CellPress
REVIEWS

Box 1. #ArtGenetics and Crowdsourcing

Museums all over the world are full of interesting artwork. However, their catalogues or online databases rarely provide information regarding fruits and vegetables. For example, the painting below by Frans Snyders is full of fruits and vegetables, but the entry in the museum catalogue simply states: 'Still life with monkeys' (Figure IA). In other instances, interesting species are depicted in a corner of the painting and are easily missed, as is often the case for the tiny strawberry plant at the feet of the virgin mother Mary. Therefore, it remains essential to take a close look at each and every painting. In addition to incorporating digital art collections, we must not lose sight of less accessible museums or private collections. That is why we call upon the general public to assist us, by providing useful iconographic material, collected from their own trips to a museum, castle, or mansion (Figure IB). The collected images will be ultimately incorporated in an open access database.

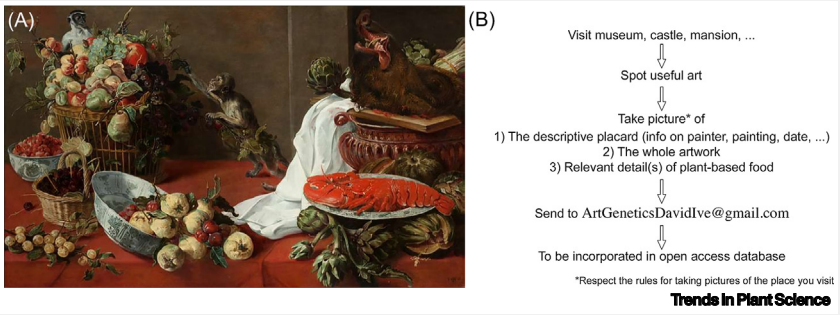

Figure I. Art Genetics and Crowdsourcing.

history and molecular biology, into the #ArtGenetics concept, leads to valuable insight in the evolution and domestication of our plant-based food. In the future, it is hoped that iconographic material worldwide is comprehended in this way, and that a novel insight into the appearance, development, and distribution of fruits, vegetables, legumes, grains, nuts, and seeds is gained. For this, the assistance of the general public in the context of Citizen Science is called upon (Box 1).

Acknowledgments

We thank the public for sending us images for our studies.

[1]Amarant vzw, B-9000 Ghent, Belgium
[2]Ghent University, Department of Plant Biotechnology and Bioinformatics, 9052 Ghent, Belgium
[3]VIB Center for Plant Systems Biology, 9052 Ghent, Belgium

*Correspondence:
ive.desmet@psb.vib-ugent.be (I. De Smet).
@Twitter: @IveDeSmet1978

https://doi.org/10.1016/j.tplants.2020.05.010

References

1. Zhou, X. *et al.* (2020) 5200-year-old cereal grains from the eastern Altai Mountains redate the trans-Eurasian crop exchange. *Nat. Plants* 6, 78–87
2. Duran, N. and MacDonald, K. (2006) Information sources for food studies research. *Food Cult. Soc.* 9, 233–243
3. Vergauwen, D. and De Smet, I. (2016) Down the rabbit hole–carrots, genetics and art. *Trends Plant Sci.* 21, 895–898
4. Vergauwen, D. and De Smet, I. (2017) From early farmers to Norman Borlaug – the making of modern wheat. *Curr. Biol.* 27, R858–R862
5. Vergauwen, D. and De Smet, I. (2019) Watermelons versus melons: a matter of taste. *Trends Plant Sci.* 24, 973–976
6. Vergauwen, D. and De Smet, I. (2019) The strawberry tales: size matters. *Trends Plant Sci.* 24, 1–3
7. Janick, J. *et al.* (2007) The cucurbits of mediterranean antiquity: identification of taxa from ancient images and descriptions. *Ann. Bot.* 100, 1441–1457
8. Paris, H.S. *et al.* (2013) Medieval iconography of watermelons in Mediterranean Europe. *Ann. Bot.* 112, 867–879
9. Martin, M. *et al.* (2001) The domestication process of the Modern Rose: genetic structure and allelic composition of the rose complex. *Theor. Appl. Genet.* 102, 398–404
10. Guo, S. *et al.* (2019) Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. *Nat. Genet.* 51, 1616–1623
11. Zhao, G. *et al.* (2019) A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. *Nat. Genet.* 51, 1607–1615
12. Zhou, Y. *et al.* (2016) Convergence and divergence of bitterness biosynthesis and regulation in Cucurbitaceae. *Nat. Plants* 2, 16183
13. Iorizzo, M. *et al.* (2016) A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. *Nat. Genet.* 48, 657–666
14. Scott, M.F. *et al.* (2019) A 3000-year-old Egyptian emmer wheat genome reveals dispersal and domestication history. *Nat. Plants* 5, 1120–1128
15. Renner, S.S. *et al.* (2019) A 3500-year-old leaf from a Pharaonic tomb reveals that New Kingdom Egyptians were cultivating domesticated watermelon. *bioRxiv*, 642785

Spotlight

Pectin Drives Cell Wall Morphogenesis without Turgor Pressure

Dangquan Zhang[1,*] and Baohong Zhang[2,*]

How the plant cell wall expands and forms shapes is a long-standing mystery. Traditional thought is that turgor pressure drives these processes. However, a recent study by Haas and colleagues shows for the first time that the expansion of pectin homogalacturonan nanofilaments drives morphogenesis without turgor pressure in plant epidermal cells.

Plants Have an Intricate Cell Wall with Diverse Functions

The cell wall is the rigid and semipermeable biphasic outer layer of plant cells. It is an essential component of plant cells and represents the major difference between plant and animal cells (animal cells lack a cell wall). In addition to its major function as a framework for the cell to prevent overexpansion, the plant cell wall (i) controls and directs cell growth, (ii) provides mechanical support and strength for the plant, (iii) mediates cell communication, (iv) plays a significant role in defense against pathogen attack and external abiotic stress, (v) serves as a translocation channel to govern the entry and egress of molecules, and (vi) acts as a storage site for carbohydrates and other molecules.

To maintain these diverse functions, plants have evolved a complex cell wall primarily composed of polysaccharides. These are generally classified into three groups: cellulose, hemicellulose, and pectin. Cellulose

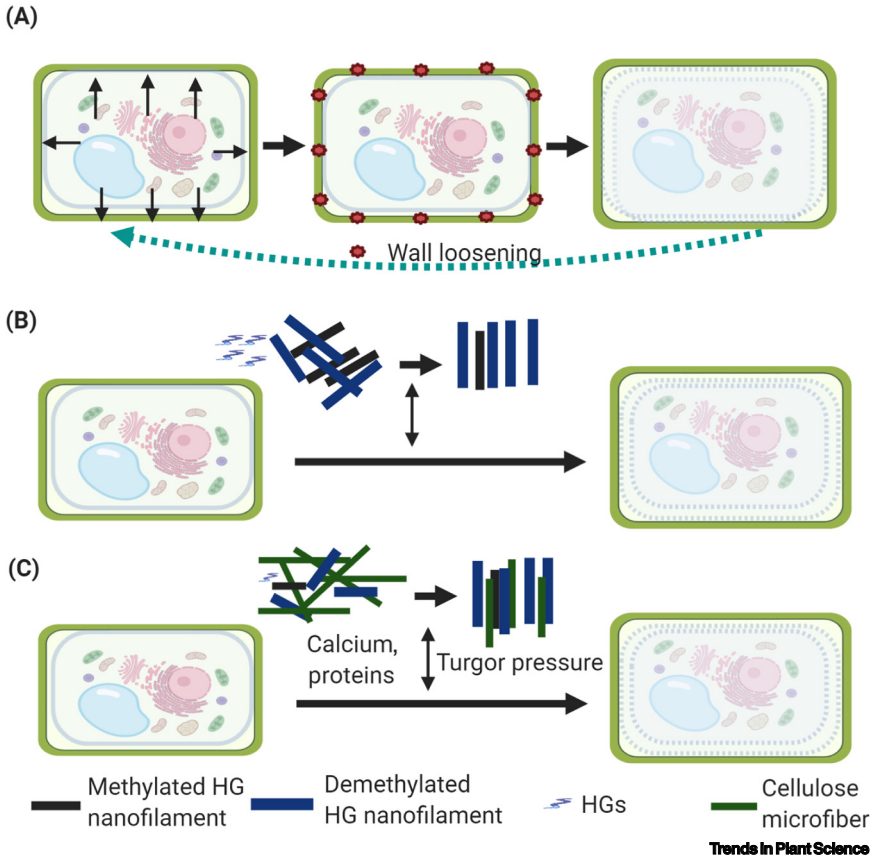

Figure 1. Models of Forces Governing Plant Cell Wall Expansion. (A) The traditional model in which turgor pressure drives cell wall expansion and morphogenesis [2]. When turgor pressure is generated inside the cells, it causes cell wall loosening and expansion, and returns the cell wall to its original state when the turgor pressure is released [2]. (B) The model proposed by Haas and colleagues [3] in which pectin homogalacturonan (HG) methylation/demethylation and nanofilament remodeling provide the force that drives cell wall expansion. Pectin HG forms a quaternary nanofilament structure in the cell wall, and its methylation/demethylation determines the quaternary structure. Methylated HG is packed in hexagonal lattices whereas demethylated HG is packed in rectangular lattices [3]. HG demethylation thus drives HG nanofilament expansion and cell wall growth. (C) The model of cell wall expansion proposed in this paper. Cell wall molecules interact with each other during the remodeling of cellulose microfibers and pectin HG nanofilaments which drives cell wall expansion and cell growth. Other molecules, including soluble proteins and calcium, may serve as signaling molecules in response to turgor pressure and internal/external stresses. The pectin–cellulose network plus turgor pressure together drive cell wall growth and control cell wall shape. This figure was generated using BioRender (https://biorender.com/).

forms organized crystalline microfibrils that are embedded in a gel-like matrix of pectins, hemicelluloses, and a small number of proteins. In the current model, isotropic turgor pressure drives plant cell expansion, and the network of aligned cellulose locally restricts growth, thus controlling shape formation and plant morphogenesis (Figure 1A) [1,2]. However, a recent study by Haas and colleagues may change the textbook concept and oblige us to rethink the driving force behind cell wall shape and cell growth [3].

Nanofilament Structure of Pectin Homogalacturonan

Pectins constitute a diverse class of polysaccharides characterized by 1,4-linked α-D-galactosyluronic acid (GalpA, also known as galacturonic acid), including homogalacturonan (HG), xylogalacturonan (XGA), apiogalacturonan, rhamnogalacturonan I (RGI), and rhamnogalacturonan II (RGII). The ratios of these pectins is variable. HG (60%) is the most abundant, followed by RGI, and together these two classes constitute >75% of total pectins [4]. Pectins in the extracellular matrix were previously thought to adopt a disorganized hydrated gel-like structure within the cellulose–hemicellulose network. However, a recent study by Haas and colleagues has completely changed this view. Using advanced nanoimaging microscopy, they observed for the first time that pectins form organized filamentous structures, termed HG nanofilaments, in the intact cell wall [3].

In 1981, an X-ray diffraction study showed that pectin HG may form crystalline fibrous structures *in vitro* [5]. *In vitro* helical tertiary structures of the galacturonic acid arrange uniaxially into fibrous quaternary structures [5]. However, pectin crystalline helical structures have not been observed in intact cells. For a long time, scientists have thought that pectin forms an amorphous collection of polymers in plant cell walls. Using super-resolution 3D direct stochastic optical reconstruction microscopy (3D-dSTORM) and cryo-scanning electron microscopy (cryo-SEM), Haas and colleagues observed the quaternary nanostructure of the homoglycan polymer HG. They demonstrated that, in the anticlinal walls of arabidopsis (*Arabidopsis thaliana*) pavement cells, pectin HG assembles into discrete nanofilaments instead of forming a continuously interlinked network. These HG nanofilaments align perpendicular to the cotyledon surface, and their estimated width measured by cryo-SEM is about 15–30 nm [3].

Demethylation-Mediated Pectin Nanofilament Inflation Drives Plant Cell Anisotropic Growth without Turgor Pressure

Pectin methylation and demethylation play important roles in pectin structure and

function. Pectin is biosynthesized in a methylated form in Golgi vesicles, and is generally converted into the demethylated form on insertion into the cell wall [6]. To study the behavior of methylated and demethylated HG, and their impact on cell wall shape and cell growth, Haas and colleagues first generated transgenic arabidopsis plants overexpressing either PECTIN METHYLESTERASE (PME) or PECTIN METHYLESTERASE INHIBITOR (PMEI). They then employed cryo-SEM to determine the nanofilament architecture. This revealed that HG nanofilaments are present in organized structures in anticlinal but not in periclinal walls [3]. The cross-sections of HG nanofilaments were ~1.4 longer in HG-demethylated walls (PME overexpression) than in HG-methylated walls (PMEI overexpression) [3]. This suggests that (i) HG demethylation causes the HG nanofilament to expand, and (ii) spatial and temporal HG demethylation in the cell wall is likely to produce local radial expansion of the HG nanofilaments, leading to cell wall expansion. This conclusion is consistent with a previous *in vitro* study [5]. The quaternary structures switched from methylated to demethylated HG and this resulted in 1.42-fold expansion of the HG nanofilaments in transgenic plants by cryo-SEM measurements [3]. HG demethylation alone can cause cell and tissue expansion as shown in the *in vitro* study of Haas and colleagues. This suggests that HG demethylation can drive cell wall morphogenesis and cell growth in the absence of turgor pressure. This conclusion is further supported by a reversible dehydration study and 3D nonlinear finite element method (FEM) modeling [3].

Concluding Remarks and Future Perspectives

This is the first observation that pectin forms nanofilament structures in intact cells. Based on the observation and their FEM model, Haas *et al.* demonstrate that HG methylation and demethylation result in switching of quaternary structures of HG nanofilaments, from a hexagonal to a rectangular lattice in the cell wall, leading to HG filament expansion, and that this is the primary force that shapes the cell wall and cell growth (Figure 1B) [3]. However, there are still many questions to be answered to fully understand the mechanisms driving cell wall biosynthesis and morphogenesis. Although HG nanofilament expansion provides power for cell wall expansion, it may not be the only driving force. Other forces such as traditionally hypothesized turgor pressure, as well as cellulose microfiber remodeling, may operate together with HG remodeling to control cell wall expansion and cell shape (Figure 1C). Observing the HG nanofilament quaternary is important, and finer (e.g., crystalline) structures will need studies to confirm the natural structure of each component and their interactions in the cell wall. In addition, although methylation/demethylation of pectin HG is a key driver of pectin filament remodeling and function, the molecular mechanism driving the switch between these two forms is unclear.

Different studies and experimental technologies show that cellulose and pectin coexist and are directly associated with each other [3,7]. This may suggest that components of the complex extracellular matrix, particularly cellulose microfibers and pectin HG nanofilaments, form a single cohesive network rather than two separate networks. Both cellulose microfibers and pectin nanofilaments actively participate in cell wall development [3,8]. The most likely scenario is that cellulose microfibers and pectin nanofilaments work together, as well as with other components such as hemicelluloses, to form a pectin–cellulose network. This network, plus turgor pressure, drives cell wall growth and controls cell wall shape (Figure 1C). During this process, soluble extracellular matrix proteins and/or other molecules such as calcium [9] also play important roles [10], and these may serve as signaling molecules to communicate information from inside and outside of cells, including turgor pressure, and thus guide pectin demethylation and cellulose microfiber/pectin nanofilament remodeling. To elucidate the exact mechanism controlling cell wall formation and shape, more technologies will need to be employed (e.g. the CRISPR/Cas9 genome editing tool can be used to silence, overexpress, or even monitor a specific sequence [11]). These tools may be employed to knock out some important genes in pectin and cellulose biosynthesis and assembly to study their functions. Wang and colleagues [12] recently employed CRISP/Cas9 to knock out genes associated with pectin degradation. Their work demonstrated that there are extensive interactions between pectin and cellulose, and that pectin chains may intercalate within, or between, nascent cellulose microfibrils [7,12]. This further suggests that pectin nanofilaments and cellulose microfibers may work together to control cell wall development.

Acknowledgments

We greatly appreciate Dr Claudia Jolls for her excellent comments and for proofreading this paper. D.Z. is supported by the National Natural Science Foundation of China (31172257), the Science Research Project in Henan Province of China (192102110174), and the Talent Project of Henan Agriculture University. B.Z. is supported in part by Cotton Incorporated and the National Science Foundation (award 1658709).

[1]Henan Province Engineering Research Center for Forest Biomass Value-Added Products, College of Forestry, Henan Agricultural University, Zhengzhou, Henan 450002, China
[2]Department of Biology, East Carolina University, Greenville, NC 27858, USA

*Correspondence:
zhangdangquan@163.com (D. Zhang) and
zhangb@ecu.edu (B. Zhang).

https://doi.org/10.1016/j.tplants.2020.05.007

References

1. Altartouri, B. *et al.* (2019) Pectin chemistry and cellulose crystallinity govern pavement cell morphogenesis in a multi-step mechanism. *Plant Physiol.* 181, 127–141
2. Cosgrove, D.J. (2016) Plant cell wall extensibility: connecting plant cell growth with cell wall structure,

mechanics, and the action of wall-modifying enzymes. *J. Exp. Botany* 67, 463–476
3. Haas, K.T. *et al.* (2020) Pectin homogalacturonan nanofilament expansion drives morphogenesis in plant epidermal cells. *Science* 367, 1003–1007
4. Harholt, J. *et al.* (2010) Biosynthesis of pectin. *Plant Physiol.* 153, 384–395
5. Walkinshaw, M.D. and Arnott, S. (1981) Conformations and interactions of pectins: II. Models for junction zones in pectinic acid and calcium pectate gels. *J. Mol. Biol.* 153, 1075–1085
6. Anderson, C.T. (2016) We be jammin': an update on pectin biosynthesis, trafficking and dynamics. *J. Exp. Botany* 67, 495–502
7. Wang, T. and Hong, M. (2016) Solid-state NMR investigations of cellulose structure and interactions with matrix polysaccharides in plant primary cell walls. *J. Exp. Botany* 67, 503–514
8. Sampathkumar, A. *et al.* (2019) Primary wall cellulose synthase regulates shoot apical meristem mechanics and growth. *Development* 146, dev179036
9. Wang, X. *et al.* (2020) Pectin methylesterase selectively softens the onion epidermal wall yet reduces acid-induced creep. *J. Exp. Botany* 71, 2629–2649
10. Francoz, E. *et al.* (2019) Pectin demethylesterification generates platforms that anchor peroxidases to remodel plant cell wall D domains. *Dev. Cell* 48, 261–276
11. Adli, M. (2018) The CRISPR tool kit for genome editing and beyond. *Nat. Commun.* 9, 1911
12. Wang, D. *et al.* (2019) Characterization of CRISPR mutants targeting genes modulating pectin degradation in ripening tomato. *Plant Physiol.* 179, 544–557

Spotlight

Prime Editing: Game Changer for Modifying Plant Genomes

Marek Marzec [1,*] and Goetz Hensel[2,3]

Prime editing, developed by Anzalone *et al.*, brings genome editing to a new level, because this approach allows introduction of all mutation types, including insertions, deletions, and all putative 12 types of base-to-base conversions. Previously tested in human cells, this technique has been adapted for use in plants by Lin *et al.*

Targeted genome modification is a powerful tool in research, which opens new possibilities of directly targeting and modifying genomic sequences in a simple and effective way, thereby accelerating gene function analysis and, by introducing favorable alleles, can speed up the breeding process. A great breakthrough in this area was the adaptation of the prokaryote immune system clustered regularly interspaced short palindromic repeats and CRISPR-associated protein (CRISPR/Cas) for targeted genome editing [1]. In this approach, RNA-guided endonuclease(s) are guided by a short RNA sequence (sgRNA) to introduce double-strand DNA breaks in a specific region of the genome [1]. When the breaks are repaired via the predominant nonhomologous end-joining DNA repair pathway, random insertions or deletions (indels) are introduced in the target sequence [2]. Further modifications of the system have increased the specificity and efficiency of introducing indels [3]. The next milestone in genome editing was the utilization of RNA-guided endonucleases for base editing, including all four transitions: C → T, T → C, A → G, and G → A [4]. Recently, Anzalone *et al*. described a new technique, called prime editing, that enables the introduction of indels and all 12 base-to-base conversions (both transitions and transversions) without inducing a DNA double-strand break [5]. This is a real game changer because previous techniques either allowed only one base edit at a time or needed the challenging simultaneous delivery of a custom repair template. By combining a fusion protein with dual function and a modified RNA, prime editing will now allow easier modification of promoter or untranslated regions and will make allele replacement more feasible for targets, which will not confer a selection benefit like herbicide resistance. In this new approach, sgRNA was replaced by prime editing guide RNA (pegRNA), which not only drives the endonuclease but also contains a primer binding site (PBS) region and the sequence that will be introduced to the targeted gene. The PBS region will create a primer for the reverse transcriptase (RT), which was fused to the modified nCas9 nickase protein and uses the sequence from the pegRNA as a template. Information that is copied directly from the pegRNA might be introduced into the target sequence (Figure 1). The prime editing technique allows the introduction of all 12 point mutations to target genes at locations ranging from 3 bp upstream to 29 bp downstream of a protospacer adjacent motif (PAM), as well enabling insertions up to 44 bp and deletions up to 80 bp in human cells [5]. It should be noted that the efficiency of prime editing was similar to that obtained by base editing, but the specificity of prime editing was higher in comparison with previous systems. The number of observed off-targets was lower in the case of prime editing, even if the same protospacer was used. The explanation for this increased specificity is that in the case of the standard CRISPR/Cas system only one hybridization, between target DNA and protospacer from sgRNA, occurs. Whereas in the prime editing system, three hybridization events are present: between target DNA and spacer from pegRNA; between target DNA and PBS from pegRNA; and between target DNA and RT product (Figure 1).

Lin and coworkers have adapted this groundbreaking prime editing system for plants. It was proved that, using prime editing, it was possible to generate all types of single base substitutions (with efficiencies of 0.2–8.0%), insertions up to 15 nt, and deletions up to 40 nt in rice endogenous genes [6]. Plant prime editing is less efficient than base editors for making transition point mutations, however, it provides a method for generating changes that cannot be made with other genome editing tools. This is a first description for prime editing in plants and the authors suggest solutions that may increase the efficiency of genome editing using this new technique. First of all, it was proved that the RT used in the original prime editing system [Moloney murine leukemia virus reverse transcriptase