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Review article

Hierarchies of plant stiffness

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ABSTRACT

Plants must meet mechanical as well as physiological and reproductive requirements for survival. Management of internal and external stresses is achieved through their unique hierarchical architecture. Stiffness is determined by a combination of morphological (geometrical) and compositional variables that vary across multiple length scales ranging from the whole plant to organ, tissue, cell and cell wall levels. These parameters include, among others, organ diameter, tissue organization, cell size, density and turgor pressure, and the thickness and composition of cell walls. These structural parameters and their consequences on plant stiffness are reviewed in the context of work on stems of the genetic reference plant *Arabidopsis thaliana* (Arabidopsis), and the suitability of Arabidopsis as a model system for consistent investigation of factors controlling plant stiffness is put forward. Moving beyond Arabidopsis, the presence of morphological parameters causing stiffness gradients across length-scales leads to beneficial emergent properties such as increased load-bearing capacity and reversible actuation. Tailoring of plant stiffness for old and new purposes in agriculture and forestry can be achieved through bioengineering based on the knowledge of the morphological and compositional parameters of plant stiffness in combination with gene identification through the use of genetics.

Contents

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1. Introduction

Physiological and mechanical requirements, as well as the physical environment, are among the most important factors that contribute to shaping plant organs and anatomy during growth, and hence the stiffness of plants and their organs. It is essential to first recall the physical and chemical laws to which a plant is subjected to understand the different morphological features that a plant develops throughout its body. Physiological functions dictated by growth, survival and reproduction, as well as mechanical demands, are subject to habitat conditions that determine plant morphology, anatomy and each of its constitutive tissues.

The primary functions a plant must perform include photosynthesis, fluid movement, reproduction, and mechanics that withstand the static and variable forces encountered during the plant's life span. Thus, internal (supporting self) and external forces (e.g., gravity, wind) must be balanced with the metabolic needs of life (e.g., acquisition of sufficient sunlight, water and nutrients; prevention of water loss), postembryonic growth and environmental responses within the ecological context of a particular plant species. Their ability to do this is linked to their unique, hierarchical architecture, in which the morphological and compositional parameters governing stiffness are developed across multiple length scales from organ to tissue to cells to cell walls (Fig. 1) [1–4]. To clarify our use of the term parameters (variables, properties) influencing stiffness, as well as to define various mechanical terms used throughout the review, we have provided further description in Box 1 and Fig. 1. In addition, techniques commonly used to mechanically test the stiffness parameters described throughout the review have been outlined in Box 2 and Fig. 2.

In this review, we will focus on mature plant organs (stems) and their structural and mechanical properties, rather than on the biomechanics of cell and tissue initiation, growth and development, as these recently have been extensively reviewed (e.g., [1,5,6]). We investigate parameters affecting plant stiffness from a top-down perspective, considering the work that has been performed on plant biomechanics in the genetic reference plant *Arabidopsis thaliana* (Arabidopsis), and address the question of whether Arabidopsis could be used as an appropriate model system in which to elucidate the roles of various structural parameters that affect plant stiffness. Having discussed a series of controlling factors across multiple length scales, we next consider the emergent functional properties observed in plant species where gradients in morphological parameters are present. These properties are often found in plant species that are used as reference plants for engineered, bio-inspired actuation prototypes. Finally, we briefly address the need for and basic techniques for the bioengineering of plant stiffness to improve agricultural success and develop better functional products.

Box 1: Terminology related to mechanics of biological systems in the context of this paper

Stiffness parameters: these are inherent morphological and compositional features that predetermine the extent to which an object will deform in response to an applied force. In the case of plants, the contribution of different parameters to overall stiffness depends on the species type, developmental stage and environmental history of the plant.

Length scale: this describes the different orders of magnitude at which parameters of stiffness appear (Fig. 1) [1,111].

Load: an applied force (internal or external) that generates mechanical stress in an object [4,23,27].

Stress: the amount of force (F) applied across a given area (A) of an object (stress = F/A, N m^2) [4,23,27].

Strain: the change in length (l) of an object in response to an applied stress (strain = $\Delta l/l_o$, dimensionless) [4,23,27].

Stiffness: measures the resistance offered by an elastic object to deformation. A stiff object resists deformation in response to large stresses while a compliant (i.e., less stiff) object deforms in response to relatively small stresses [4,23,27].

Flexural rigidity: the ability of an object to resist bending in response to an applied force. Rigid objects resist bending deformation in response to large stresses and flexible objects bend in response to small stresses [4,27].

Strength: the amount of load required to cause an object to break (e.g., tear, snap, lose cohesiveness, etc.) [4,23,27].

Failure: the point at which an object breaks (e.g., tears, snaps, buckles, etc.) [4,23,27].

Tension, compression and shear (Fig. 2A): types of forces that can act upon an object. Tensile forces pull and cause lengthening; compressive forces push and cause shortening; shear forces are misaligned tensile or compressive forces that can cause tearing [4,27].

Actuation: the generation of mechanical energy and a subsequent change in an object's shape in response to an external, non-mechanical stimulus [57,112].

Reversible actuation: the release of energy that allows an object to return to its original shape when a stimulus is removed [96,97].

Functional gradients: transitions in morphology or composition along the length or cross-section of an object that predetermine the manner in which the object will deform in response to a stimulus [92].

Smart materials and structures: this refers to synthetic materials or structures that have built-in actuating properties based on concepts including those derived from studying biological actuators (e.g., pinecones) [97].

2. Top-down investigation of plant stem stiffness hierarchies in the reference plant Arabidopsis

Plant stiffness is a mechanical property whose governing parameters span across multiple levels. The interaction between these parameters at different length scales makes it difficult to distinguish in what proportion individual parameters contribute to overall plant stiffness. In this section, we examine the data available with regard to the hierarchical levels of plant structural organization and their biomechanical properties as studied in Arabidopsis, and consider the merit of using Arabidopsis as a system in which to investigate the geometrical and compositional parameters at different length scales to contribute to an integrated, multiscale model of plant stiffness.

2.1. Whole plant

A discussion of plant biomechanics, in particular plant stiffness, cannot avoid examining plant structure as a whole. Key factors that control plant stiffness include the force of gravity upon the stem, the

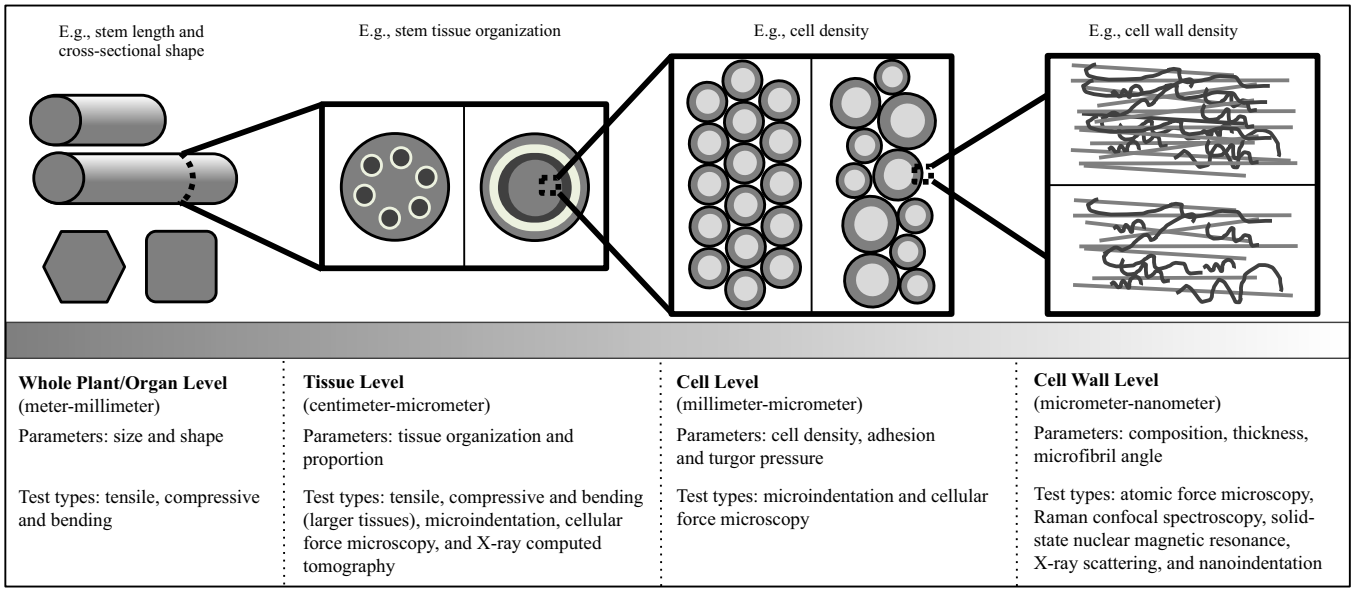

Fig. 1. A schematic of the hierarchy of mechanical parameters influencing stiffness across different length scales in plants.
Examples of the different parameters are depicted and each length scale is described. The whole plant and organ level (meter-millimeter) includes examining the mechanical properties of both the entire plant as a whole structure, as well as individually studying its various organs (e.g., stem, petiole, leaf, root, etc.). The tissue level (centimeter-micrometer) involves examining individual tissues for their mechanical properties as well as how the organization of different tissues as a collective whole can influence plant/organ stiffness. The cell level (millimeter-micrometer) includes studying isolated cells for their mechanical properties as well as how cells interact with neighbouring cells to influence the mechanical parameters observed at the tissue level. Finally, the cell wall level (micrometer-nanometer) involves studying the mechanical properties of the cell wall in terms of its composition, structure and interaction between its various components in order to understand how this building block of plant material ultimately affects the mechanical parameters observed at higher length scales (cell, tissue, organ and whole plant). Adapted from [3,111].

weight of branches and leaf canopy, and the flow-induced stresses upon the whole plant due to wind acting upon the canopy [7,8]. The effect of these forces is particularly strong in large, long-lived plants such as trees, and the ability of plants to withstand these stresses is dependent on properties of the stem (Section 2.2. below) as well as the distribution of weight and formation of overall leaf canopy shape by the pattern of branching [3,8]. However, in a small herbaceous annual such as Arabidopsis, these factors are less important, and have not been studied. For some background on stiffness at the whole plant level, we refer you to [8–12].

2.2. Organ level

As primary load-bearing structures for the plant, stems must be able to support both themselves and other organs (e.g., fruit, flowers and leaves) [3,13]. Through the alteration of geometric variables such as stem shape, height, cross sectional diameter or a combination thereof, it is possible for plant growth to be tailored, resulting in the final morphology accommodating mechanical requirements, including stiffness, imposed by both static and dynamic environmental factors (e.g., gravity and wind, respectively) [3,7,13].

2.2.1. Environmental history

Variation in mechanical performance naturally exists among accessions of Arabidopsis. An investigation of the tensile stiffness of 12 accessions of Arabidopsis identified correlations with stem diameter and cell wall composition [14]. Natural variation in stiffness most likely exists due to the different habitats in which the accessions have evolved. This includes factors such as nutrient availability, environmental conditions and mechanical perturbation, the latter of which directly impacts stiffness in Arabidopsis. Stems of plants grown under mechanical perturbation mimicking wind disturbance were 50% shorter than control plants (Fig. 3A) and demonstrated alterations in tissue shape and proportions (Fig. 3E), as well as in cell wall thickness. Further, mechanically-perturbed stems were 70% less stiff than unperturbed stems (Table 1), demonstrating that external mechanical stimulation can result in adaptive growth that modulates the final stiffness properties of a plant [13].

2.2.2. Height, diameter and cross-sectional stem shape

Organ-level variables that modulate stem stiffness are interrelated and include stem diameter and stem shape as predetermined through adaptive growth and evolution. Depending upon the species, both stem diameter and shape can in part affect stem height [7,9]. In larger species, for example trees, height becomes a more critical parameter that can affect stiffness due to self-weight from large branching canopies and increased tissue mass [4,9]. Height has little impact on plant stiffness in Arabidopsis, however, since this species is quite small. Thus, at the organ level for Arabidopsis, stem diameter and cross-sectional shape are the main parameters to consider in relation to plant stiffness as they alter the ability of the stem to bend and hence its flexural rigidity.

The Arabidopsis dominant gain-of-function mutant *STURDY* has a qualitative increase in stiffness with 40% thicker stems than wild type plants and a concomitant increase in cell number and reinforced/lignified tissue (Table 1) [101]. The results seen with *STURDY* mutants contrast with those observed from mechanical testing of dried stems of natural accessions of Arabidopsis, where a negative correlation was determined between tensile stiffness and diameter. Instead, diameter was correlated with length of vegetative growth: i.e., time to flowering [14]. These results cannot be directly compared – quantitative mechanical testing was not performed on *STURDY* stems. However, this contrast highlights the point that stem diameter cannot be separated from sub-organ level morphological variables such as turgor, cell number (tissue mass), cell type and cell size. This is also exemplified in the transgenic Arabidopsis line MYB87-SRDX, which has thicker stems accompanied both by changes in cell number and cell size (Fig. 3B and Table 1) [15].

Box 2: Mechanical test types commonly used for testing plants

This is not intended to be an exhaustive list of techniques, but rather a general overview of techniques commonly used to study mechanical properties in plants. For a comprehensive overview of the available techniques along with their advantages and caveats, please refer to [24,36,40].

Uniaxial testing (Fig. 2B): mechanical testing performed along a single axis of an object [4,23].

Tensile test (Fig. 2B): this involves securing a sample at both ends (usually along the longitudinal axis in the case of plants) and pulling with an applied amount of force to cause the object to lengthen. Tensile tests can measure tensile stiffness and strength [4,23].

Compression test (Fig. 2B): the opposite of a tensile test, this involves holding a sample in place between two plates and pushing with an applied force to cause an object to shorten. Compression tests can measure compressive stiffness and strength [4,23].

Three-point and four-point bending tests (Fig. 2B): these test types involve placing a sample between three (3-point) or four (4-point) fixtures and bending it. This test type applies tensile and compressive forces to an object and can be used to measure flexural stiffness and strength [78,113].

Monotonic and cyclic testing: monotonic testing refers to a test in which an increasing amount of force is applied to an object and is usually continued until it fails. In contrast, cyclic testing refers to a test in which a force (below the amount needed to fail an object) is applied and subsequently removed over multiple cycles. Unlike monotonic testing, cyclic testing can measure structural fatigue, providing an idea of the behaviour of an object under repeatedly applied amounts of force [4,114].

Micro- and nano-indentation: indentation assays utilize indenter probes with varying tip geometries in order to indent tissues and cells. The amount of deformation of cell surfaces as a result of indentation can then be used to measure the apparent stiffness of the sample [23,24,40,115].

Mechanical tests can be combined with microscopy and spectroscopy techniques in order to provide structure-function relationships between observed mechanical behaviours and morphological features of a given plant, organ, tissue, cell or cell wall. Below are a few examples commonly used to simultaneously study structure and function in plants:

Atomic and cellular force microscopy (AFM and CFM): both AFM and CFM use a probe to scan the surface of specimens, and can isolate cell wall stiffness from combined cell wall and turgor stiffness. These two indentation techniques combined with microscopy allow simultaneous collection of morphological and mechanical information that can be overlaid to create topographical maps of cell and tissue surface stiffness, providing insight into the relationship between structure and mechanical function [24,115,116].

Small angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS): samples are exposed to X-rays and X-ray photons bounce off the electrons in the specimen. The resulting photon scatter is a reflection of the electron density of the sample, and is used as a measurement to provide information on the structure of the specimen. SAXS measures small scattering angles (near 0°) whereas WAXS measures large scattering angles (5°>). Cell wall polymers can be differentiated from each other based on their electron density (SAXS), or by atomic changes in polymer structure (WAXS). SAXS and WAXS can both be used to measure cellulose microfibril angle in plant cell walls [89,117,118].

X-ray computed tomography: this non-invasive technique utilizes X-ray beams to scan samples from different angles. This two-dimensional information can be compiled using computer software to generate three-dimensional reconstructions of the sample. Thus X-ray computed tomography provides structural information including sample density, as well as transverse and longitudinal morphology [119,120].

Raman confocal spectroscopy: this non-destructive technique is able to chemically image the cell wall, identifying cell wall polymers based on their specific chemical signature. Samples are exposed to a laser beam and photons are deflected off the sample surface. Scattered photons can then be detected and can be used to measure changes in the vibration of chemical functional groups of different polymers in the sample. When Raman spectroscopy is combined with confocal microscopy, it is possible to generate chemical image maps that combine compositional and structural information in order to spatially identify the relative proportion of specific cell wall polymers across a cell or tissue. Mechanical testing stages can be used in combination with a Raman confocal spectroscope in order to map real time changes in chemical spectra during mechanical testing [23,121,122].

Solid-state nuclear magnetic resonance: nuclear magnetic resonance is a non-destructive, spectroscopic technique that examines electron distribution within a sample in order to identify specific chemical components. The nucleus of an atom has a small magnetic field that is generated by electrons orbiting the nucleus. Differences in the orientation of this magnetic field and dipolar interactions between atoms give rise to unique signatures for different chemicals, and hence unique, detectable signatures for specific plant cell wall polymers. Solid-state nuclear magnetic resonance can provide information on the structure of individual cell wall polymers, similar to Raman spectroscopy, as well as the overall *in vivo* architecture of the cell wall. Changes in electron distribution can also be used to infer mechanical properties of the cell wall based on its overall structure and polymer makeup [45,123,124].

Stem shape has been studied for its contributing role to overall plant stiffness in multiple species [8,16], but has not been mechanically tested yet in Arabidopsis. Several Arabidopsis meristem mutants have been identified with fasciated (flattened, broader) stems (i.e., *clavata1*, *fasciata1 and 2*, *meristem enlargement1*) (Fig. 3C and Table 1) [17,18]. These mutants could be interesting to mechanically test to determine if stem shape contributes to overall plant stiffness in Arabidopsis.

2.3. Tissue level

Tissues are not distinct entities that operate independently within a plant organ; their physical adhesion to one another facilitates biochemical crosstalk, among other processes, and influences the manner in which tissues respond both individually and as a unit to local and global mechanical stresses [3]. Therefore, the spatial arrangement, density and proportion of tissue types relative to each other within the plant stem must be tightly controlled, as modifications in these parameters have mechanical ramifications, including changes to stem stiffness.

Arabidopsis stems have a radially symmetric pattern typical of many dicots in which the vascular bundles are arranged in a collateral manner with interfascicular fibers forming in the areas between bundles. This creates a ring of vascular and vascular-supporting tissue that separates the central pith from the cortex and epidermal tissues (Fig. 3D-i/iii) [19]. The role of interfascicular fibers as a key load bearing tissue has been demonstrated by the ~80% decrease in tensile strength of the basal stem of *interfascicular fiberless1/revoluta (ifl1/rev)* mutants that lack interfascicular fibers compared to wild type stems (Tables 1 and 2) [20]. Though not tested for *ifl1* stems, it would be expected that stiffness would also be decreased as seen for cellulose mutants that affect the thickness of interfascicular fiber cell walls (see Section 2.5.2 and Tables 1 and 2).

Table 1
Mechanical properties of Arabidopsis stem structure and cell wall mutants.

Gene	Function/Predicted Function	Mutant Phenotype	Tissue Tested[a]	Test Type[b]	Basic Result[c]		References[f]
					Stiffness[d]	Strength[e]	
Structural mutants							
STURDY	Patatin-like protein	Increased cell number and stem diameter	Stem	Qualitative observation	↑		[101]
MYB87	Transcription factor	OE of MYB87 fused to SRDX transcriptional repressor domain	Stem	Not tested			[15]
CLAVATA1	Receptor kinase with leucine-rich repeat	Fasciated stem	Stem	Not tested			[17]
FASCIATA1	Chromatin Assembly Factor-1 subunit	Fasciated stem	Stem	Not tested			[17]
FASCIATA2	Chromatin Assembly Factor-1 subunit	Fasciated stem	Stem	Not tested			[17]
MERISTEM ENLARGEMENT1	MicroRNA miR166a	Fasciated stem	Stem	Not tested			[18]
IFL1/REV[g]	Transcription factor	Lack IF	Stem	Tensile		↓	[20]
		avb1 OE allele: Amphivasal vascular bundles; reduced IF cell wall thickness	Stem	Tensile		↓	[19]
HCA1	Unknown	Altered vascular bundle organization; reduction in IF tissue	Stem	Not tested			[21]
HCA2	Transcription factor	Altered vascular bundle organization; reduction in IF tissue	Stem	Not tested			[22]
(Natural accessions x12)	n/a	Natural variation in morphology and composition	Stem	Tensile	~↑↓[h]	~↑↓[h]	[14]
(WT after mechanical perturbation)	n/a	Altered stem morphology	Stem	3-Point Bending	↓		[13]
Primary Cell Walls							
CESA6/PROCUSTE	PCW cellulose synthase subunit	Reduced cellulose	Hypocotyl	Tensile	↓		[50]
AtKTN1/FRA2/BOT	Katanin MT severing protein	Altered MF organization; reduced cellulose	Hypocotyl	Tensile	↓	↓	[29]
MUR2	XyG fucosyltransferase	Reduced XyG Fuc	Hypocotyl	Tensile	↓	↓	[23,29,35,125]
			Pedicel	Tensile	~	~	[29]
MUR3	XyG galactosyltransferase	Reduced XyG Gal	Hypocotyl	Tensile	↓	↓	[23,29,125]
			Pedicel	Tensile	~	~	[29]
XXT1 XXT2[i]	XyG xylosyltransferases	Lack detectable XyG	Hypocotyl	Tensile	↓	↓	[51]
			Pedicel	Tensile	↓		[126]
MUR1	GDP-L-fucose synthase	Reduced Fuc in XyG and RG II	Hypocotyl	Tensile	↓	↓/~	[29,35]
			Pedicel	Tensile	~	↓	[29]
QUA2	HG methyltransferase	Reduced HG	Hypocotyl	Tensile	↓	~	[35]
PME5	Pectin methylesterase	OE: decreased HG esterification	Hypocotyl	AFM	↓		[54]
			Meristem	AFM	↓		[53]
PMEI3	Pectin methylesterase inhibitor	OE: increased HG esterification	Hypocotyl	AFM	↑		[54]
			Meristem	AFM	↑		[53]
ARAD1 ARAD2	Arabinan arabinosyltransferases	Decreased arabinans	Stem	Compression		↑	[52]
			Stem	Indentation		~	[52]
AtKINESIN-4A/FRA1 AtKINESIN-4C	Kinesin MT motor protein	Altered matrix polysaccharide secretion (MF organization?)	Leaf	AFM	↑		[127]
Secondary Cell Walls							
CESA7/IRX3/FRA5	SCW cellulose synthase subunit	Reduced cellulose	Stem	3-Point Bending	↓	↓	[58]
			Stem	Tensile		↓	[64]
CESA8/IRX1/FRA6	SCW cellulose synthase subunit	Reduced cellulose	Stem	3-Point Bending	↓	~	[58]
KORRIGAN/IRX2	β-glucanase in cellulose synthase complex	*irx2* SCW-specific allele: reduced cellulose	Stem	3-Point Bending	↓	↓	[58]
AtKTN1/FRA2/BOT	Katanin MT severing protein	Altered MF organization; reduced cellulose	Stem	Tensile	↓	↓	[59,66,128]

Table 1 (*Continued*)

Gene	Function/Predicted Function	Mutant Phenotype	Tissue Tested[a]	Test Type[b]	Basic Result[c]		References[f]
					Stiffness[d]	Strength[e]	
			Stem	3-Point Bending	↓	↓	[129]
AtKINESIN-4A/FRA1	Kinesin MT motor protein	Altered matrix polysaccharide secretion (MF organization?)	Stem	Tensile		↓	[130]
			Stem	3-Point Bending	↓	↓	[131]
IRX9	Xylosyltransferase in xylan backbone elongation	Severely reduced xylan; reduced cellulose & lignin	Stem	Tensile		↓	[68]
GUX1 GUX2	Xylan glucuronyltransferases	Lack substitution of xylan backbone	Stem	4-Point Bending		↓	[75]
FRA8/IRX7	Glycosyltransferase in the synthesis of xylan reducing end tetrasaccharide	Severely reduced xylan; reduced cellulose & lignin	Stem	Tensile		↓	[67]
IRX8/GAUT12	Glycosyltransferase in the synthesis of xylan reducing end tetrasaccharide	Severely reduced xylan; reduced cellulose & lignin	Stem	Tensile		↓	[68]
PARVUS/GATL1	Glycosyltransferase in the synthesis of xylan reducing end tetrasaccharide	Reduced xylan	Stem	Tensile		↓	[132]
RWA1 RWA2 RWA3 RWA4	Acetyl Coenzyme A transporters	Reduced acetylation of xylan; altered proportion of GlcA vs Me-GlcA substitution	Stem	Tensile		↓	[70]
ESK1/TBL29	Xylan acetyltransferase	Reduced acetylation of xylan	Stem	Tensile		↓	[62,71–73]
[ESK1/TBL29] TBL3 TBL31[j]	Xylan acetyltransferases	Reduced acetylation of xylan	Stem	Tensile		↓	[73]
[ESK1/TBL29] TBL34	Xylan acetyltransferases	Reduced acetylation of xylan; reduced cellulose	Stem	Tensile		↓	[72]
[ESK1/TBL29] TBL34 TBL35	Xylan acetyltransferases	Reduced acetylation of xylan; reduced cellulose	Stem	Tensile		↓	[72]
[ESK1/TBL29] TBL33	Xylan acetyltransferases	Reduced acetylation of xylan; reduced xylan & cellulose	Stem	Tensile		↓	[62]
[ESK1/TBL29] TBL32 TBL33	Xylan acetyltransferases	Reduced acetylation of xylan; reduced xylan & cellulose	Stem	Tensile		↓	[62]
CSLA2 CSLA3 CSLA9	Glycosyltransferases for glucomannan backbone synthesis	Lack detectable glucomannan	Stem	4-Point Bending	~	~	[76]
IRX4/CCR1	Cinnamoyl-CoenzymeA reductase for lignin monomer synthesis	Reduced lignin	Stem	Tensile	↓	↓	[128]
			Stem	3-Point Bending	↓	↓	[63]
FLA11/IRX13 FLA12	Fasciclin-like arabinogalactan proteins (AGPs) with possible roles in cellulose synthesis	Reduced cellulose, slightly increased lignin, slightly higher MFA, reduced AGPs	Stem	Tensile	↓	↓	[133]
			Stem	3-Point Bending	~	~	[133]
PME35	Pectin methylesterase	Increased methyl-esterification of HG	Stem	Compression		↓	[134]

PCW = primary cell wall; SCW = secondary cell wall; XyG = xyloglucan; HG = homogalacturonan; RG II = rhamnogalacturonan II; Fuc = fucose; Gal = galactose; GlcA = glucuronic acid; Me-GlcA = methylated glucuronic acid; AGP = arabinogalactan protein; MT = microtubules; MF = cellulose microfibrils; MFA = microfibril angle; IF = interfascicular fiber; OE = overexpression; AFM = atomic force microscopy; n/a = not applicable; WT = wild type.

[a] Simplified to basic type, see reference for details of region and age.

[b] Simplified to basic type, see references for details of testing regime as well as Box 2 and Fig. 2 for a description of the mechanical test types.

[c] Results noted as ↑ = increased compared to wild type, ↓ = decreased compared to wild type, ~ = similar to wild type, blank = not reported; note that since most testing experiments are not comparable, not showing degree of increase or decrease.

[d] Stiffness = Modulus of Elasticity (stress/strain).

[e] Strength = Ultimate Strength (stress or force at failure).

[f] References for the mechanical testing of these mutants- for recent reviews on the molecular function of PCW & SCW genes see [42,31,55,41,56,135], for cloning of CLAVATA1 and FASCIATED1 and 2 see [136]; [137].

[g] Gene names separated by backslash (/) indicate alternate names.

[h] Results either showed no variation, or an increase or decrease in stiffness and strength between natural accessions.

[i] Gene names separated with space represent independent genes; results described regard double (triple, quadruple. . .) mutants.

[j] Square brackets indicate that the *esk1/tbl29* phenotype is enhanced (worsened) by the creation of double and/or triple mutants with the other listed *TBL* genes.

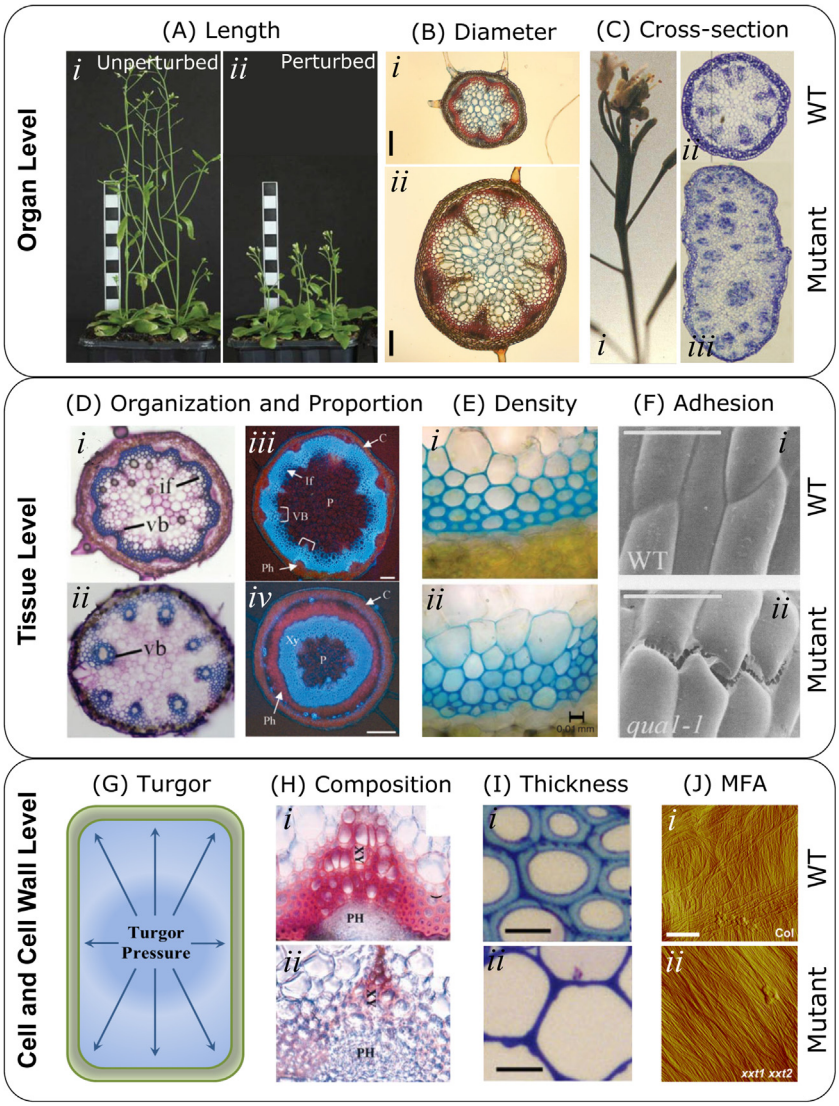

Fig. 3. Examples of Arabidopsis inflorescence stem treatments and mutants that affect hierarchical parameters governing stiffness. Parameters that have been mechanically tested and shown to affect stiffness are marked with an asterisk (*). (A*) Mechanically unperturbed (i) and mechanically perturbed (ii) mature WT Col-0 stems; a reduction in stem height often accompanies changes in stiffness (scale: each square is 1 cm) (reproduced with permission from [13]). (B) Variation in stem diameter is correlated with altered stiffness [14]; mutants such as MYB87-SRDX (ii) that alter diameter compared to WT (i Col-0) could possibly be used to investigate this parameter further (scale bar: 200 μm, reproduced with permission from [15]). (C) Mutants that could potentially be used to examine the influence of cross-sectional shape on stiffness include the oblong-shaped *fasciata1* (i) and *men1/+* (iii) stems when compared with rounded WT (ii Col-0) stems (reproduced with permission from [17] (i) and [18] (ii, iii)). (D*) The organizational conversion of collateral vascular bundles in WT Ler (i) to amphivasal bundles and the change in interfascicular fibre tissue proportion in the *amphivasal vascular bundle1* overexpression mutant (ii) accompanied a reduction in stem stiffness (reproduced with permission from [19]). Mutants such as *high cambial activity1* (iii-WT/iv- mutant) that also display organizational and tissue proportion phenotypes could be used to further explore the influence of these parameters on stiffness (scale bar: 100 μm) (reproduced with permission from [21]). (E*) Changes in tissue density were found to reduce bending stiffness in mechanically perturbed Col-0 plants (ii) by 50% as compared to unperturbed WT plants (i) (scale bar: 0.01 mm) (reproduced with permission from [13]). (F) Mutants, such as *quasimodo1* that displays reduced adhesion (ii) compared to WT Ws (i) hypocotyls, could be used to investigate the mechanical implications of adhesion in relation to stem stiffness (scale bar: 50 μm) (reproduced with permission from [33]). (G*) Turgor pressure has been previously demonstrated to impart stiffness to plant tissue. (H*) Changes in cell wall composition affect stiffness; mutants, such as *irregular xylem4* (ii), show reduced bending stiffness compared to WT (i Ler) as a result of decreased lignin content (visualized with phloroglucinol staining) (reproduced with permission from [63]). (I*) Cell wall thickness is correlated with stiffness; *amphivasal vascular bundle1* (ii) whose walls were thinner than WT Col-0 (i) exhibited a reduction in stiffness (scale bar: 13 μm) (reproduced with permission from [19]). (J) Microfibril angle (MFA) is known to affect stiffness in other plant species; mutants such as *xyloglucan xylosyltransferase (xxt)1/xxt2* could potentially provide the opportunity to study the effect of MFA on stiffness in Arabidopsis (scale bar: 500 nm) (reproduced with permission from [50]). IF = interfascicular fiber, VB = vascular bundle, P = pith, Ph = phloem, Xy = xylem, C/Co = cortex, Ep = epidermis.

2.3.1. Tissue organization and relative tissue proportions

While mutations in the *IFL1/REV* gene affect the presence of interfascicular fiber tissue, other mutants have been identified that affect the organization of vascular and other tissues in Arabidopsis stems. This includes, coincidentally, the dominant *amphivasal vascular bundle1* (*avb1*) allele of *REV/IFL1*, whose mutants have amphivasal vascular bundles more typical of those that are found in monocots (i.e., xylem tissue completely surrounds the phloem tissue) (Fig. 3D-ii, Tables 1 and 2) [19]. Breaking force tests demonstrated a 60% decrease in *avb1* stem strength compared with wild type stems. The reduction in cell wall thickness observed for *avb1* interfascicular fibers (Fig. 3I) suggests that this mechanical defect is most likely due to lack of stem reinforcement rather than altered distribution of vascular bundles as a load-bearing tissue [19]. However, the potential contribution of tissue organization to stem stiffness in Arabidopsis cannot be excluded until definitively proven to have no effect. This would be best studied in mutants in

Table 2
Comparison of mechanical properties of primary cell wall, secondary cell wall and stem structural mutants of Arabidopsis.

Gene	Phenotype	Stiffness[a]	Strength[a]	References
Primary Cell Wall Mutants: Hypocotyl Tensile Tests – Ryden et al. 2003[b]				
Atktn1/fra2/bot[c]	Reduced cellulose	+++	+++	[29]
WT with 0.25 μM DCB[d]	~60% cellulose	++	++	[29]
mur2	Reduced XyG Fuc	WT	++++	[29]
mur3	Reduced XyG Gal	++	+++	[29]
mur1	Reduced XyG & RGII Fuc	+++	+++	[29]
Primary Cell Wall Mutants: Hypocotyl Tensile Tests – Burgert & Dunlop 2011[e]				
mur2	Reduced XyG Fuc	++++	+++++	[23,35,39]
mur3	Reduced XyG Gal	++	++++	[23,39]
xxt1 xxt2[f]	No XyG	+++	++++	[39,51]
mur1	Reduced XyG & RGII Fuc	++++	+++++	[35,39]
qua2	Reduced HG (50%)	++++	+++++	[35,39]
Secondary Cell Wall Mutants: Basal Stem Three-Point Bend Tests[g]				
cesa8/irx1/fra6[g]	40% cellulose	++	+++++	[58]
korrigan/irx2	36% cellulose	++	++++	[58]
cesa7/irx3/fra5	18% cellulose	+	+++	[58]
irx4/ccr1	50% lignin (Fig. 3H)	++	++	[63]
Secondary Cell Wall and Structural Mutants: Basal Stem Breaking Force Tests[h]				
cesa7/irx3/fra5	45% cellulose	nd	+	[64]
Atktn1/fra2/bot	80% cellulose	nd	+++	[59]
Atkinesin-4a/fra1	MFA, reduced matrix	nd	+++	[130]
irx9	45% xylan, reduced cellulose & lignin	nd	+	[68]
fra8/irx7	42% xylan, reduced cellulose & lignin	nd	+	[67]
irx8/gaut12	25% xylan, reduced cellulose & lignin	nd	+	[68]
parvus/gatl1	~50% xylan (reduced cellulose & lignin?)	nd	+	[69]
rwa1 rwa2 rwa3 rwa4	60% acetylation of xylan	nd	++++	[70]
esk1/tbl29	70% acetylation of xylan	nd	+++	[62,71–73]
esk1/tbl29 tbl3 tbl31	56% acetylation of xylan	nd	++	[73]
esk1/tbl29 tbl34	63% acetylation of xylan, reduced cellulose	nd	+++	[72]
esk1/tbl29 tbl34 tbl35	63% acetylation of xylan, reduced cellulose	nd	++	[72]
esk1/tbl29 tbl33	37% acetylation, reduced cellulose & xylan	nd	+	[62]
esk1/tbl29 tbl32 tbl33	20% acetylation, reduced cellulose & xylan	nd	+	[62]
ifl1/rev	Missing IF	nd	+	[20]
avb1/ifl1/rev	Amphivasal vascular bundles, reduced IF cell wall thickness (Fig. 3I)	nd	+++	[19]

WT = wild type; DCB = 2,6-dichlorobenzonitrile; XyG = xyloglucan; HG = homogalacturonan; RG II = rhamnogalacturonan II; Fuc = fucose; Gal = galactose; MFA = microfibril angle; IF = interfascicular fiber.

[a] Absolute stiffness and strength measurements from the referenced papers were converted to percent of wild type, then noted on a 5+ scale where each + represents 20% of the total WT stiffness or strength value; note that all mutants listed are significantly different than wild type, thus a rating of +++++ means within a 20% range of the WT value. If the mutant is the same as WT, "WT" is noted.

[b] All data estimated from Ryden et al., 2003 from different figures.

[c] Gene names separated by backslash (/) indicate alternate names.

[d] Wild type hypocotyls grown in the presence of cellulose synthesis inhibitor 2,6-dichlorobenzonitrile (DCB; 0.25 μM).

[e] Note that tests were performed on 4 and 6 day old hypocotyls and are not absolutely comparable, comparisons listed here are based on Burgert & Dunlop 2011, see Burgert & Dunlop 2011 plus listed primary papers for details.

[f] Gene names separated with space represent separate genes, results described regard double mutant.

[g] Note that tests were performed by the same group with the same apparatus, but in two different publications; 48d results taken.

[h] Note that tests were performed by the same group with the same apparatus, but in multiple publications, and the age of the stem can differ, see references for details.

which stem reinforcement remained constant, but tissue organization was altered.

Other tissue organization mutants include *high cambial activity (hca) 1* and *hca2* that lack (or have very few) interfascicular fibers and, instead, have a continuous ring of vascular tissue (Fig. 3D-iv and Table 1) [21,22]. These mutants are also affected in the relative proportions of vascular versus other tissues, e.g., *hca1* mutants have an increase in vascular versus pith tissue, and a reduction in cell size that correlates with decreased stem diameter despite the overproliferation of vascular tissue (Fig. 3D-iv) [21]. While neither of these stem organization mutants has been mechanically tested, changes in the proportion of stem tissues has been linked to the mechanical properties of Arabidopsis. The 70% reduction in stem stiffness observed in the mechanical perturbation experiments mentioned previously (Section 2.2.1) was accompanied by reductions in the proportion of pith and interfascicular fibers tissue, while the area of cortex tissue increased. While thinner interfascicular fiber cell walls were also observed in the perturbed plants, it was suggested that altered tissue proportions were responsible for ~20% of the decrease in stem rigidity [13]. Obviously, it would be of interest to compare the effects on plant stiffness of various tissue organization mutants with those resulting from mechanical perturbation.

2.3.2. Tissue density

Tissue density is affected by cell number, size, degree of cell-to-cell adhesion, cell wall thickness (i.e., thicker walls can increase density), and cell wall composition, making it difficult to discern density's direct contribution to plant stiffness. However, tissue density is predicted to alter stiffness in stems, and its contribution can be approximated if variables such as wall composition are assumed to remain unchanged. For example, using the assumption that cell wall composition did not change, the reduced density of interfascicular fiber tissue of mechanically wind-perturbed Arabidopsis plants resulting from thinner cell walls was calculated to decrease bending stiffness by ~60% in perturbed plant stems (Fig. 3E) [13]. The increased cell density seen in the thinner stems of the *hca1*

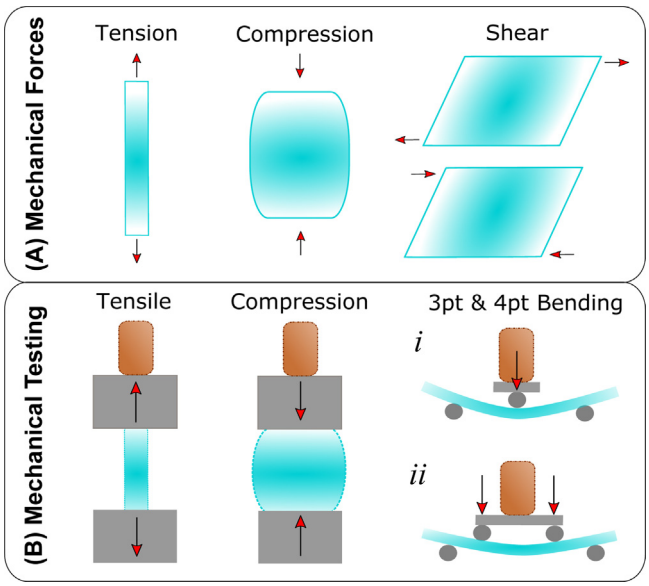

Fig. 2. Examples of uniaxial mechanical forces that act on objects, and commonly used mechanical tests used to investigate the mechanical properties of objects. (A) Mechanical forces include: tension that pulls and can lengthen or break a material/object; compression that pushes and can shorten or crush/buckle a material/object; and shear that comprises two misaligned tensile or compressive forces that causes a material/object to slide against itself in opposite directions, usually leading to tearing. (B) Mechanical tests commonly used to analyze materials include: tensile tests in which a specimen is secured at both ends and pulled until failure or until a specific amount of force is reached (e.g., in the case of cyclic testing), and compression tests in which a specimen is held in place between two plates and is pressed until failure or until a specific amount of force is reached. If the holders or plates keeping the specimen in place are not perfectly aligned, shear tensile and compressive tests can be performed. Three-point and four-point bending tests hold a sample in place between three and four fixtures, respectively, and the specimen is bent until failure or a specific amount of force is reached. Bending involves tensile and compressive forces acting on a specimen, and can be measured by calculating flexural rigidity from both three and four-point bending tests. In the schematic above, the arrows show the direction that the forces act in (A) and the direction in which each force type is applied (B). The grey rectangles and circles represent the different fixtures and holders that are used to keep samples in place during testing. The brown rectangle represents a load cell that reads the change in the amount of force applied to a specimen during the course of a mechanical test. Schematic adapted from [23,40,78,113]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mutant noted above also makes it an attractive candidate for future mechanical investigation [21].

2.4. Cell level

The base unit of plant structure is the cell; usually a living protoplast performing metabolic functions surrounded by the cell wall, an extracellular matrix that forms the boundary of the cell and mediates adhesion and interaction with other cells, as well as with other biotic and abiotic factors (exceptions would include, for example, woody tissues where cells have lost their protoplast following secondary cell wall reinforcement) [23,24]. As mechanical entities in their own rights, plant cells have balanced external forces (typically tensile and compressive) that result from interaction with other cells with internal forces such as turgor to maintain plant structure in response to forces exerted on the organism [24]. This balance is mediated by modulating cell shape and size, as well as cell wall production and cell-to-cell adhesion, allowing cells to be sufficiently stiff that they withstand mechanical stress while simultaneously permitting growth [23,25]. In this section, we will focus on cell geometry and cell-to-cell adhesion. Properties of plant cell walls beyond those relating cell-to-cell adhesion will be discussed in detail in Section 2.5.

2.4.1. Cell geometry

Plant tissues are cohesive units formed from specialized cell types whose individual physical attributes combine and impart unique properties, including stiffness, that dictate the manner in which a given tissue type responds to mechanical stress [26]. Pith, vascular, cortex, and interfascicular stem tissues are composed of cells with different shapes that modify the mechanical properties of the stem, including stiffness, in a species-dependent manner [2,24]. Different conformational shapes along the transverse and longitudinal axes of the plant cell can change its bending stiffness [4,27]. Shape also affects how cells fit together both within a single tissue and between different tissue types. Cells that share large contact surfaces are packed tightly together with little airspace between them, allowing them to form a cohesive unit that can better distribute mechanical stress. For cells with the same elasticity, this would generate stiffer tissues than those in which cells are more loosely packed [2,28].

In Arabidopsis, stems most tissues are typically made up of the same polyhedral-shaped cells when observed in cross-section. Thus, there is little to no change in shape in the transverse plane among the different tissues. However, several biochemically and structurally perturbed Arabidopsis mutants have a dwarfed plant phenotype with shortening in the length of load bearing and non-load bearing cell types along the longitudinal axis of the plant [13,19,21,29]. These mutants could be used to help elucidate the contribution of cell shape to stiffness if other parameters (e.g., changes in cell wall thickness) that may also change are taken into consideration.

The other facet of cell geometry is size, which takes into account the volume of a cell. Changes in cell size alter the amount of surface area available for contact with adjoining cells and also changes the total volume through which a mechanical force can be dissipated in individual cells [28]. If other variables (e.g., cell number) remain constant, increases or decreases in cell size can have consequences on both tissue size and overall stem diameter, thereby altering tissue and organ stiffness. To date there is no literature that directly investigates the contribution of cell size in relation to cell mechanical stiffness in Arabidopsis.

2.4.2. Cell-cell adhesion

Plant cells generally adhere to one another by the middle lamella that creates a physical connection between neighbouring cells through which biochemical and mechanical crosstalk can occur [30]. The middle lamella is initially composed primarily of pectins, but in tissues having secondary cell wall deposition may become lignified [31,32]. The level of adhesion between cells can be altered by modifying the biochemical composition of the middle lamella, as seen in various Arabidopsis mutants [30]. Cell adhesion is dependent upon other properties that affect stiffness (e.g., cell shape and cell wall composition). The role of adhesion as a mechanical property has not yet been specifically tested in Arabidopsis. It may be possible to approximate the contribution of cell adhesion to an observed value of stiffness by calculating how much other variables contribute to total stiffness, as has been performed with tissue density. For example, mutants in both the *QUASIMODO1 (QUA1)* gene and the *QUA2* gene of Arabidopsis have less pectic homogalacturonan in the primary cell wall and are defective in cell adhesion (Fig. 3F) [33,34]. While *qua1* plants have not yet been tested, hypocotyls (embryonic stem, see Section 2.5.1) mutant in the *qua2* gene have been mechanically tested and demonstrate reduced tensile stiffness. It is likely that cell separation may be partly responsible for the observed reduction in stiffness [35].

2.4.3. Turgor pressure

Turgor pressure, the hydrostatic force imposed by the plant cell protoplast upon the cell wall, has been extensively studied and

reviewed with regard to its significant mechanical role in driving cell expansion and imparting stiffness to cells, tissues and plant organs that lack secondary cell walls (Fig. 3G) [5,36,37]. Turgor varies among plant organs, tissues, cells and the developmental stages of the plant, as well as with the water status of the plant [38]. As a non-directional force, turgor acts upon regions of differing mechanical strength and stiffness within plant cell walls, establishing cell shape, size and direction of expansion [37,38].

Although turgor pressure is responsible for much of the stiffness observed in plant cells that lack a secondary cell wall, its total contribution has been challenging to quantify, as previous efforts lacked the resolving power to distinguish between the input of cell wall properties and turgor on measured stiffness values in living cells [36–38]. Plasmolysis prior to testing via treatment with solutions of high osmolarity eliminates the stiffening effects of turgor pressure. Comparison of mechanical tests of Arabidopsis wild type and cell wall mutant hypocotyls revealed reduced stiffness and increased differential of mechanical properties after plasmolysis [29]. However, potential alterations to cell wall mechanical properties due to the plasmolysis treatment makes it preferable to do comparisons with hydrated tissues [39]. Emerging experimental methods and novel approaches using modified micro- and nanoindentation methods such as atomic and cellular force microscopy combined with modelling have helped to separate the effects of cell wall composition from turgor pressure and their impact on cell stiffness (reviewed in [24,36,38]).

2.5. Cell wall level

As mechanical entities, living plant cells must balance external forces with the internal osmotic force of turgor pressure. The ability to withstand such forces is largely conferred by the cell wall. Similarly, the mechanical properties of whole plants have a significant origin in the presence and specific compositional make-up of both primary and secondary cell walls [6,39].

2.5.1. Primary cell walls

Young and growing primary cell walls require both strength to maintain the structural integrity of their cells and compliance to allow for changes in cell size and shape. Many cell types undergo secondary cell wall thickening after growth is completed. This provides structural support (further stiffness and strength) to the cell and the organism (e.g., vascular tissue and interfascicular fibers). In both cases, walls are comprised of stiff, semi-crystalline microfibrils of the β-1,4-D-glucose polymer cellulose that wrap around the cell like cables and interact with a matrix of other polysaccharides (hemicelluloses and pectins) and a small amount of proteins [39,40]. This structure has led to the simplistic comparison of plant cell walls to fiber-reinforced composites (e.g., [6,39]). In the primary cell walls of many dicots, such as Arabidopsis, the most significant hemicellulose is xyloglucan, which shares the β-1,4-D-glucose backbone of cellulose, but is substituted with short side chains containing xylose, galactose and fucose. The type and degree of interaction between xyloglucan and cellulose is currently debated, as is the degree of its role as a load-bearing polymer in the primary cell wall [37,41]. Major pectins include homogalacturonan, xylogalacturonan, and rhamnogalacturonan II, as well as rhamnogalacturonan I. The first three polymers share a backbone of α-1,4-D-galacturonic acid residues that differ in their degree of substitution: homogalacturonan lacks side chains, xylogalacturonan is substituted with xylose and rhamnogalacturonan II has a set of four complex, evolutionarily-conserved side chains. Rhamnogalacturonan I has a backbone of repeating units of α-1,4-D-galacturonic acid – α-1,2-L-rhamnose that can be substituted on the rhamnose residues with arabinan, galactan and arabinogalactan side chains [42]. The structural properties of pectins within the wall, specifically gel stiffness and porosity, result from the proportion and degree of branching, as well as the amount of interaction between pectin molecules through the formation of linkages such as boron diesters between rhamnogalacturonan II side chains and calcium bridges between the free galacturonic acid residues of homogalacturonan molecules. The latter is modulated through the in-wall activity of pectin methylesterases, as homogalacturonan is thought to be synthesized and deposited into the wall in a relatively neutral, highly-esterified state [6,42,43]. As pectin is hydrophilic in nature, its structure is also correlated with the hydration state of the wall. For example, homogalacturonan demethylation (leading to the presence of free acid groups) and the presence of certain rhamnogalacturonan I side chains such as arabinans have been correlated with increased cell wall hydration and decreased stiffness (reviewed in [6,44]). The porosity of the wall regulated by pectin structure also directly affects the water-holding capacity of cell walls and the ability of water to move within the wall, both of which affect biomechanical responses (reviewed in [6,43]). It is unclear whether the different types of pectins represent individual polymers, linear structural domains or branches of larger heterogeneous polymers. Pectins can also be complexed with structural cell wall proteins such as the recently described ARABINOXYLAN PECTIN ARABINOGALACTAN PROTEIN1 (APAP1) of Arabidopsis [37,42]. An increasing number of studies, including ones using solid-state nuclear magnetic resonance [45,46], have demonstrated the significant degree of physical interaction between pectins and cellulose (reviewed in [6,47]).

Arabidopsis is increasingly employed as an easily manipulated genetic model system to determine and dissect details of cell wall polymer synthesis and deposition. It is also being used to elucidate the interactions and mechanical roles of the various components during different aspects of plant development. This has included the selection of mutants affecting polymer synthesis, growth in the presence of chemical modulators, and bioengineering of plants with altered polymer synthesis and enzymatic modifications. With respect to the mechanics of the primary cell wall, research largely has focused on uniaxial testing of elongated hypocotyls of dark-grown seedlings. This early seedling stem-like tissue is preferred due to its cylindrical shape and the simplicity of its anatomical structure (Tables 1 and 2) [29,48]. Since hypocotyls can dry out rapidly, testing has been performed with samples bathed in liquid or water vapour. While this ensures consistency between samples with respect to having undamaged, hydrated primary cell walls, it means that absolute values obtained include the stiffening effect of turgor pressure [39]. In the discussion below and in Table 2, the focus is comparative, based on the relative mechanical properties for cell wall and structural mutants tested under conditions that allowed for reasonable comparison. For a more comprehensive list of Arabidopsis mutants that have had mechanical investigations and their basic results, see Table 1.

Hypocotyl tensile tests have demonstrated that overall reductions in cellulose, xyloglucan and pectins all lead to decreases in both stiffness and strength (cellulose synthase inhibitor [49] herbicide 2,6-dichlorobenzonitrile-treated seedlings, *cesa6/prc, Atktn/fra2/bot, xxt1 xxt2* and *qua2* mutants [29,35,50,51]; Tables 1 and 2). From the mutants tested in a comparable manner (Table 2; [29,39]), it is unsurprising to see that a significant reduction in cellulose content (~40%) due to inhibitor treatment led to the most drastic decreases in hypocotyl stiffness and strength [29]. Further, it appears that pectins, both in terms of quantity of homogalacturonan (*qua2*) and ability to form rhamnogalacturonan II cross-links (*mur1*), have a moderate effect on stiffness and limited effect on strength [29,35]. The effects of altering xyloglucan side chains are more complex and can be greater than the effects of diminished pectins, with the loss of galactose substitution leading to the most severe reduction in stiffness and strength

of any of the matrix polymer mutants, including one completely lacking xyloglucan. This result suggests a significant mechanical role for the galactose side chains of xyloglucan. However, the limited effect of the complete loss of xyloglucan on plant growth and mechanical properties was surprising and has led to debate on the exact role of xyloglucan as a load-bearing polymer within primary cell walls. This result is further complicated by changes in proportions and organization of other cell wall components that appear to allow their assumption of a greater load-bearing role within the wall in these mutants (discussed further in Section 2.6) [23,50,51]. Side chains of pectins also can affect primary cell wall mechanical properties. Stems of *arabinan deficient* mutants (*arad 1 arad2*) were stronger under compression and had decreased compliance in indentation tests, consistent with predictions that arabinans act as cell wall plasticizers [6,52]. The degree of homogalacturonan methylesterification modulates both hypocotyl and meristem cell wall stiffness, as seen with atomic force microscopy analysis of surface properties. Plants overexpressing PECTIN METHYLESTERASE5 have decreased homogalacturonan esterification and reduced cell wall stiffness, while those overexpressing the PECTIN METHYLESTERASE INHIBITOR3 have increased homogalacturonan esterification and increased stiffness. There is a correlation between the degree of homogalacturonan methylesterification and consequent stiffness in both hypocotyl and meristematic tissues. In both cases, these changes are under developmental regulation [53,54]. The effect of the degree of methylesterification on pectin gel stiffness is complicated by the pattern of esterification, such that pectin methylesterase activity can increase or decrease the degree of calcium-crosslinking of homogalacturonan molecules [6,43].

2.5.2. Secondary cell walls

Plant secondary cell walls are cellulose-reinforced primarily polysaccharide-based composites, similar to primary cell walls. However, secondary cell walls are stiffer at least in part due to their higher cellulose content, longer cellulose chains, increased diameter and/or bundling of microfibrils and a greater proportion of crystalline (versus amorphous) cellulose [55]. Secondary wall material is deposited after growth interior to the primary cell wall. In dicots and gymnosperms, secondary cell walls are generally observed to have three layers specified as S1, S2 and S3 moving from earlier to later stages of secondary cell wall production. Interestingly, these layers can differ significantly in thickness, with the S2 layer generally being substantially the thickest and thus responsible for the majority of secondary cell wall mechanical properties [2,39,56]. Secondary cell walls also differ from primary cell walls in having a parallel arrangement of microfibrils that can be described by their angle relative to the longitudinal axis of the cell, which is different among the three layers. In fiber cells such as those of stems, the microfibril angle is correlated with cell mechanics such that cells with a lower angle (more longitudinal to the cell axis) are more stiff in axial loading, while those with a higher angle (more transverse to the cell axis) are less stiff [39,57]. Secondary cell walls also differ from primary cell walls in their matrix composition: there is little pectin or protein, different hemicelluloses predominate and walls are impregnated to varying degrees (depending upon the species) with the polyphenolic compound lignin. In many dicots, including Arabidopsis, the main secondary cell wall hemicelluloses are xylans, which have a β-1,4-D-xylose backbone that tends to be decorated with glucuronic acid (unmodified or methylated) and smaller quantities of arabinose. Lesser quantities of glucomannans are also found in dicotylenous secondary cell walls, polymers with a backbone of both β-1,4-D-mannose and β-1,4-D-glucose [31,56]. As seen in pectins, the backbone residues of xylans and glucomannans can be acetylated [31,43,56]. After the production of the polysaccharide secondary cell walls, lignification can occur through the deposition and polymerization of monolignols within the wall. For example, dicot secondary cell walls are rich in guaiacyl (G) and syringyl (S) lignin, formed from coniferyl alcohol and sinapyl alcohol, respectively [31,32,56]. While the monomers present are consistent in dicots, the proportions and degree of lignification can vary among species. Lignin acts to strengthen cell walls, and, as a hydrophobic polymer, leads to the exclusion of water ([31,32,56]).

A number of genes involved in secondary cell wall polymer synthesis have been identified in Arabidopsis by screening for plants with thinner-walled, collapsed xylem cells (*irregular xylem [irx]* mutants) and reduced stem strength (*fragile fiber [fra]* mutants), gene co-expression with previously identified secondary cell wall cellulose synthase genes (added to *irx* series) and protein homology to other secondary cell wall biosynthetic enzymes (e.g., IRX10-like) [58–61]. In most cases, secondary cell wall mutants have thinner or unevenly-deposited secondary cell walls in both xylem and interfascicular fibers. This correlates with their reduced polysaccharide content [31,62], and weaker/less stiff stems under external testing conditions (Tables 1 and 2). While testing has not been done on isolated fibers or xylem cells from Arabidopsis due to their small dimensions, the collapsing of xylem cells seen in these mutants is thought to reflect the loss of strength in these cell walls that makes them unable to withstand the normal compressive forces from the negative pressure exerted by transpiration [58,63]. The organ-level phenotype of these mutants is shortened plant stature, probably resulting from inefficient water transport through these collapsed cells (e.g., [31,58,62]). For a comprehensive overview of the *irx* mutants discussing the complexity of their roles and phenotypes, as well as xylan and lignin synthesis in general, see [31]. As with primary cell walls, the discussion below is comparative. See Table 2 for a gross comparison of results that were obtained in a similar manner, and Table 1 for a full listing of secondary cell wall experiments.

Differing levels of cellulose (*cesa8/irx1/fra6, korrigan/irx2, cesa7/irx3/fra5*) and lignin (*irx4/ccr1*, plus transgenic plants with a range of lignin production) were positively correlated with both bending stiffness and strength in three-point bending tests on stems of four of the original *irx* mutants (Fig. 3H and Table 2). Comparison between cellulose and lignin results suggests that the amount of lignin present in the cell wall has a greater impact on stem bending strength than does the cellulose content of the wall. *irx3 (cesa7/irx3/fra5)* mutants that have an 82% reduction in cellulose content (compared to wild type) have stronger stems than *irx4 (irx4/ccr1)* mutants with a 50% reduction in lignin [58,63]. The majority of available mechanical testing results for secondary cell wall mutants consist only of uniaxial tension breaking-strength tests. However, since these were performed by the same research group using the same instrument, it is broadly possible to compare the results across a number of mutants affecting cellulose and xylan synthesis (Table 2). Unsurprisingly, a similar positive correlation between cellulose content and strength is seen with these tests as for three-point bending, however, the severity of the strength decreases appear to be greater. For example, stems of the *fra5* allele (65% reduction in cellulose) of CESA7/IRX3/FRA5 have 10% of wild type tensile strength, while the *irx3* allele (82% reduction in cellulose) has only a 50% decrease in bending strength, presumably due to the fact that bending involves both tensile and compressive forces ([58,64]). The breaking force strength of the loss-of-function *ifl1* allele of *IFL1/REV* that lacks interfascicular fibers is similar to that of *fra5* mutants that have a 65% reduction in stem cellulose. This hints at the considerable amount of cellulose found in interfascicular fiber secondary cell walls as a proportion of the total found in stems [20,64]. While it has been possible to study changes in cellulose quantity versus mechanical properties, no mutants affecting only microfibril angle have yet been identified, and it seems like it

may be difficult to separate cellulose, and perhaps other polysaccharide synthesis, from microfibril angle [50,65,66].

A large number of genes affecting different aspects of xylan synthesis have been tested for breaking force (Tables 1 and 2). Disruption of genes involved in xylan backbone synthesis or the production of the xylan reducing end tetrasacchride (*irx9, fra8/irx7, irx8/gaut12* and *parvus*) led to plants containing 25–50% of wild type xylan levels, all of which have very severe decreases in tensile strength (~85% reduction). While it appears that changes in xylan have a similar effect on strength as the loss of cellulose, this is unlikely, as lesser reductions in both cellulose and lignin in addition to xylan have been proposed for at least the *irx9, fra8/irx7* and *irx8/gaut12* mutants, suggesting that the decrease in xylan is only responsible for part of what is seen [67–69]. The xylan backbone can be *O*-acetylated in a number of positions, and recently a number of genes from the *REDUCED WALL ACETYLATION (RWA)* and *TRICHOME BIREFRINGENCE LIKE (TBL)* gene families have been identified for their overlapping roles in that process [62,70–73]. Comparison among mutants that specifically affect xylan acetylation (*eskimo1(esk1)/tbl29* single, *esk1/tbl29 tbl3 tbl31* triple and *rwa1 rwa2 rwa3 rwa4* quadruple mutants) demonstrates a correlation between reduced stem strength and loss of acetylation. However, the reductions in strength do not necessarily track with the absolute level of acetylation, reflecting the contribution of differences in the pattern of acetylation (i.e., position on the xylose residue and presence of glucuronic acid substitution on the same monosaccharide) in these different mutants [62,70–73]. While the loss of strength seen with a significant decrease in acetylation (56% of wild type acetylation in *esk1/tbl29 tbl3 tbl31* triple mutants) is not as severe as that seen for loss of cellulose or xylan noted above (4-fold versus 5-fold, respectively), it is still quite significant [64,67,68,73]. This demonstrates the important mechanical role of xylan acetylation in the secondary cell walls. Acetylation increases the hydrophobicity of xylan and has been suggested to increase interaction between xylan, cellulose and lignin [62,72–74]. Unlike acetylation, the presence of glucuronic acid side chains seems to have a minor effect on xylan interactions in secondary cell wall, as *glucuronic acid substitution of xylan1 (gux1) gux2* double mutants have only a small decrease in stem strength as assessed via four-point bending (Table 1) [75]. However, since even strongly cellulose deficient mutants demonstrate much less significant changes in strength in bending tests than tensile tests (Table 2), it is hard to compare this result to those of other xylan mutants. Reductions of xylan quantity and/or acetylation probably lower stiffness as well as strength as seen for cellulose. Still, this needs to be confirmed and quantified. Glucomannans are also found in Arabidopsis stems, and triple mutants defective for the backbone synthases CSLA2 CSLA3 and CSLA9 that lack detectable mannans had no change in stem stiffness or strength in four-point bending tests (Table 1) [76]. This suggests that, unlike xylans, mannans do not have a significant role in the mechanical properties of Arabidopsis stems.

2.6. Can Arabidopsis be used as a model to understand the role of parameters that govern plant stiffness?

In the sections above, we have outlined examples of structural and mechanical properties of the genetic reference plant Arabidopsis with regard to parameters that affect plant stiffness across multiple length scales (organ, tissue, cell and cell wall). Now we return to the question that we posed at the beginning of this review paper: *Can Arabidopsis be used as a model to understand the role of parameters that govern plant stiffness?*

In our opinion, the answer is *yes*, with some obvious caveats that will be discussed below.

First, we would like to highlight the need for such a model system in which consistent and systematic investigations of the different morphological and compositional parameters affecting plant stiffness and biomechanics can be performed. While a significant amount of work has been done and much learned about the factors that modulate plant biomechanics, there is a great deal of heterogeneity to the research that has been completed in this field. This heterogeneity ranges from the organs, tissues and cells investigated (from isolated wood fibers, to stems, to growing pollen tubes) to the species employed (from the herbaceous dicot Arabidopsis, to gymnosperm trees, to monocotyledonous grasses) to the techniques employed for mechanical testing (uniaxial tensile, to three-point bending, to nanoindentation) (for general reviews on plant mechanics see [2,4,39,40]). Background structural differences, including organ size, tissue organization, cell number, cell type and cell wall composition, render it difficult to compare these studies and to develop a correct and cohesive model of the interaction of controlling factors across length scales that give rise to particular plant stiffness properties. It also makes it difficult to discern how they could be modulated for human benefit.

With this need for a model system in which to investigate structural parameters contributing to plant stiffness, we believe that Arabidopsis is a useful candidate because it is a very well studied and easy to manipulate plant system (reviewed in [77] and references therein). In particular, its widespread use as the predominant plant genetic and genomic model for all aspects of plant growth and development has led to the continuing identification of mutants and genes modulating key factors expected to affect stiffness such as stem size, tissue organization and cell wall biosynthesis, as well as the ability to manipulate these factors through genetic engineering (Table 1).

Obviously, not all aspects of plant stiffness and biomechanics can be studied in Arabidopsis, and information is needed on other species for economic purposes, among others. To that end, Arabidopsis, though a promising system, cannot be the sole model or the only species studied. Arabidopsis is a small, herbaceous, annual dicotyledon in which the hierarchies of plant stiffness are somewhat simplified. For example, bulk features of wood or whole plant issues faced by large species and trees such as the mechanical forces imposed by large size and leaf canopies cannot be addressed in Arabidopsis, though properties of vascular tissues, fiber cells and secondary cell walls that make up wood can be studied [78,79]. Arabidopsis is neither a monocot nor a gymnosperm, so specific aspects of tissue organization and cell wall composition that are unique to these plant groups cannot be analyzed. However, once again, some aspects of these groups potentially can be addressed in Arabidopsis: monocot-type tissue organization can be mimicked through mutants (e.g., *avb1*–Section 2.3.1) [19] and, while the specifics of interactions between different cell wall polymers do need to be addressed in their primary systems, it has been suggested that the matrix polymers that vary between plant groups mainly have the same physicochemical role to form a gel-like component in the cell wall and/or to reduce aggregation of cellulose microfibrils [50,80,81].

Caveats must also be raised for the use of genetic dissection as a tool to analyze the contribution of specific parameters. Changes to individual gene function can lead to complex and unpredictable consequences on the biology of organisms, making it difficult and perhaps unlikely to be able to target changes to a particular component or process by making selective changes to particular genes. This is due to a number of causes, including redundancy in gene function, the multiple functions of certain gene products (pleiotropy), and organismal stress-sensing systems that lead to compensatory changes in plant structure and physiological responses at multiple levels [82,83]. Examples of unexpected consequences of genetic manipulation that can have biomechanical ramifications, and thus complicate studies, include: (1) Multiple tissue and/or cell level defects in mutants identified for their struc-

tural changes at the tissue level. For example, *avb1* mutants have altered organization of their vascular bundles, but their interfascicular fibers also have thinner secondary cell walls [19], while *hca1* mutants not only lack interfascicular fibers, but also have changes in tissue proportion, cell size and stem diameter [21]. (2) Compensatory changes in composition and cell wall polymer organization in cell wall biosynthesis mutants. For example, while *xxt1 xxt2* mutants lacking detectable xyloglucan have a subtle increase in other matrix polymers that allow greater load-bearing in the wall, it has recently been shown that there also is a decreased amount of cellulose that is more aggregated and aligned than found in wild type cell walls (Fig. 3J) [50,51]. Many of the *irx* mutants affecting secondary cell wall production have effects on multiple polymers, pattern of secondary cell wall deposition and plant height (reviewed in [31]). (3) Stress-sensing systems (e.g., the cell wall integrity signalling pathway) can be activated leading to unpredictable physiological responses including hormone responses, activation of plant defence mechanisms and ectopic deposition of cell wall material (reviewed in [84–86]). Such changes have been observed for mutants of multiple cellulose synthase subunits, among others (reviewed in [84]). This caveat and multiple examples highlight the necessity to ensure that lines chosen for study are well-characterized biologically at multiple levels and across all tissues to be studied so that all morphological and compositional parameters with biomechanical consequences can be accounted for in the production of future models. It may also suggest that a search for well-characterized mutants with structural changes of interest may be more practical than trying to target particular genes or pathways.

While we raise these issues related to genetic dissection, it must be pointed out that similar caveats of the complexity of differences exist when comparing samples from widely differing species and tissues. Considerations include scale, organ geometry, tissue organization, cell structure and cell wall composition. Physical dissection and chemical treatments (e.g., to remove certain cell wall components) also need to be considered carefully. With these in mind, an advantage of the use of Arabidopsis as a model system is that one is still working in the same species, with tissues and organs of largely the same structure, organization and composition. That said, in all cases, it is important to perform a detailed characterization of the system from tissue organization through to cell wall composition and organization prior to mechanical testing and interpretation. Only then can all morphological and compositional parameters be incorporated into models. For an interesting discussion of the many requirements for analysis required to put together a comprehensive and multiscale model of plant cell walls alone, see reference [87].

3. Functional gradients in plant stiffness

While Arabidopsis is an useful system in which to generate data to add to an integrated model of plant stiffness parameters, as a small, short life-spanned, dicotyledonous, herbaceous annual, it cannot address all levels of plant stiffness or all structural or compositional peculiarities of different types of plants (e.g., trees, grasses, gymnosperms). In addition to the above subsets of basic factors that cannot be studied directly using Arabidopsis are functional gradients of plant structural properties across length-scales. These functional gradients enhance plant mechanical performance and are a source of inspiration for engineers (e.g., architecture, smart synthetic materials). These gradients will be discussed below along with their further functionalization in certain species that allow emergent properties such as hydration-based actuation.

3.1. Stiffness gradients and their effects on load-bearing capacity

The hierarchical architecture of plants is optimized in a manner that tackles environmental challenges and realizes complex functions. Spatial gradients have been evolutionarily incorporated into compositional and structural features of plant organs, allowing notably lower stress concentrations and greater adhesion to be achieved at the interfaces of different tissue types. This evolutionary adaptation strategy is well suited for resisting severe mechanical forces and accommodating larger deformations.

From a structural point of view, the stems of monocotyledonous plants can be regarded as fiber-reinforced composites where stiff vascular bundles with fibrous caps are embedded in a soft parenchymatous tissue [88]. The discontinuous transitions in stiffness at the interfaces between these tissue types generate a highly non-uniform distribution of stresses, which can cause critical shear stresses at cell ends, and eventually lead to stem breaking In stems of the Mexican fan palm *Washingtonia robusta* (Fig. 4A), a gradual transition in stiffness from the central part of fiber caps to the surrounding parenchymatous tissue has been hypothesized to be an evolutionary adaptation strategy to mechanical forces. These changes in stiffness are correlated with a gradient in the degree of lignification in the cell walls of the fiber caps. This gradient of cell wall composition is predicted to alter both shear modulus and shear strength of the cell wall matrix, and eventually the axial tensile stiffness of the fibers [89]. The profiles of stiffness in the individual tissue types in the culms of the giant reed *Arundo donax* (Fig. 4B), indicate that gradual changes in stiffness exist within parenchymatous and sclerenchymatous tissues that are attributed to alterations at the tissue level. The absorbance pattern of ultra-violet microspectrophotometry scans on toluidine blue cross-sections (Fig. 4B i) and the intensity of stained lignin (Fig. 4B ii) revealed that the fiber rings enclosing the vascular bundles had lignification gradually decreasing from the outer side towards the pith parenchyma. Thus, a gradual transition between the stiffness of the sclerenchymatous fibers and the parenchyma is expected; however, the profile of microfibril angle exhibits an abrupt change and thus it does not contribute to the stiffness gradient at the interface [88]. In Moso bamboo, *Phyllostachys pubescens* (Fig. 4C), a stiffness gradient across the fiber cap is developed by differential cell wall thickening which affects tissue density and thereby axial tissue stiffness in the different regions of the cap [90]. Radial gradients in tissue parameters (tissue density, vascular bundle volume and solid fractions) of bamboo give rise to an increase in the axial stiffness, module of rupture and axial compressive strength from inside toward outside of the tissue [91,92].

3.2. Plant stiffness gradients and plant actuation

The juxtaposition of cells having walls of differing hydration capacities allows for the creation of plant actuators. Those that act as bilayers whereby differential swelling (extent or direction) of neighbouring tissues with dissimilar microfibril angle and/or degree of matrix swelling (type of matrix present and degree of lignification) leads to reversible shape changes are of particular interest. While differential swelling is the main mechanism commonly used to describe moisture-responsive actuation in plants, stiffness and compliance also come into play. In mechanical terms, even in the simplest case of a bilayer that is extensively used for analysis of plant movement, the net bending of the structure is a function of both the swelling ratio and the stiffness ratio of two constituents. Heavily reinforced tissues are less able to bend, while those that swell are usually less stiff. Below we focus on an example from our own laboratories and discuss how stiffness gradients shape the stem deformation patterns we have observed in the species *Selaginella lepidophylla*.

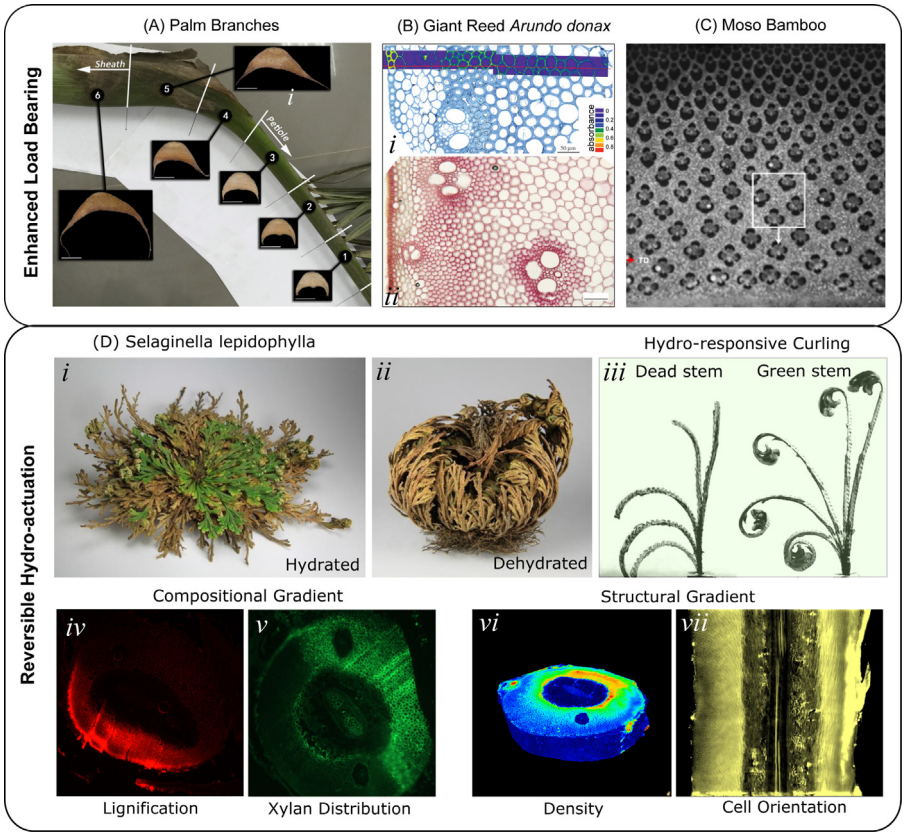

Fig. 4. The role of stiffness gradients in plant function. (A) Cross sectional gradients in petiole and sheath of palm branch (reproduced with permission from [16]). (B) (*i*) Composite image of the giant reed *Arundo donax* cross section stained with toluidine blue and visualized with two-dimensional UV absorbance scans; and (*ii*) cross section stained with phloroglucinol/hydrochloric acid that stains lignified tissues in red. The gradient in absorbance indicates variation in the degree of lignification (reproduced with permission from [88]). (C) The functionally graded structure of moso bamboo (reproduced with permission from [92]). (D) The spikemoss *Selaginella lepidophylla* in (*i*) hydrated and (*ii*) dried states and (*iii*) the curling pattern of its dead and living stems (reproduced with permission from [96]). Compositional gradients in the apical cross-section of a living stem in (*iv*) the degree of lignification and (*v*) xylan distribution and the structural gradients reflected in (*vi*) the density gradients in the cross section and (*vii*) the variation of cells orientations observed in the longitudinal section. The synchrotron-based X-ray tomographic microscopy experiments in (*vi-vii*) were performed at the TOMCAT beamline of the Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland (Rafsanjani et al., unpublished data) [138].

The spirally arranged stems of *S. lepidophylla* (Fig. 4D i) compactly curl into a nest-ball shape upon dehydration (Fig. 4D ii), limiting the photo-inhibitory and thermal damages the plant might experience in arid environments [93]. There is a dichotomy between the curling patterns of the stems located in the center of the plant and those at the exterior of the spiral phyllotaxy of the plant. Upon dehydration, the outer dead stems act as classical bilayers [94,95] and bend into arcs after a relatively short period of desiccation, whereas the axially graded inner green stems curl slowly into spirals due to a hydro-actuated strain gradient along their length (Fig. 4D iii). The reversible curling/uncurling of stems is attributed to both compositional and structural gradients giving rise to functionally graded stiffness within the transverse and longitudinal sections of the stems (Fig. 4D iv–vii) [96]. In inner stems, secondary cell wall lignification occurs throughout the whole cortex in the basal sections, whereas the middle of the stem has lignified tissues only in the abaxial cortex, and in a narrow abaxial strip at the apical tip (Fig. 4D iv) [96]. There is an abundance of xylan mostly in the adaxial cortex of the stem (Fig. 4D v), giving rise to a second compositional gradient along the length of the stem, as elucidated using antibodies (Brule et al., unpublished data). The morphology of cortical cells also exhibits a smooth gradient in tissue density, which is reflected in cell size and cell wall thickness (Fig. 4D vi). Finally, using X-ray tomography, the orientation of cortical cells was found to vary gradually from axially oriented cells in the abaxial side of the stem to almost 45° inclination in the adaxial side (Fig. 4D vii) (Rafsanjani et al., unpublished data). The presence of multiple gradients in the coiling inner stems of *S. lepidophylla* suggests that it is a useful model for studying the contribution of structural and compositional gradients to plant stiffness, as well as to investigate how functional gradients predetermine the direction and magnitude of deformation observed in this species.

4. Harnessing of plant stiffness: biomechanical tailoring of plants

Agricultural and forestry engineering has historically focused on product quality, yield, and resistance to biotic and abiotic challenges (e.g., pests, pathogens, soil quality, drought). More recently, attention has been paid to the development of new products and usages for current crops and crop residues, such as fibers for functional materials and optimized degradation for use in biofuels. Explicit discussion of the manipulation of plant stiffness is not necessarily present, but there are many new and old agricultural and product needs that require or would benefit from modulation of plant stiffness properties. As much recently has been published regarding the details of plant cell wall and tissue structure optimization for biofuel and forestry purposes [97,98], we will restrict our discussion to examples of these needs and basic strategies for their manipulation, and refer readers to the following reviews for the details of bioengineering [98,99].

Agricultural issues related to plant stiffness include growth fitness (e.g., lodging) and food quality (e.g., texture, shape maintenance). Stem and root lodging cause irreversible damage leading

to breakage in response wind and rain forces. Lodging is a global problem that causes significant yearly food production losses [7]. While conventional methods of lodging resistance have included creating dwarfed crop species that are small and subsequently less prone to bending and breakage, not all crop species are amenable to dwarfing [100]. Here knowledge of stiffness parameters could be useful to breed crop species (dwarfed or not) with stronger stems to improve both lodging resistance and crop yield [7,24,101].

On the crop quality side, texture is critical: crops must obtain a desired state of firmness, which can vary upon the end use (e.g., food crops used raw or cooked), as well as the need to store and/or transport them without damage [102,103]. An important area of study thus includes fruit ripening, which includes the developmentally-regulated softening of plant tissues. This is achieved primarily through the loss of cell adhesion and cell wall integrity via the activity of polysaccharide active enzymes on the middle lamella and cell wall proper including pectin methylesterases that can loosen pectin gels and allow the entrance of degradation enzymes such as pectate lyases and polygalacturonases [102,103]. Texture is also affected by the rheological properties of the cell wall components present, particularly of matrix polymers such as pectins and arabinoxylans, and highly glycosylated cell wall proteins. These matrix polymers are of particular interest when extracted from cell walls or obtained as extruded mucilages or gums, as they are used in for food, industrial and medical purposes as emulsifiers, glues and coatings [104,105]. Thus, texture properties can be modulated by manipulation of the composition of cell walls, or the timing and degree of cell wall degradation via wall active enzymes [103–105].

The stiffness of plant fibers is also of significant interest, ranging from individual textile fibers such as cotton and linen (flax), to those that make up wood, to the use of fibers derived from plant residues for the creation of novel composite materials. Unlike the fruit ripening and pectins mentioned above that relate to primary cell walls, fibers are generally derived from cells that have undergone secondary cell wall thickening. For example, cotton fibers elongate as hairs from the seed coat and, at maturity, are composed of a secondary cell wall that is mostly crystalline cellulose, while flax bast fibers are derived from reinforced phloem cells of flax stems that have a more complex, gelatinous secondary cell wall [106–108]. The length, strength and stiffness of the mature fibers are critical for the production of textiles and for composite materials. Fibers with different biomechanical properties are required for different uses [106]. Wood is made up of many physically connected files of reinforced vascular and/or interfascicular fiber cells, depending on the type of tree, and the required structural parameters depend on the end use of the wood for construction or furniture versus providing fibers for products such as paper [2,106]. The length and stiffness properties of individual fibers (or wood) can largely be modulated via alteration of cell wall properties (primary cell wall compliance for growth; degree and type of secondary cell wall reinforcement for stiffness and strength), either directly through manipulation of their biosynthesis or through the regulation of the timing and location of cells/cell types with desired primary cell wall or secondary cell wall properties [106,109]. One could also envision for wood, and/or other plant products composed of more than individual fibers, the ability to create gradients of tissue, cell or cell wall properties to create actuator-type "smart" materials (i.e., that are capable of reacting to stimuli in their environment and responding in an adaptive manner). By studying how specific plant species take advantage of compositional and structural gradients to affect changes in their conformation through manipulating of tissue stiffness, it is possible to learn how to integrate these properties into the design of engineered plants with functional properties for advanced materials or sustainable construction (reviewed in [97,110]) [96].

5. Concluding remarks and future directions

In this paper, we have laid out and discussed a number of morphological and compositional parameters affecting plant stiffness across multiple length scales ranging from organs to tissues to cells and cell walls. It is clear from this consideration that these properties are complex, intermingled and interdependent, making it difficult to determine the exact contribution of each. What is needed is a comprehensive, systematic and consistent multiscale mechanical analysis of structural parameters across length scales to feed into an integrated model of the development of plant stiffness.

In an attempt to look at these factors in a consistent background and avoid complications from considerations across organ type and species and plant groups, we have reviewed the literature of stem stiffness within the reference plant Arabidopsis, where the use of genetic dissection and transgenic plants has allowed some consideration of individual or related groups of variables. It is clear from this review that there are a number of useful mutant lines affecting key parameters. However, most either have not been mechanically tested, or tested with a very limited set of techniques (e.g., only tensile breaking force), or not in a comparable manner in terms of tissue and plant stage (see Tables 1 and 2). While Arabidopsis is not a perfect model for examining all morphological and compositional variables affecting plant stiffness, it has the advantage of ease of use and the availability of mutant lines affecting multiple stiffness parameters. As such, comprehensive analysis in Arabidopsis could be used in combination with current and new data from other plant systems to develop an integrated model of plant stiffness.

The next step is a systematic investigation of stiffness parameters across length scales in Arabidopsis using a combination of relevant tissues (e.g., starting with mature stems) and mutants such as those described earlier in this paper (listed in Tables 1 and 2). It is imperative, as discussed in Section 2.6, to have a comprehensive analysis of mutant plant morphological, structural and biochemical phenotypes to understand the full biological consequences of the genetic alterations in question in addition to mechanical testing to inform the interpretation of the results. Further, for mechanical experiments to be comparable, it is critical to ensure that there is consistency not only in the tissue used, but also in such things as the Arabidopsis accession studied, plant developmental stage, dimensions/location of sample within that tissue, and hydration state (e.g., for stems, it would be useful to consider their mechanical properties both in hydrated and dry states to allow consideration of the mechanics in living plants versus materials derived from dry, harvested plants). The mechanical experiments performed also need to be comprehensive and include different loading conditions (e.g., cyclic tension and compression, strength and fracture characterization, micro- and nanoindentation, and atomic force microscopy [Box 2]). Molecule-level monitoring tools such as X-ray scattering or Raman microspectroscopy in combination with mechanical testing would also inform structure-function analysis (see [39,40], and Box 2). Finally, tests should move beyond one or two dimensions, take turgor pressure into consideration, combine in-situ mechanical testing with imaging techniques such as X-ray computed tomography (Box 2), and/or be performed in a non-destructive fashion to ensure that the properties measured are true to native plant conditions [24,36].

Finally, there is a good deal of scope for addressing agricultural and new biodegradable and biocompatible material needs through the tailoring of stiffness of plants and products derived from them such as fibers and wood. The creation and harnessing of gradients of stiffness parameters to create adaptive or 'smart' materials is of particular interest. In addition to the use of genetic engineering of genes related to tissue organization, cellular differentiation and cell wall synthesis indicated above, it is possible to adapt plant structure through other means. These include taking advantage

of plant responses to plant spacing, mechanical perturbation, and growth or treatment under certain nutrient or hormone conditions [13,99,106].

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