieved by the operation of the nervous and endocrine systems. Both ensure functional integration among organs, tissues and, ultimately, individual cells. No such system exists in plants, but *plant hormones*, a group of chemical compounds, fulfill an analogous role through short- and long-distance transport.

Plant hormones effect changes in cell division and expansion and are thus the main contributors to plant elongation, branching and growth. The modulation of plant hormones through metabolism (biosynthesis, inactivation and degradation), transport, and response (perception and signaling) ensures integrated control of organ growth. For example, auxin is mainly produced in shoot meristems and developing leaves and is transported basipetally toward the bottom of the plant, where it promotes root initiation. Conversely, cytokinins (CKs) are mainly produced in roots and are transported to the shoots, where they promote bud growth. This mechanism produces an interdependence between shoot and root growth. Hence, more shoot growth means more production of the root-inducing hormone and vice versa. Besides integrating plant growth, plant hormones are key players in various, if not all, aspects of plant development. We will look at how specific hormones contribute to plant architecture in subsequent sections; note that separate Teaching Tools are available that describe additional roles for each of these hormones.

THE STRUCTURE OF THE PLANT SHOOT

Alexander von Humboldt's (1769-1859) "colossal dragon-tree (*Dracaena draco*) of Orotava" in Tenerife, was estimated to be 6000 years old, but still bore "the blossom and fruit of perpetual youth". The extraordinary lifespan of *D. draco*, and other equally long-lived species, like the Great Basin bristlecone pine (*Pinus longaeva*), which can exceed the 5000 year mark, is due to the persistence and function of meristems.

Molecular determinants of the shoot apical meristem

Continuous post-embryonic growth is the result of meristematic activity in the shoot and root apices, influenced by the integration of chemical and physical signals. Genetic instructions combined with environmental cues signal some cells of the meristem to differentiate and give rise to tissues and organs, while some cells are set aside and maintained as a population of undifferentiated 'stem cells'. Thus, the apical meristems fulfill two functions: organ initiation and stem cell maintenance. Zones of differential functions can be identified through differential rates of cell division and differential gene expression patterns.

In the shoot apical meristem, stem cells are maintained in an uncommitted state through a signaling loop controlled by the CLAVATA3 (CLV3) and WUSCHEL (WUS) proteins in the central zone (CZ). In the CLV3-WUS signaling loop, WUS is expressed in the *organizing center* (OC) and diffuses to the CZ, where it promotes proliferation of stem cells. Stem cells produce CLV3, a small peptide that diffuses back to the OC and repress expression of WUS through binding to specific receptors (CLV1). Auxin and other hormones, such as cytokinins (CKs) and gibberellins (GAs) are also fundamental, as their concentrations create a demarcation for each region in the SAM. High CK levels have been proposed to position the domain of expression of WUS in the OC and thus promote stem cell identity and maintenance in the CZ. CK levels, in turn, are maintained high by the action of transcription factors of the *KNOX* family, *KNAT1* and *STM*. KNOX proteins drive down the levels of GA the SAM. Auxin levels are also kept low in this region. Differentiation occurs when cells leave the CZ and are exposed to different chemical and physical influences. Organogenesis at the shoot apex thus occurs through a dynamic balance between cells maintaining their uncommitted state and committing to a specific cell fate.

Leaves: Phyllotaxis and phytomers

Cells that leave the CZ can commit to differentiate, in which case auxin and GAs act synergistically to promote expansion and differentiation. Auxin activates transcription factors like AS1, which represses *KNOX* genes in the organ primordia, and stimulates GA biosynthesis. The budding primordium forms a leaf, an organ that (usually) functions to capture light and exchange gases with the atmosphere.

The arrangement of leaves on the stem follows a very precise, regular and repetitive pattern called *phyllotaxis*. Two basic geometric parameters describe different phyllotaxies: the angle between successive leaves and the vertical distance between them. The most common phyllotactic pattern is the spiral one; its mechanistic basis has been the subject of intense research and debate for many decades. Recent work has shown that the spatial periodicity of leaf initiation is controlled by a combination of chemical (mainly auxin) and physical forces (cell wall mechanics). Phyllotaxis is under strong genetic control and is seldom affected by environmental disturbances. It thus represents a good taxonomic character, although its adaptive value has never been proven, an apparent paradox first observed by Charles Darwin (1809–1882).

Leaves, in turn, can be arranged on branches, a very successful evolutionary innovation optimizing the disposition of leaves in

space. Side branches originate from a second type of meristem arising from leaf axils (hence called axillary or lateral meristems) which appears as a bud, and which can eventually grow to produce a side branch. The module formed by (1) a node which a particular leaf is attached to, (2) the axillary bud on that leaf, and (3) the subtending (lower) internode, defines a modular functional unit called *phytomer*. Variation of phytomer size and number allows plants to achieve great flexibility in final size.

Branches: Bud dormancy and apical dominance

If all meristems produced new phytomers incessantly, a plant would soon grow out of control and lose all semblance of organization. Plants use a neat trick to avoid this, called *dormancy*. Dormant meristems retain their potential for growth and differentiation, but are only activated under specific conditions, for instance, upon the plant reaching a certain developmental threshold (such as the time to flowering) or as a means of reconstructing a damaged structure. It has thus been proposed that lateral branches are formed after some sort of cost-benefit analysis has occurred in the plant. For instance, new branches would only be formed if the benefit of increased light capture would outweigh their construction cost. Lateral bud outgrowth is generally inhibited by the shoot apex, a phenomenon called 'apical dominance'. Auxin is a key player in the inhibition of lateral bud outgrowth. The picture, however, is not yet complete, in spite of decades of intensive research. Inorganic nutrients, sugars and other hormones have successively been added to a long list of both inhibitors and stimulators of axillary bud outgrowth. A thorough understanding of the molecular basis of apical dominance is still missing, but it will most likely consist of an integrated combination of multiple endogenous and environmental factors such as daylength, light quality, temperature and nutrition.

Branching patterns have physiological and ecological consequences. The number of branches in annual plants tends to be low, whereas perennial plants tend to branch profusely. A trade-off exists between investment in vertical growth (height) and lateral growth (branching), as height requires more mass investment in main stems compared with lateral branches. Conifer trees invest more mass in their trunk than do eudicot trees and tend to be taller, yet less branched. As described below, branching also affects the number of reproductive structures formed, so has implications for crop yields.

MOLECULAR CONTROL OF PLANT STATURE

The trend to grow vertically can be traced back to the end of the Devonian period, 400 million years ago. The transition of plant life from the aquatic to the terrestrial environment set off a 'race to the top', initially to increase the potential range of dispersal of spores (later, pollen and seeds) and then in search of light to avoid shading by nearby objects. While growing tall provides considerable adaptive advantages, it also brings new challenges, such as building an appropriate support structure to cope with self- and wind-loading and transporting water and nutrients over long vertical distances against a strong gravitational pull. Some plants have been extremely successful at growing tall as can be attested by species reaching the theoretical maximum height for trees, calculated at around 120 m.

As in many other areas of plant biology, the first hints at the molecular control of plant height came through the study of spontaneous and artificially induced mutants. 'Dwarf' plants have long attracted interest both as botanical curiosities and as subjects for scientific inquiry. One of the seven discrete traits that Mendel famously characterized in his garden peas was height (he classified the observed phenotypes of his plants as tall/short). Shortness in Mendel's *le* pea mutant was a recessive trait produced by a spontaneous mutation that was characterized, almost a century later, as impairing the GA biosynthesis at the step of the *GA_3-oxidase* (or *GA_3-β-hydroxylase*) gene, which converts inactive GA_{20} into active GA_1.

Gibberellins and the control of plant height

GAs are a family of diterpenoid compounds that participate in the control of various physiological and developmental processes. GAs can affect internode elongation through both cell expansion and division, particularly in grasses, which have internode (intercalary) meristems. The effect of GAs is nicely illustrated by comparison of two GA-related mutants in maize and tomato with opposite phenotypes. Whereas the GA-deficient maize *dwarf1* (*d1*) mutant shows stunted growth and reduced final height, the tomato *procera* (*pro*) mutant is a slender, tall plant (from Latin, *procerus* = 'tall') due to a constitutive GA response. Significantly, exogenous GA application will revert the stunted phenotype of biosynthesis mutants, but the application of this hormone, or its biosynthesis inhibitors (*e.g.*, Paclobutrazol), will not affect the phenotype of signaling mutants. Similar phenotypes have been described for GA-related mutants in Arabidopsis and pea.

The Green Revolution in the 1960s provides a dramatic demonstration of the power of manipulating plant hormonal balance to achieve increases in crop yield. In cereals, shorter plants respond better to nitrogen fertilization, because their shorter stature avoids excessive vegetative growth and prevents lodging (falling over to one side). Thus, shorter plants can potentially support higher grain yield, especially under high fertilizer input. A large international consortium led by Normal Borlaug (1914-2009) and Gurdev Khush (born 1935) bred high-yielding semi-dwarf wheat and rice varieties harboring mutations in the *Reduced height 1* (*Rht1*) and *semidwarf1* (*sd1*) genes, respectively. One important feature of semi-dwarf plants harboring the *sd1* and *Rht1* alleles is that their reduction in vegetative growth (leaves and stem) is more severe than in reproductive growth (grain-bearing spikes and panicles), leading to higher grain yields.

Later studies found that both of these important green-revolution genes are involved in the GA pathway. The *sd1* gene is a loss-of-function mutation in a key GA biosynthesis gene, *GA_{20}-oxidase*. The GA signaling pathway combines positively and negatively acting components. Binding of an active form of GA (GA_1, GA_3, GA_4 and GA_7) to its receptor protein (GIBBERELLIN INSENSITIVE DWARF 1, GID1) triggers degradation of the transcriptional repressor DELLA. *Rht1* is a dominant gain-of-function mutation in the coding sequence of a DELLA repressor protein, similar to the Arabidopsis *gai* mutant. Gain-of-function mutations

with mild effects in the DELLA protein can lead to partial 'insensitivity' to GA, and produce semi-dwarf phenotypes as found in the wheat *Rht1* mutant. More severe mutant alleles tend to exhibit negative pleiotropic effects, such as partial sterility and severely reduced growth. Severe dwarf mutants will actually reduce photosynthesis and productivity. Systematic analysis and characterization of different alleles of DELLA and other GA-related proteins is a promising avenue for crop breeding; alleles that produce plants with desirable heights without yield penalty can undergo breeding potential evaluation and then be introduced into elite varieties.

GA, along with the gaseous hormone ethylene, also plays a role in helping some types of rice called deepwater rice survive periods of prolonged flooding. Expression of the *GA_{20}-oxidase* gene is upregulated during flooding, increasing the concentration of the active gibberellins GA_1 and GA_4 and promoting internode elongation. This response is partially mediated by ethylene, whose enhanced biosynthesis during flooding triggers signaling machinery leading to GA biosynthesis and thus internode elongation. Elongated internodes keep the top of the plant above water while the formation of air channels (aerenchyma) in the internodes helps avoid oxygen deprivation in the submerged portion of the plant.

Brassinosteroids and the control of shoot architecture

Brassinosteroids (BRs), a large family of sterol derivatives, are another class of hormones that act in an additive way with GAs to control stem elongation, particularly in seedlings and young plants. This is the basis for one of the most widely used BR sensitivity bioassays: the bean second internode elongation assay. Mutants with reduced BR levels in Arabidopsis, pea and tomato are all phenotypically short. Both cell expansion and cell division are affected by reduced levels of BRs.

Brassinosteroid synthesis mutants in many species have been identified that affect plant height. A classic rice dwarf mutant, *dwarf2*, also known as *ebisu*, harbors a mutation in a P450 family protein involved in BR biosynthesis. The *DWARF* (*D*) gene in tomato encodes another a P450 protein that catalyzes the oxidation of 6-deoxocastasterone to castasterone (CS), an active BR but also the immediate precursor of brassinolide (BL), the most active BR found to date. CS is mostly active in vegetative tissues and BL in fruits. Loss of function in the *D* gene leads to dwarfism in tomato. The mutation in *D* is one of the three major allelic variations responsible for the reduced size of the tomato model cultivar MicroTom (MT). Introgression of the functional *D* allele restores height in MT plants, but introgressing other BR biosynthesis or sensitivity mutations can further reduce height dramatically. Thus, manipulation of BRs allows fine-tuning of plant height.

In cereals, BR deficiency is also associated with a change in leaf insertion angle, an important agronomic trait. A rice mutant deficient in brassinosteroid biosynthesis, *Osdwarf4-1*, causes a steeper leaf insertion angle, which increase photosynthesis in lower leaves and allows planting at higher densities. In modern varieties of maize, steeper leaf insertion angles similarly allow for greater planting density and therefore increased yield per unit area.

The BR signaling pathway was first characterized in Arabidopsis, largely through the identification of plants insensitive to exogenous brassinosteroids. Through these genetic studies, the plasma-membrane localized BRI1 receptor and BAK1 co-receptor were identified, as well as downstream kinases and phosphatases that transduce the signal to regulate transcription factor activities. Several rice dwarf mutants including in the *OsBRI1* receptor gene have been shown to be affected in BR perception and signaling.

Auxin and the control of shoot architecture

Since auxin affects cells division and expansion, it is not surprising that it can also influence vertical growth. The effect of auxin on stem elongation (and branching – see below) has been characterized mostly through the use of mutants altering polar auxin transport (PAT). Indole-acetic acid (IAA), the most common auxin molecule, is synthesized at the shoot apex and transported basipetally toward the roots. At the low extra-cellular pH, IAA is protonated and hence nonpolar, so can freely diffuse into cells, with diffusion facilitated by the AUX/LAX family of transmembrane proteins. The higher cytosolic pH results in IAA ionization to IAA^-, which can only leave the cell through active transport via specific transporters including PIN-FORMED (PIN) and ATP binding cassette type B/ P-glycoprotein/ multidrug resistance (ABCB/ PGP/MDR) proteins.

Transporter proteins of the PIN family, with eight members in Arabidopsis and ten in tomato, are the main mediators of auxin transport in the plant. PIN1 is the most ubiquitous among them, although some redundancy exists in their function. Auxin transport is said to be *canalized*, as it can positively reinforce its own flow, first by rearranging the cellular localization of PIN1 efflux transporters and later by inducing the differentiation of cells along the transport pathway into vascular tissue. Another positive feedback loop exists between auxin transport and mechanical forces, as tension in the plasma membrane generated during growth in young organ primordia influences PIN1 polarity and levels at the membrane, as well as auxin accumulation.

Alterations in auxin transport underpin another dwarfing mechanism that has been successfully exploited in agriculture. The shortness of the maize *brachytic2* (*br2*) mutant, characterized by compact lower stalk internodes, is not reversible by exogenous treatment with auxins, brassinosteroids, cytokinins or GAs, hinting that a process other than hormone biosynthesis is involved. Cloning and molecular characterization of *br2* showed that its phenotype is caused by loss of function in an ABCB/PGP/MDR protein. PAT is thus reduced in *br2* plants, which also causes other traits of agronomic interest such as more erect, darker leaves of the 'stay-green' (i.e., delayed senescence) type. Nevertheless, the *br2* mutation has not been used commercially due to the severe nature of the mutation.

A mutation in *dwarf3* (*dw3*), the sorghum ortholog of *br2* that also encodes an ABCB/PGP/MDR protein, has attracted agronomic interest since the 1950s. The *dw3* mutant decreases leaf insertion angle and improves vertical distribution of solar radiation in the canopy. This, in turn, optimizes canopy photosynthesis, reduces excessive heat loads due to infrared radiation and increases nitrogen accumulation in leaves, which taken together improve overall performance of the crop. The *dw3* short phenotype, however, is unstable, and spontaneous reversion of the mutant phenotype can occur due to a direct duplication within the

dw3 gene that allows unequal crossing-over and reversion at the locus.

As mentioned above, PAT depends on a pH gradient between the apoplastic and symplastic compartments resulting from the action of proton-pump transporter proteins. Overexpression of the Arabidopsis vacuolar pyrophosphatase 1 (AVP1) transporter, a vacuolar proton pump, increases PAT and internode elongation in many species, revealing another promising avenue for manipulation of plant height.

In summary, short-stature plants have been identified in many species and can confer yield advantages. In many cases, the contributing genes have been identified as being involved in hormone synthesis, signaling or transport. New methods of gene editing offer the opportunities to fine-tune these processes and further improve crop productivity.

MOLECULAR CONTROL OF SHOOT BRANCHING

Branching is an almost universal feature of vascular plants, with few exceptions such as tree ferns and most members of the Arecaceae (coconut) family, which seldom or never branch. The molecular mechanisms controlling side branching have been explored using branching mutants in model species such as pea, tomato and rice. For instance, the *lateral suppressor* (*ls*) tomato mutant fails to form axillary meristems. Side branches can form from the main axis by either subdivision of the apical meristem or, more commonly, from axillary meristems (AMs) located in the leaf axils (the axil is the point where the leaf meets the stem). Axillary branching patterns can vary considerably between species, leading to dramatic changes in shoot architecture.

Where do axillary meristems come from?

The ontogenetic origin of the AMs is still unclear. Are they clonally produced as remnants from the apical meristem left behind during vertical growth, or are they formed *de novo* via positional information in the leaf axil? Two alternative models have been proposed to account for either possibility. The 'detached meristem' model proposes that a few pluripotent (capable of adopting multiple fates) cells detach from the primary SAM and associate with the leaf axil as the leaf differentiates from the SAM. The alternative 'de novo induction' model is mainly supported by the analysis of the *phabulosa-1d* (and related HD-ZIPIII transcription factors) mutants, in which an AM initiates from relatively more differentiated leaf cells. A major difference between these models is whether AM initiation requires a meristematic cell lineage. Recent live-imaging and laser ablation experiments have shown that a group of *SHOOTMERISTEMLESS* (*STM*)-expressing cells is required for AM initiation. *STM* expression is sufficient for the production of meristematic cell identity. The progeny of these meristematic cells form the axillary buds, and whether high STM expression persists or is induced later appears to vary between species.

AM initiation can be followed by either dormancy or outgrowth into branches upon receiving different combinations of endogenous and environmental cues. In many species, three broad regions of the shoot can be distinguished on the basis of how a branch behaves at each position. In the basal zone (closer to the root), the branches will grow and replicate the structure of the main shoot. In the middle zone buds tend to stay dormant unless activated by some specific cue, such as loss of the apical bud (release of apical dominance), and in the upper zone they will contribute to the formation of reproductive structures, usually after flowering has been triggered.

Hormonal control of side branch outgrowth

Apical dominance by the shoot apex is controlled by auxin, as proven by classical experiments of shoot decapitation. Application of auxin to the cut tip of shoot a can restore apical dominance, otherwise axillary buds branch out and one of them may eventually grow vigorously enough to become dominant.

Auxin maintenance of axillary bud dormancy depends on PAT. Chemical inhibitors of PAT such as 2,3,5-triiodobenzoic acid (TIBA) or N-1-naphthylphthalamic acid (NPA) induce lateral bud outgrowth. A local depletion of auxin (auxin minimum) is necessary for the establishment of AMs. Auxin minima fail to form in mutants of the influx and efflux transporters *aux1* and *pin1*, precluding AM initiation in the leaf axil.

Bud outgrowth is strongly correlated with auxin export from the bud. The "auxin canalization hypothesis" states that buds must export auxin to grow. The efflux carrier PIN1 mediates PAT and acts a central integrator of developmental information along the axis of the plant. Hence, removal of the apical bud is believed to reduce the competition between apical and axillary buds to export auxin through PIN1 carriers.

Cytokinins act antagonistically to auxin, suppressing apical dominance. Emerging evidence suggests that CKs might influence auxin at the level of PAT. Cytokinin treatment results in increased accumulation of PIN transporters in shoots. CKs, or their precursors, synthesized in the roots and transported acropetally through the xylem can reach arrested side buds and break their dormancy. Additionally, auxin modulates CK concentration by repressing its biosynthesis. CKs are also main controllers of sink establishment, a condition necessary to stimulate the vigorous growth of the side shoot.

The whole picture of control of branch outgrowth was rendered more complex by the discovery of altered branching pattern mutants that are neither auxin nor cytokinin mutants, including *max* (*more axillaries*) in Arabidopsis, *rms* (*ramosus*) in pea and *dad* (*decreased apical dominance*) in petunia. Cloning of the *MAX* genes in Arabidopsis showed that *MAX1*, *3* and *4* are involved in the biosynthesis of an acropetally mobile signal, whereas MAX2 is present in the shoot and acts in the signal transduction of this signal. The mobile compounds are strigolactones, a group of sesquiterpenes derived from carotenoids. Strigolactones promote bud inhibition by modulating auxin transport, and control the amount of PIN transporters in the shoot. Some interplay between strigolactones and CKs also appears to exist. For instance, strigolactone-deficient mutants show very low levels of root-derived cytokinins in the xylem sap. Nutrient deficiency induces the biosynthesis of strigolactones, inhibiting shoot branching.

Strigolactones were first identified as seed germination stumulents, and then also shown to promote the development of beneficial symbioses in the soils. Strigolactones are exuded by the roots as a signal from plants to arbuscular mycorrhizal fungi in the

soil. Spores of these fungi germinate and branch in response to the presence of strigolactones, fostering the development of the beneficial mycorrhizal symbiosis with the host plants. Root parasitic plants such as *Orobanche* and *Striga* have hijacked the system for their own benefit as a means to detect the presence of potential hosts for colonization.

Side branching in monocot species

Monocots, such as grasses and cereals, show two types of vegetative branching. The first type occurs from AMs at lower levels in the plant, which elongate parallel to the *culm* (primary stem) and become *tillers*. The tillers are indistinguishable from the culm in shape and height and can produce their own adventitious roots and thus achieve some functional independence from the culm. The second type of branching can occur from AMs in the top internodes, but this seldom occurs, as these branches tend to remain suppressed at the bud stage.

Cereals have been subjected to divergent selective pressure during domestication. Wheat and rice have been selected for low apical dominance leading to multiple tillers that distribute grain production evenly, with relatively simultaneous maturation. As discussed above, their height has also been reduced, as a means to avoid lodging and grain losses before harvest. A trade-off between height and branching has been demonstrated in rice, where plant height is negatively correlated with tillering. The molecular basis governing this allometric (differential relative size) relationship is yet unknown.

Selection in crops with lateral seed-bearing branches such as maize, sorghum and pearl millet proceeded along the opposite pathway to that in wheat and rice, resulting in tiller suppression. This phenomenon is what the agronomist Jack Harlan (1917-1998) called the "sunflower effect": a strong increase in apical dominance, suppressing side branching and concentrating seed production on a single, large terminal head. In maize, axillary branch number and length also decreased during domestication, leading to the formation of the lateral ear (which is itself an axillary branch).

One of the most outstanding discoveries toward understanding the genetic basis of domestication was achieved from studying maize. The dramatic morphological difference between maize and its wild relative teosinte was mapped to only six regions in the genome. One of them harbors *teosinte branched1* (*tb1*) a TCP-family transcription factor controlling the number and length of axillary branches. Domestication of maize entailed the selection of genotypes with higher expression of *TB1*, a negative regulator of growth, leading to a decline in shoot tillering. *tb1* loss-of-function mutants, on the other hand, show increased branching (i.e., reduced apical dominance). *TB1* is conserved in mono- and eudicots and mutants in Arabidopsis (*branched*) and rice (*fineculm1*) show similar phenotypic effects as in maize. Planting density can also affect branching, as even modern maize cultivars with strong apical dominance will display certain levels of side branching if planted at lower densities.

A complex network of transcription factors and hormones interact to regulate both the initiation and outgrowth of tiller buds. Although at first glance the outgrowth mechanism is different between eudicots and monocots, some commonalities are beginning to emerge. The strigolactone signaling module, for instance, appears to be conserved. There are orthologs of the MAX genes in rice, and plants deficient in *MAX1*, *3* or *4* give rise to more tillers, indicating functional equivalence in the strigolactone pathway in cereals. The *LS* gene mentioned above is a key transcriptional regulator of AM initiation, whose function is also conserved between eudicots and monocots.

THE TRANSITION FROM VEGETATIVE TO REPRODUCTIVE GROWTH

Plant architecture can influence yield by altering the relative growth and position of vegetative and reproductive organs, fruit location on the plant and ease of harvest. A key event that determines the balance between vegetative and reproductive growth is the transition to flowering, which therefore has attracted immense interest over many decades from breeders and basic researchers alike. The modular structure of plants implies that a certain number of vegetative modules has to be produced to support, both structurally and physiologically, the subsequent reproductive modules. The genetic programming of a plant is therefore initially locked in on 'vegetative mode' to ensure that flowering does not occur prematurely, which would compromise the plant's ability to survive and reproduce. A developmental 'switch' needs to occur for a plant to acquire the capacity to respond to flowering cues. These cues are a combination of endogenous (usually related to plant size and nutritional status) and exogenous (including seasonal cues such as light quantity and quality, temperature, and other) signals.

Molecular control of vegetative phase change

Before the actual transition from vegetative to reproductive growth, the shoot has to become competent to respond to reproduction-inducing signals. The acquisition of competency is called the 'juvenile-to-adult phase transition' or 'vegetative phase change'. It is marked by changes in various traits such as leaf and stem morphology, growth rate and even resistance to herbivores and disease. The classic example of this phenomenon is seen in the ivy (*Hedera helix*) plant, in which stem and leaf morphology change dramatically after phase transition. This phase change is stable, in that if a cutting or explant is taken from the juvenile part of the plant, the new regenerating plant will have a stable juvenile phenotype, whereas if the explant is taken from the adult part of the plant, the regenerant will likewise display an adult phenotype. Hormones (mainly GAs) and microRNAs (miR156 and miR172) are fundamental regulators of phase change in many plant species.

Florigen and the control of flowering

Once a plant is competent to respond, the appropriate molecular signal needs to reach the apical meristem, which will switch from vegetative to reproductive programming. The notable German botanist Julius von Sachs (1832-1897) was the first to propose the existence of a leaf-derived chemical compound capable of inducing flowering. It was, however, only after the discovery of photoperiodism (the response of plants to the relative duration of

light and dark) that this hypothetical compound was placed on more solid theoretical ground. Mikhail Chailakhyan (1901-1991) introduced the term 'florigen' for the elusive chemical substance and listed a series of postulates it had to fulfill to act as the flower-forming signal.

Decades of biochemical pursuit proved fruitless and the florigen concept fell out of favor in the plant biology community, particularly after a series of flowering pathways were described in Arabidopsis that did not necessitate the existence of a biochemical silver bullet: the photoperiod, vernalization, autonomous and gibberellin flowering pathways. Deeper probing into the photoperiod pathway, however, revealed the existence of a phloem-mobile signal capable of inducing flowering encoded by the gene *FLOWERING LOCUS T* (*FT*), which abides by some of the tenets of the 'florigen' hypothesis. Originally believed to be transported via its mRNA, it is now known that the FT protein moves with the assimilate flow from the leaf to the shoot apex, where it binds to a receptor protein and triggers the conversion of the meristem from vegetative to reproductive. The *FT* ortholog in tomato is *SINGLE FLOWER TRUSS* (*SFT*) in tomato.

Florigenic and anti-florigenic signals

FT is part of a gene family (*CETS*, named after the orthologs *CE*NTRORADIALIS from Antirrhinum, *T*ERMINAL *F*LOWER-*1* from Arabidopsis and *S*ELF-*P*RUNING from tomato) with homology to phosphatidylethanolamine binding proteins, which function as master regulators of developmental processes in plants and animals. In Arabidopsis, the *CETS* gene family is composed of six genes, one of which, *TERMINAL FLOWER1* (*TFL1*), antagonizes the flower-inducing effects of *FT*. TFL1 and its orthologs in other species are thus named 'anti-florigenic' signals.

This pair of regulatory proteins (FT, TFL1) is strongly conserved across angiosperms, and has been shown to participate in other developmental processes besides flowering regulation. The TFL1 protein competes with FT for the binding site of a bZIP transcription factor (FD) and thus the FT/TFL1 balance determines the identity of the meristem. A meristem that entirely transitions to reproductive development is called determinate, because vegetative growth ceases. By contrast, when the meristem gives rise to both vegetative and reproductive tissues, as described below for tomato, growth is said to be indeterminate, or ongoing.

The FT/TFL1 module is integrated with multiple flowering pathways and other proteins to induce or repress flowering in response to specific environmental cues. In Arabidopsis, for instance, the transcription factor CONSTANS (CO) is stabilized by light and, upon reaching a certain concentration threshold, activates expression of the *FT* gene in the phloem. A similar system operates in rice, where *Heading date 1* (*Hd1*, the *CO* ortholog) is expressed in a diurnal pattern, but instead represses *Hd3a* (the *FT* ortholog). Thus, in Arabidopsis the duration of the light period has to exceed a certain threshold for flowering to occur ('long day species'), whereas in rice flowering will proceed only when the light period is short enough ('short day species') to avoid accumulation of the Hd1 flowering repressor. This synchronization with external cues allow plants to keep track of seasons and adjust their life cycle accordingly.

Growth responses to the transition to reproductive growth

The transition to flowering is mediated at the apical meristem, where endogenous and external cues are integrated and the appropriate developmental responses take place. Shoot meristems can be described as vegetative (producing leaves), inflorescence (producing flowers) or floral (the flowers themselves are meristems and the organs they produce are floral organs).

When a single apical meristem forms all of the primary organs, the resulting growth habit is called *monopodial* ("single foot") as in Arabidopsis or *Capsella bursa-pastoris*. Vegetative and reproductive growth thus can be clearly separated in two phases: vegetative growth, in which the meristem produces leaves, and reproductive growth in which the meristem produces floral meristems with their associated leaves called bracts. (Flower development is covered in a separate Teaching Tool).

Alternatively, several meristems can be involved in the construction of the plant, with the resulting organism a concatenation of alternating vegetative and reproductive structures. This growth pattern is called *sympodial* ("joined feet"), of which tomato is the classic example. In tomato, the vegetative apical meristem converts into a floral meristem after producing a series of 6-12 internodes with leaves. Vegetative growth, however, continues through the top-most axillary meristem, which grows vigorously displacing the inflorescence to the side and producing a new sympodium with three leaves and an inflorescence. The remarkable variability in growth habit in tomato and its wild relatives is fine-tuned by genes that control meristem identity and determinacy and by their functional specificity in apical or lateral meristems.

'MOLECULAR TAILORING' OF PLANT ARCHITECTURE

Alteration of plant architecture has been one of the main drivers of crop domestication, as can be easily confirmed by visual comparison of many crop plants and their respective wild relatives. Height, branching, time of flowering, relative organ size and position have all been scrupulously altered over centuries of careful observation and selection. Genome editing technologies, coupled with decades of invaluable physiological and agronomical knowledge, may bring about the revolutionary approach of molecularly 'tailoring' new crops with traits currently difficult or impossible to acquire through classical breeding. Advances in establishing the molecular basis for domestication traits could pave the way for an accelerated achievement of the crop *ideotypes* – the theoretical model of what an optimal crop plant could be in a given environment. A few examples of how plant breeding is contributing to improvements in crop architecture are provided below. These include manipulation of cytokinin levels to control height and grain production in rice, balancing vegetative and reproductive growth in tomato, and determinacy in soybean.

Adjusting plant height

As mentioned above, the reduction of internode length and thus plant height, which is a generally advantageous trait, has been achieved in grasses by selecting and breeding mutations for the

gibberellin biosynthesis and signaling pathways (*sd1* and *Rht1* mutations in rice and wheat, respectively). In rice, additional improvement in plant architecture and yield was achieved by adding in the mutation *Grain number1* (*Gn1*), which codes for CYTOKININ OXIDASE (CKX), an enzyme that inactivates cytokinins. In the *Gn1* mutant the reduced expression of the *OsCKX2* gene causes cytokinin accumulation in the inflorescence meristems and increases the number of rice reproductive organs (spikelets). The resulting *sd1* and *Gn1* double mutant plants have a favorable plant height (semi-dwarf) and inflorescence architecture (many), because *sd1* reduces excessive vegetative growth and *Gn1* improves the number of grains per panicle. Thus, combining (also known as *pyramiding*) *sd1* and *Gn1* increases the grain yield even further than what had been obtained with the Green-Revolution *sd1* mutation, and points to the potential of plant architecture modification through plant hormone manipulation for molecular breeding.

Optimizing growth habit

In tomato, a molecular balance exists between *SELF-PRUNING* (*SP*), the *TFL1* (flowering repressor) ortholog, and *SINGLE FLOWER TRUSS* (*SFT*), the *FT* (flowering promoter) ortholog. This balance leads to the formation of repeating sympodial units of three leaves and one inflorescence indefinitely, the hallmark of indeterminate growth. A recessive mutation in *sp* alters the balance in favor of *SFT*, conferring a determinate growth habit. The sympodial units produce successively fewer leaves until the plant eventually terminates in two inflorescences. Plants harboring the *sp* mutation show limited growth of the shoot, a bushy aspect, compact constitution and more synchronous fruit set. This trait has been of supreme importance to allow mechanical harvest in the tomato crop and is now present in most tomato varieties grown on the field for processing (as opposed to eating fresh).

An optimal balance between vegetative and reproductive growth can be achieved by more subtle manipulation of the *SFT* dosage in an *sp* mutant background or by genome editing of the promoter region of the *SP* gene. For instance, tomato plants homozygous for the *sp* mutation and heterozygous for the *sft* mutation are semi-determinate, instead of determinate. Semi-determinate plants are advantageous over determinate plants, which are too reproductive and have fewer sources of assimilates; and over indeterminate plants, which tend to be too vegetative and have fewer harvestable sinks (fruits). Targeted manipulation of both *SP* and *SFT* genes could lead to tomatoes with the optimal amount of vegetative and reproductive growth to boost potential yield. Of course actual yield is determined by agronomic practices and adequate management of the crop.

Could further manipulation of those orthologs lead to the production of even more advantageous plant growth habits? Some evidence in tomato indicates that this is possible, as allelic variation for two other members of the *CETS* family, *SP5G* and *SP9D,* leads to a subtle extension of determinate growth (hence called semi-determinate) which improves some agronomic traits. Loss-of-function mutations in *SP5G* lead to more compact plants with earlier flowering and fruit ripening. Tomato plants harboring the *SP9D* allele from the wild tomato relative *S. pennellii* show increased vegetative growth, which in turn acts as a source for greater sugar content in fruits.

The soybean *TFL1/SP* ortholog (*Dt1*) has also been a target of domestication. Four independent nucleotide substitutions leading to amino acid changes in the protein sequence have been identified in different accessions of the domesticate *Glycine max*, all of them determinate, but not in the wild relative *G. soja*, which has indeterminate growth habit. Soybean harbors at least another four *CETS*-family genes besides *Dt1*. A second locus affecting growth habit, *Dt2*, has been described. In *Dt1*/*Dt1* homozygous backgrounds, the wild-type allele of *Dt2* produces semi-determinate growth, whereas the homozygous mutant *dt2*/*dt2* produces indeterminate phenotypes.

Manipulation of growth habit could help adjust soybean to the agricultural calendar. Soybean is now cultivated across a long latitudinal gradient, where daylength and temperatures can vary considerably. Varieties belonging to different 'maturity groups' have been bred for each climate and latitude, with indeterminate varieties found predominantly in regions with short growing seasons. Determinate growth varieties, on the other hand, tend to flower earlier, but a more subtle adjustment would be desirable to allow the old adage of 'sowing in the rain and harvesting in the sun' to be true in each particular location. Ideally, more vegetative growth before the transition to flowering would also be advantageous, but the fact that soybean leaves senesce after flowering make this a difficult breeding goal using conventional breeding.

Tailoring side branching

Elimination of side branching is a long-sought goal of breeding in many horticultural and woody species, as it removes the need for regular pruning of 'suckers' in the former and improves timber quality in the latter. The complexity of the molecular pathways involved in axillary meristem establishment and outgrowth make this a particularly challenging goal. Few, if any, candidate genes are amenable to manipulation without large negative pleiotropic (additional) effects. This is an area where more basic research is needed, particularly on the link between environmental and endogenous cues determining the phenotypic outcome of side branching. This is especially true in rice and wheat breeding, where a smaller number of sturdier tillers would be advantageous for high-density planting. As already discussed, the genetic control of tillering in cereals is an active area of research and many genes have been shown to be involved in controlling this trait. This knowledge, however, is not yet sufficient to produce rice or wheat varieties with a pre-set, fixed number of tillers, due to the strong influence of the environment and agronomic practices on the final plant phenotype.

Adjusting the harvest index

As can be deduced from the previous sections, an overarching theme of plant architecture improvements in agriculture is optimization of the balance between vegetative and reproductive growth. That a golden-mean (Aristotle's *medium virtus*) should be reached to balance vegetative and reproductive growth is not

obvious at first glance. This is because vegetative growth, which represents the main source of photoassimilates, and reproductive growth (the sink that provides fruits and seeds to be harvested) are equally important and can compete with one another for resources. In cereals, 'harvest index' (HI), *i.e*., the ratio of grain to total shoot dry mass, has been a trait of paramount importance in crop breeding. Currently, the HI of rice and wheat approach values near 0.6, meaning that 60% of the final dry weight of a plant is harvestable grain, or in other words, that only 40% of photoassimilates are left behind in organs necessary to do photosynthesis. Any further improvement in HI will involve changes in plant architecture that should optimize the amount of photoassimilates destined for reproductive growth without compromising the capacity for photosynthesis in the first place. HI initially appeared to be a complicated, quantitatively controlled trait. The tenacious work of plant geneticists and physiologists has revealed that individual genes of large effect underlie many of the most important domestication and breeding traits related to HI increase. In contrast to the patient work of able agronomists over many years, breeding could soon entail the exploitation of novel physiological and genetic knowledge to rapidly engineer and combine traits of interest in crops.

CONCLUSION AND FUTURE DIRECTIONS

Post-embryonic development allows plants to adjust their shoot architecture to a constantly changing environment. The suite of adaptations that allow such plasticity evolved for survival and reproductive fitness, mainly by enhancing light interception, mechanical stability, water economy and seed dispersal. As we have shown, plant shoot architecture also has a huge impact on crop productivity and has therefore been, to varying extents, manipulated to suit the needs and desires of humans, through domestication and improvement of crops. Almost every achievement in this respect has been attained using conventional breeding techniques of crossing and screening for traits of interest. Although very effective, this method has two limitations: it usually takes a long time, and it reduces genetic variation. The excessive focus on specific traits can lead to negative effects on others.

For many years, plant biologists and agronomists have harnessed genetic variation available in wild species related to crops. If a certain trait is monogenic, it can be transferred to the crop plant through conventional breeding. However, if the trait is controlled by multiple genetic loci, as is quite common for abiotic stress resistance, nutritional quality and flavor, it is difficult to transfer it to the target crop without concurrent losses in other traits. An approach called "*de novo* domestication" provides a way to conquer this problem. Instead of introducing alleles from wild relatives into cultivated crops, as has been conventionally done in classical breeding, it exploits the possibility of precisely engineering genome sequences of wild species to "domesticate" them from scratch ('*de novo*'). In other words, plants spanning the breadth of abiotic tolerances and flavors can be rapidly growth-optimized to increase yield and quality. Knowledge of hormone biosynthesis and signaling pathways, genetic networks controlling organogenesis, and biochemical determinants of energy flow and metabolism could be used for targeted engineering of 'ideal', better-looking or higher yielding plants. A sustained basic research effort to unveil the fundamentals of plant growth and development is crucial for the success of this endeavor.

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RECOMMENDED READING

(This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included.)

General Background

Arber, A. (1950). The Natural Philosophy of Plant Form. (Cambridge, UK: Cambridge University Press).

Berleth, T., and Sachs, T. (2001). Plant morphogenesis: long-distance coordination and local patterning. Curr. Opin. Plant Biol. **4:** 57–62. https://doi.org/10.1016/S1369-5266(00)00136-9.

Coen, E., Rolland-Lagan, A.G., Matthews, M., Bangham, J.A., and Prusinkiewicz, P. (2004). The genetics of geometry. Proc. Natl. Acad. Sci. USA **101:** 4728–4735. https://doi.org/10.1073/pnas.0306308101.

Dawkins, R. (2009). The Greatest Show on Earth: The Evidence for Evolution. (London: Bantam).

Davies, P., ed. (2010). Plant Hormones. (New York: Springer International Publishing). https://doi.org/10.1007/978-1-4020-2686-7

Evans, L.T. (1996). Crop Evolution, Adaptation and Yield. (Cambridge, UK: Cambridge University Press).

Fosket, D.E. (1994). Plant Growth and Development: A Molecular Approach. (New York: Academic Press).

Friedman, W.E., Moore, R.C., and Purugganan, M.D. (2004). The evolution of plant development. Am. J. Bot. **91:** 1726–1741. https://doi.org/10.3732/ajb.91.10.1726.

Friml, J., et al. (2006). Apical-basal polarity: why plant cells don't stand on their heads. Trends Plant Sci. **11:** 12–14. https://doi.org/10.1016/j.tplants.2005.11.010.

Harlan, J.R. (1975). Crops and Man. (Madison, USA: American Society of Agronomy).

Jones, R., Ougham, H., Thomas, H., and Waaland, S. (2013). The Molecular Life of Plants. (Oxford, UK: Wiley-Blackwell).

Meyer, R.S., and Purugganan, M.D. (2013). Evolution of crop species: genetics of domestication and diversification. Nat. Rev. Genet. **14:** 840–852. https://doi.org/10.1038/nrg3605.

Monod, J. (1968). Chance and Necessity. (New York: Vintage Books).
Niklas, K.J. (2000). The Evolution of Plant Body Plants – A Biomechanical Perspective. Ann. Bot. **85:** 411–438. https://doi.org/10.1006/anbo.1999.1100.
Niklas, K.J. (2016). Plant Evolution. (Chicago: Chicago University Press). https://doi.org/10.7208/chicago/9780226342283.001.0001
Pollan, M. (2001). The Botany of Desire. (New York: Random House).
Sachs, T. (1991). Pattern Formation in Plant Tissues. (Cambridge, UK: Cambridge University Press). https://doi.org/10.1017/CBO9780511574535b
Schwanitz, F. (1996). The Origin of Cultivated Plants. (Cambridge, MA: Harvard University Press).
Shepard, K.A., and Purugganan, M.D. (2002). The genetics of plant morphological evolution. Curr. Opin. Plant Biol. **5:** 49–55. https://doi.org/10.1016/S1369-5266(01)00227-8.
Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development, (Cambridge, UK: Cambridge University Press). https://doi.org/10.1017/CBO9780511626227
Sussex, I.M., and Kerk, N.M. (2001). The evolution of plant architecture. Curr. Opin. Plant Biol. **4:** 33–37. https://doi.org/10.1016/S1369-5266(00)00132-1.
Taiz, L., Zeiger, E., Moller, I.M., and Murphy, A.S. (2015). Plant Physiology and Development, (Sunderland, Mass.: Sinauer Associates).
Takhtajan, A. (1991). Evolutionary Trends in Flowering Plants. (New York: Columbia University Press).
Turnbull, C., ed. (2005). Plant Architecture and Its Manipulation. (Oxford, UK: Wiley-Blackwell).
Ueda, M., and Laux, T. (2012). The origin of the plant body axis. Curr. Opin. Plant Biol. **15:** 578–584. https://doi.org/10.1016/j.pbi.2012.08.001.
Wang, Y., and Li, J. (2008). Molecular basis of plant architecture. Annu. Rev. Plant Biol. **59:** 253–279. https://doi.org/10.1146/annurev.arplant.59.032607.092902.

Structure of the plant shoot and molecular control of plant stature and shoot branching

Bainbridge, K., Guyomarc'h, S., Bayer, E., Swarup, R., Bennett, M., Mandel, T., and Kuhlemeier, C. (2008). Auxin influx carriers stabilize phyllotactic patterning. Genes Dev. **22:** 810–823. https://doi.org/10.1101/gad.462608.
Barthélémy, D., and Caraglio, Y. (2007). Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Ann. Bot. **99:** 375–407. https://doi.org/10.1093/aob/mcl260.
Bäurle, I., and Laux, T. (2003). Apical meristems: the plant's fountain of youth. BioEssays **25:** 961–970. https://doi.org/10.1002/bies.10341.
Bennett, T., Hines, G., van Rongen, M., Waldie, T., Sawchuk, M.G., Scarpella, E., Ljung, K., and Leyser, O. (2016). Connective auxin transport in the shoot facilitates communication between shoot apices. PLoS Biol. **14:** e1002446. https://doi.org/10.1371/journal.pbio.1002446.
Booker, J., Sieberer, T., Wright, W., Williamson, L., Willett, B., Stirnberg, P., Turnbull, C., Srinivasan, M., Goddard, P., and Leyser, O. (2005). MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. Dev. Cell **8:** 443–449. https://doi.org/10.1016/j.devcel.2005.01.009.
Burian, A., Barbier de Reuille, P., and Kuhlemeier, C. (2016). Patterns of stem cell divisions contribute to plant longevity. Curr. Biol. **26:** 1385–1394. https://doi.org/10.1016/j.cub.2016.03.067.
Choe, S., Fujioka, S., Noguchi, T., Takatsuto, S., Yoshida, S., and Feldmann, K.A. (2001). Overexpression of DWARF4 in the brassinosteroid biosynthetic pathway results in increased vegetative growth and seed yield in Arabidopsis. Plant J. **26:** 573–582. https://doi.org/10.1046/j.1365-313x.2001.01055.x.
Cook, M.G., and Evans, L.T. (1983). Some physiological aspects of the domestication and improvement of rice (*Oryza* spp). Field Crops Res. **6:** 219–238. https://doi.org/10.1016/0378-4290(83)90062-X.
Crawford, S., Shinohara, N., Sieberer, T., Williamson, L., George, G., Hepworth, J., Müller, D., Domagalska, M.A., and Leyser, O. (2010). Strigolactones enhance competition between shoot branches by dampening auxin transport. Development **137:** 2905–2913. https://doi.org/10.1242/dev.051987.
Doebley, J.F., Gaut, B.S., and Smith, B.D. (2006). The molecular genetics of crop domestication. Cell **127:** 1309–1321. https://doi.org/10.1016/j.cell.2006.12.006.
Doebley, J., Stec, A., and Hubbard, L. (1997). The evolution of apical dominance in maize. Nature **386:** 485–488. https://doi.org/10.1038/386485a0.
Domagalska, M.A., and Leyser, O. (2011). Signal integration in the control of shoot branching. Nat. Rev. Mol. Cell Biol. **12:** 211–221. https://doi.org/10.1038/nrm3088.
Doust, A. (2007a). Architectural evolution and its implications for domestication in grasses. Ann. Bot. **100:** 941–950. https://doi.org/10.1093/aob/mcm040.
Doust, A.N. (2007b). Grass architecture: genetic and environmental control of branching. Curr. Opin. Plant Biol. **10:** 21–25. https://doi.org/10.1016/j.pbi.2006.11.015.
Garrison, R. (1955). Studies in the development of axillary buds. Am. J. Bot. **42:** 257–266. https://doi.org/10.1002/j.1537-2197.1955.tb11117.x.
Greb, T., Clarenz, O., Schafer, E., Muller, D., Herrero, R., Schmitz, G., and Theres, K. (2003). Molecular analysis of the *LATERAL SUPPRESSOR* gene in Arabidopsis reveals a conserved control mechanism for axillary meristem formation. Genes Dev. **17:** 1175–1187. https://doi.org/10.1101/gad.260703.
Hayward, A., Stirnberg, P., Beveridge, C., and Leyser, O. (2009). Interactions between auxin and strigolactone in shoot branching control. Plant Physiol. **151:** 400–412. https://doi.org/10.1104/pp.109.137646.
Jasinski, S., Tattersall, A., Piazza, P., Hay, A., Martinez-Garcia, J. F., Schmitz, G., Theres, K., McCormick, S., and Tsiantis, M. (2008). *PROCERA* encodes a DELLA protein that mediates control of dissected leaf form in tomato. Plant J. **56:** 603–612. https://doi.org/10.1111/j.1365-313X.2008.03628.x.
Lee, J., Park, J.-J., Kim, S.L., Yim, J., and An, G. (2007). Mutations in the rice *liguleless* gene result in a complete loss of the auricle, ligule, and laminar joint. Plant Mol. Biol. **65:** 487–499. https://doi.org/10.1007/s11103-007-9196-1.
McConnell, J.R., and Barton, M.K. (1998). Leaf polarity and meristem formation in Arabidopsis. Development **125:** 2935–2942.
Melnyk, C.W., Schuster, C., Leyser, O., and Meyerowitz, E.M. (2015). A developmental framework for graft formation and vascular reconnection in *Arabidopsis thaliana*. Curr. Biol. **25:** 1306–1318. https://doi.org/10.1016/j.cub.2015.03.032.
Ongaro, V., and Leyser, O. (2008). Hormonal control of shoot branching. J. Exp. Bot. **59:** 67–74. https://doi.org/10.1093/jxb/erm134.
Sachs, T. (2002). Consequences of the inherent developmental plasticity of organ and tissue relations. Evol. Ecol. **16:** 243–265. https://doi.org/10.1023/A:1019628526880.
Sakamoto, T., Morinaka, Y., Ohnishi, T., Sunohara, H., Fujioka, S., Ueguchi-Tanaka, M., Mizutani, M., Sakata, K., Takatsuto, S., Yoshida, S., Tanaka, H., and Kitano, H., et al. (2006). Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. Nat. Biotechnol. **24:** 105–109. https://doi.org/10.1038/nbt1173.
Sampathkumar, A., Yan, A., Krupinski, P., and Meyerowitz, E.M. (2014). Physical forces regulate plant development and morphogenesis.

Curr. Biol. **24:** R475–R483. https://doi.org/10.1016/j.cub.2014.03.014.

Sanchez, P., Nehlin, L., and Greb, T. (2012). From thin to thick: major transitions during stem development. Trends Plant Sci. **17:** 113–121. https://doi.org/10.1016/j.tplants.2011.11.004.

Schmitz, G., and Theres, K. (1999). Genetic control of branching in Arabidopsis and tomato. Curr. Opin. Plant Biol. **2:** 51–55. https://doi.org/10.1016/S1369-5266(99)80010-7.

Schmitz, G., and Theres, K. (2005). Shoot and inflorescence branching. Curr. Opin. Plant Biol. **8:** 506–511. https://doi.org/10.1016/j.pbi.2005.07.010.

Shi, B., Zhang, C., Tian, C., Wang, J., Wang, Q., Xu, T., Xu, Y., Ohno, C., Sablowski, R., Heisler, M.G., Theres, K., and Wang, Y., et al. (2016). Two-step regulation of a meristematic cell population acting in shoot branching in Arabidopsis. PLoS Genet. **12:** e1006168. https://doi.org/10.1371/journal.pgen.1006168.

Smith, R.S., Guyomarc'h, S., Mandel, T., Reinhardt, D., Kuhlemeier, C., and Prusinkiewicz, P. (2006). A plausible model of phyllotaxis. Proc. Natl. Acad. Sci. USA **103:** 1301–1306. https://doi.org/10.1073/pnas.0510457103.

Wang, H., Studer, A.J., Zhao, Q., Meeley, R., and Doebley, J.F. (2015). Evidence that the origin of naked kernels during maize domestication was caused by a single amino acid substitution in *tga1*. Genetics **200:** 965–974. https://doi.org/10.1534/genetics.115.175752.

Wang, Q., Hasson, A., Rossmann, S., and Theres, K. (2016). Divide et impera: boundaries shape the plant body and initiate new meristems. New Phytol. **209:** 485–498. https://doi.org/10.1111/nph.13641.

Wang, Q., Kohlen, W., Rossmann, S., Vernoux, T., and Theres, K. (2014). Auxin depletion from the leaf axil conditions competence for axillary meristem formation in Arabidopsis and tomato. Plant Cell **26:** 2068–2079. https://doi.org/10.1105/tpc.114.123059.

Wills, D.M., Whipple, C.J., Takuno, S., Kursel, L.E., Shannon, L.M., Ross-Ibarra, J., and Doebley, J.F. (2013). From many, one: genetic control of prolificacy during maize domestication. PLoS Genet. **9:** e1003604. https://doi.org/10.1371/journal.pgen.1003604.

Wright, S.I., Bi, I.V., Schroeder, S.G., Yamasaki, M., Doebley, J.F., McMullen, M.D., and Gaut, B.S. (2005). The effects of artificial selection on the maize genome. Science **308:** 1310–1314. https://doi.org/10.1126/science.1107891.

Yang, M., and Jiao, Y. (2016). Regulation of axillary meristem initiation by transcription factors and plant hormones. Front. Plant Sci. **7:** 183. https://doi.org/10.3389/fpls.2016.00183.

Young, N.F., Ferguson, B.J., Antoniadi, I., Bennett, M.H., Beveridge, C.A., and Turnbull, C.G.N. (2014). Conditional auxin response and differential cytokinin profiles in shoot branching mutants. Plant Physiol. **165:** 1723–1736. https://doi.org/10.1104/pp.114.239996.

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Alvarez, J., Guli, C.L., Yu, X.-H., and Smyth, D.R. (1992). *terminal flower*: A gene affecting inflorescence development in *Arabidopsis thaliana*. Plant J. **2:** 103–116. https://doi.org/10.1111/j.1365-313X.1992.00103.x.

Hanano, S., and Goto, K. (2011). Arabidopsis *TERMINAL FLOWER1* is involved in the regulation of flowering time and inflorescence development through transcriptional repression. Plant Cell **23:** 3172–3184. https://doi.org/10.1105/tpc.111.088641.

Huijser, P., and Schmid, M. (2011). The control of developmental phase transitions in plants. Development **138:** 4117–4129. https://doi.org/10.1242/dev.063511.

Karlgren, A., Gyllenstrand, N., Källman, T., Sundström, J.F., Moore, D., Lascoux, M., and Lagercrantz, U. (2011). Evolution of the PEBP gene family in plants: functional diversification in seed plant evolution. Plant Physiol. **156:** 1967–1977. https://doi.org/10.1104/pp.111.176206.

Lifschitz, E., Ayre, B.G., and Eshed, Y. (2014). Florigen and anti-florigen - a systemic mechanism for coordinating growth and termination in flowering plants. Front. Plant Sci. **5:** 465. https://doi.org/10.3389/fpls.2014.00465.

Lifschitz, E., and Eshed, Y. (2006). Universal florigenic signals triggered by FT homologues regulate growth and flowering cycles in perennial day-neutral tomato. J. Exp. Bot. **57:** 3405–3414. https://doi.org/10.1093/jxb/erl106.

Périlleux, C., Lobet, G., and Tocquin, P. (2014). Inflorescence development in tomato: gene functions within a zigzag model. Front. Plant Sci. **5:** 121.

Pin, P.A., and Nilsson, O. (2012). The multifaceted roles of FLOWERING LOCUS T in plant development. Plant Cell Environ. **35:** 1742–1755. https://doi.org/10.1111/j.1365-3040.2012.02558.x.

Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D., and Lifschitz, E. (1998). The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. Development **125:** 1979–1989.

Poethig, R.S. (2003). Phase change and the regulation of developmental timing in plants. Science **301:** 334–336. https://doi.org/10.1126/science.1085328.

Poethig, R.S. (2013). Vegetative phase change and shoot maturation in plants. Curr Top Dev Biol **105:** 125–152. https://doi.org/10.1016/B978-0-12-396968-2.00005-1

Samach, A., and Lotan, H. (2007). The transition to flowering in tomato. Plant Biotechnol. **24:** 71–82. https://doi.org/10.5511/plantbiotechnology.24.71.

Samach, A., and Smith, H.M. (2013). Constraints to obtaining consistent annual yields in perennials. II: Environment and fruit load affect induction of flowering. Plant Sci. **207:** 168–176. https://doi.org/10.1016/j.plantsci.2013.02.006.

Shalit, A., Rozman, A., Goldshmidt, A., Alvarez, J.P., Bowman, J.L., Eshed, Y., and Lifschitz, E. (2009). The flowering hormone florigen functions as a general systemic regulator of growth and termination. Proc. Natl. Acad. Sci. USA **106:** 8392–8397. https://doi.org/10.1073/pnas.0810810106.

Tian, Z., Wang, X., Lee, R., Li, Y., Specht, J.E., Nelson, R.L., McClean, P.E., Qiu, L., and Ma, J. (2010). Artificial selection for determinate growth habit in soybean. Proc. Natl. Acad. Sci. USA **107:** 8563–8568. https://doi.org/10.1073/pnas.1000088107.

Turck, F., Fornara, F., and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu. Rev. Plant Biol. **59:** 573–594. https://doi.org/10.1146/annurev.arplant.59.032607.092755.

Wickland, D.P., and Hanzawa, Y. (2015). The *FLOWERING LOCUS T/TERMINAL FLOWER 1* Gene Family: Functional Evolution and Molecular Mechanisms. Mol. Plant **8:** 983–997. https://doi.org/10.1016/j.molp.2015.01.007.

Yang, X.-C., and Hwa, C.-M. (2008). Genetic modification of plant architecture and variety improvement in rice. Heredity (Edinb) **101:** 396–404. https://doi.org/10.1038/hdy.2008.90.

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Brooks, C., Nekrasov, V., Lippman, Z., and Van Eck, J. (2014). Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR Associated9 system. Plant Physiol. **166:** 1292–1297. https://doi.org/10.1104/pp.114.247577.

Čermák, T., Baltes, N.J., Čegan, R., Zhang, Y., and Voytas, D.F. (2015). High-frequency, precise modification of the tomato genome. Genome Biol. **16:** 232. https://doi.org/10.1186/s13059-015-0796-9.

Čermák, T., Doyle, E.L., Christian, M., Wang, L., Zhang, Y., Schmidt, C., Baller, J.A., Somia, N.V., Bogdanove, A.J., and Voytas, D.F. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. **39:** e82–e82. https://doi.org/10.1093/nar/gkr218.

Donald, C.M. (1968). The Breeding of Crop Ideotypes. Euphytica **17:** 385–403. https://doi.org/10.1007/BF00056241.

Jiang, K., Liberatore, K.L., Park, S.J., Alvarez, J.P., and Lippman, Z.B. (2013). Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture. PLoS Genet. **9:** e1004043. https://doi.org/10.1371/journal.pgen.1004043.

Khush, G.S. (2001). Green revolution: the way forward. Nat. Rev. Genet. **2:** 815–822. https://doi.org/10.1038/35093585.

Krieger, U., Lippman, Z.B., and Zamir, D. (2010). The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato. Nat. Genet. **42:** 459–463. https://doi.org/10.1038/ng.550.

Lemmon, Z.H., Reem, N.T., Dalrymple, J., Soyk, S., Swartwood, K. E., Rodriguez-Leal, D., Van Eck, J., and Lippman, Z.B. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. Nat. Plants **4:** 766–770. https://doi.org/10.1038/s41477-018-0259-x.

Lor, V.S., Starker, C.G., Voytas, D.F., Weiss, D., and Olszewski, N. E. (2014). Targeted mutagenesis of the tomato *PROCERA* gene using transcription activator-like effector nucleases. Plant Physiol. **166:** 1288–1291. https://doi.org/10.1104/pp.114.247593.

McGarry, R.C., and Ayre, B.G. (2012). Manipulating plant architecture with members of the *CETS* gene family. Plant Sci. **188-189:** 71–81. https://doi.org/10.1016/j.plantsci.2012.03.002.

Park, S.J., Jiang, K., Tal, L., Yichie, Y., Gar, O., Zamir, D., Eshed, Y., and Lippman, Z.B. (2014). Optimization of crop productivity in tomato using induced mutations in the florigen pathway. Nat. Genet. **46:** 1337–1342. https://doi.org/10.1038/ng.3131.

Rodríguez-Leal, D., Lemmon, Z.H., Man, J., Bartlett, M.E., and Lippman, Z.B. (2017). Engineering quantitative trait variation for crop improvement by genome editing. Cell **171:** 470–480. https://doi.org/10.1016/j.cell.2017.08.030.

Teichmann, T., and Muhr, M. (2015). Shaping plant architecture. Front. Plant Sci. **6:** 233. https://doi.org/10.3389/fpls.2015.00233.

Vicente, M.H., Zsögön, A., de Sá, A.F.L., Ribeiro, R.V., and Peres, L.E.P. (2015). Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (*Solanum lycopersicum*). J. Plant Physiol. **177:** 11–19. https://doi.org/10.1016/j.jplph.2015.01.003.

Zsögön, A., Cermak, T., Voytas, D., and Peres, L.E.P. (2017). Genome editing as a tool to achieve the crop ideotype and *de novo* domestication of wild relatives: Case study in tomato. Plant Sci. **256:** 120–130. https://doi.org/10.1016/j.plantsci.2016.12.012.

Zsögön, A., Čermák, T., Naves, E.R., Notini, M.M., Edel, K.H., Weinl, S., Freschi, L., Voytas, D.F., Kudla, J., and Peres, L.E.P. (2018). *De novo* domestication of wild tomato using genome editing. Nat. Biotechnol. **36:** 1211–1216. https://doi.org/10.1038/nbt.4272.