Insight

Rhamnogalacturonan-I forms mucilage: behind its simplicity, a cutting-edge organization

Susana Saez-Aguayo1,* and Asier Largo-Gosens2

1 Centro de Biotecnología Vegetal, Laboratorio Mucilab, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago 8370146, Chile
2 Área de Fisiología Vegetal, Departamento de Ingeniería y Ciencias Agrarias, Universidad de León, E-24071, León, Spain

* Correspondence: susana.saez@unab.cl

This article comments on:

Zhang Y, Yin Q, Qin W, Gao H, Du J, Chen J, Li H, Zhou G, Wu H, Wu A-M. 2022. The Class II KNOX family members KNAT3 and KNAT7 redundantly participate in Arabidopsis seed coat mucilage biosynthesis. Journal of Experimental Botany 73, 3477–3495.

Arabidopsis mucilage has been used for more than two decades to investigate and characterize different factors of pectin synthesis and modification. Using cytological, transcriptomic, chemical, and molecular approaches, Zhang et al. (2022) clarify the redundant role of KNAT3 and KNAT7, two KNOX Class II transcription factors, in mucilage rhamnogalacturonan-I (RG-I) biosynthesis through the regulation of a group of genes which coordinate the formation of these specific pectic domains.

Seed mucilage is a hydrated gel-like structure produced by seed coat tegment of myxospermous seeds and participates in seed dispersion, seed germination, and the maintenance of the earth rhizosphere (Macquet et al., 2007a; Saez-Aguayo et al., 2014; Voiniciuc et al., 2015a). The Arabidopsis seed coat mucilage has been used for >20 years to elucidate the roles of genes in the extremely complex process of cell wall synthesis and remodeling (reviewed in Šola et al., 2018). Arabidopsis mucilage is mainly composed of unbranched rhamnogalacturonan-I (RG-I) and homogalacturonan (HG) pectin domains (Box 1) (Macquet et al., 2007a; Voiniciuc et al., 2015a). Both domains are enriched in the acidic sugar galacturonic acid (GaA), although these polysaccharides have quite different structures (Mohnen et al., 2008). Indeed, in the RG-I backbone, GaA is alternated with rhamnose (Rha), while in the HG domain, the backbone is formed exclusively by GaA residues that can be methylesterified in their carboxyl groups (Mohnen et al., 2008). Mucilage polysaccharides are deposited in specialized seed epidermal mucilage secretory cells (MSCs) throughout seed development. The active deposition of mucilage polysaccharides led to the formation of a central column called the columella in a highly regulated process (Box 1A; Western et al., 2004; Macquet et al., 2007a; Voiniciuc et al., 2015b; Golz et al., 2018). In their study, Zhang et al. (2022) provide evidence of the participation of KNAT3 and KNAT7 in this process by an exhaustive analysis of the mucilage phenotype of knat3 and knat7 single mutants and the knat3knat7 double mutant using cytological, immunological, and biochemical approaches. The authors determine that KNAT7 and KNAT3 mutation affects seed epidermal cell morphology and observed a flattened columella in knat3knat7 double mutants. Also, the authors determined less RG-I production and changes in HG structure which could explain the MSC phenotype.

Elucidation of the complex regulation of mucilage RG-I synthesis and structure

Thanks to the great work realized by Zhang and collaborators, the tangled ‘ball of wool’ which represents the regulation of pectin synthesis is a little bit clearer now. At the mature stage, mucilage has been characterized as a plethora of unbranched RG-I polymers with an average size of 600 kDa; therefore, it is formed by ~1800 units of Rha–GaA disaccharide (Box 2A; Macquet et al., 2007a; Williams et al., 2020; Saez-Aguayo...
et al., 2021). Elongation of this ‘giant’ polymer is initiated by the coordinated action of enzymes localized in the Golgi apparatus and in the cytosol (Fabrissin et al., 2019; Saez-Aguayo et al., 2021). MUCILAGE-MODIFIED-4 (MUM4) converts UDP-Glc to UDP-Rha in the cytosol before the transport of the latter to the Golgi lumen by URG2/4/6 transporters (Western et al., 2004; Saez-Aguayo et al., 2021). UDP-GlcA is transported from the cytosol to the Golgi apparatus by UUAT1 and, presumably,
Box 2. Current description of key factors acting on RG-I synthesis and structure.

(A) Schematic representation of mucilage RG-I and xylan synthesis in Arabidopsis

UUAT1/3, URGT2/4/6, RRT1, GATL5, GAUT11, MUCI70, MUM5, and IRX14 have been implicated in the synthesis of mucilage RG-I and xylan molecules. URGTs and UUATs are proteins that ensure the transport of UDP-Rha and UDP-GlcA—the latter of which is transformed into UDP-GalA, the precursor of RG-I, by a glucuronate epimerase (GAE)—from the cytosol to the Golgi. The coordinated action of the two glycosyltransferases RRT1, a rhamnosyltransferase, and GAUT11, a galactouronosyl-transferase, build up the mostly unbranched RG-I backbone. As the RG-I is synthesized, the putative xylosyltransferase MUM5 adds a xylose that will constitute a xylan side chain by the addition of more xyloses by IRX14. The participation of GALT5 and MUCI70 in RG-I and HG synthesis was demonstrated, but to a minor extent. To date, no GTs involved in RG-I ramifications have been described. Once pectins are secreted into the apoplast, they mature by the removal of lateral chain ramifications realized by MUM2 and BXL1 which are a galactanase and arabinase, respectively. At the mature stage, RG-I has been characterized to be a plethora of RG-I molecules with an average size of 600 kDa, and is thus formed by ~1800 units of Rha–GalA disaccharide.

(B) Schematic model of transcriptomic regulation of genes implicated in mucilage RG-I production in seed coat epidermal cells

Fourteen transcription factors (TFs) have been reported to regulate gene expression involved particularly in RG-I formation and structure (Huang et al., 2011; Ezquer et al., 2016; Saez-Aguayo et al., 2017, 2021; Golz et al., 2018; Xu et al., 2022). The complex formed by the TF TTG1–TT8–MYB5 regulates the action of TTG2 and GL2, both activators of MUM4, URGT2, URGT4, and GATL5, involved in RG-I synthesis. MUM1 has its own pathway, regulated by STK, and inhibits UUAT1 and URGT6 which have a discrete role in RG-I formation. Additionally, MUM1 is an activator of enzymes such as MUM2 and BXL1 which remove RG-I branching. The TF DE1 BINDING FACTOR (DF1) is able to bind to GL2 and thus control MUM4 and GATL5. It was also described that TTG2 could control DF1 and GL2 expression, and DF1 is also able to repress the expression of TTG2 forming a loop of regulation. Finally, the complex described in this work demonstrates that MYB75–TT8–TTG1–KNAT3/7 activate MUM4, involved in RG-I synthesis in mucilage, and, at least, KNAT7 controlled the expression of IRX14, and MUM5 which synthesizes xylan ramifications. Regulations characterized in this study are shown with red arrows.
UUAT3, and is converted to UDP-GalA by glucuronate epimerase (GAE) (Box 2A) in the lumen of this compartment (Saez-Aguayo et al., 2017, 2021; Parra-Rojas et al., 2019). When substrates are in the lumen of the Golgi, they are incorporated into the nascent RG-I polymer thanks to RRT1 (rhamnosyl transferase 1), GATL5, GAUT11, and MUCI70 (galacturonosyl transferases), up to the formation of RG-I chains (Saez-Aguayo et al., 2017, 2021; Takenaka et al., 2018; Voiniciuc et al., 2018; Fabrissin et al., 2019). To date, there are no GTs described acting on RG-I ramification (arabinans and galactans). Recently, a hypothetical xylan side chain on mucilage RG-I has been proposed based on the evidence that xylan participates in mucilage adhesion by the attachment of RG-I to cellulose. Xylan synthesis is highly coordinated with RG-I production, suggesting the existence of xylan side chains covalently linked to RG-I (Tan et al., 2013; Voiniciuc et al., 2015b; Ralet et al., 2016; Fabrissin et al., 2019; Saez-Aguayo et al., 2021). However, the exact xylan association with the RG-I structure in mucilage still remains unclear, although MUM5 and IRX14 have been characterized as xylosyl transferases adding xylose into xylan structures in mucilage polysaccharides (Voiniciuc et al., 2015b; Fabrissin et al., 2019). During RG-I maturation, arabinan and galactan side chains are removed by MUM2 and BX1 (Macquet et al., 2007b; Williams et al., 2020). No evidence clearly explains this strong change of structure but, considering that the presence of galactans and arabinans in the RG-I structure reduces the hydration ability of mucilage (Dean et al., 2007; Arsovski et al., 2009; Rautengarten et al., 2011), it seems that the synthesis of RG-I side chains is required for compaction and organization into the dehydrated mucilage pocket, and their degradation would ensure the hydration and liberation of mucilage in the presence of water.

**KNAT3 and KNAT7 regulate mucilage RG-I synthesis by the activation of MUM4 expression**

Considering all the mucilage–related genes repressed in the knat3knat7 double mutant, the authors explored whether these TFs could act as direct activators of any of these genes. By an elegant transactivation assay using a dual-luciferase reporter, the authors demonstrate that KNAT3 and KNAT7 act as redundant activators of MUM4, suggesting the existence of other activators for MUM2 and GATL5. As it was previously described that KNAT7 can physically interact with MYB75 (PAP1) to regulate secondary cell wall formation in the Arabidopsis seed coat (Bhargava et al., 2013), the authors suggest that the activation of those mucilage–related genes could be due to the formation of the complex TTG1–TT8–MYB75–KNAT7/3 mucilage MBW module. The mucilage MBW module can activate the expression of the TFs GL2 and TTG2, which in turn could modulate the expression of MUM4, GATL5, URGT2, URGT4, and other RG-I related genes (Western et al., 2001; Kong et al., 2013; Golz et al., 2018; Saez-Aguayo et al., 2021). Thus, the KNAT complex demonstrates a specific regulation of RG-I synthesis and modification, in parallel with other TFs already reported to regulate the expression of mucilage–specific genes described in detail in the Box 2B (Huang et al., 2011; Ezquer et al., 2016; Saez-Aguayo et al., 2017, 2021; Golz et al., 2018; Xu et al., 2022).

**Step forward with seed mucilage from different species**

Thanks to two decades of research, we have now made great advances in knowledge of the key enzymes and TFs in the regulation of synthesis and modification of the major mucilage component (RG-I). However, there is a lot of work still to do to understand the synthesis, modification, and, especially, the regulation of other minor mucilage components, such as HG, cellulose,
arabinans, galactans, and galactoglucomannans, among others. However, as the research in this specific field advances, maybe we can wonder if all this knowledge of how the machinery works in the synthesis of plant polymers is relevant for the future. Recent research revealed the great potential of several pectic domains for human health (Ndeh and Gilbert, 2018). Indeed, during digestion, the colon is the place where intestinal microorganisms act as decomposers of polysaccharides, providing nutrients for probiotic bacteria and influencing the gut microbiome (Ndeh and Gilbert, 2018). The degradation of polysaccharides generates certain oligosaccharides that have known anti-inflammatory activity and boost the human immunity system, helping humans to stay healthy (Thomson et al., 2018; Barbosa and de Carvalho Junior, 2021). Among the different structural domains of pectins, the HG domain seems to be the reactive domain for alleviating periodontitis, rheumatoid arthritis, and also as a novel anti-ulcer agent (Nascimento et al., 2017). The question that is raised is: are we able to transfer this knowledge to crops such as flax or chia, to produce functional foods able to boost human health? These are some of the challenges that we could consider thanks to the research carried out on mucilage to date.

**Funding**

Fondecyt Regular 1201467 to S.-A. and A.L.-G. was granted with an “Ayuda María Zambrano para la atracción del talento internacional de la Universidad de León” from Spanish “Ministerio de Universidades” financed by European Union “NextGenerationEU”.

**Keywords:** Pectin biosynthesis, rhamnogalacturonan-I, seed mucilage, transcription factors

**References**

| Author(s) | Title | Journal | Year |
|----------|-------|---------|------|
| Asrøvski AA, Popma TM, Haughn GW, Carpita NC, McCann MG, Western TL. | 2009. AtBXL1 encodes a bifunctional β-α-L-arabinofuranosidase/α-L-arabinosidase required for pectic arabinan modification in Arabidopsis mucilage secretory cells. | Plant Physiology 150, 1219–34. |
| Barbosa JR, de Carvalho Junior RN. | 2021. Polysaccharides obtained from natural edible sources and their role in modulating the immune system: biologically active potential that can be exploited against COVID-19. | Trends in Food Science & Technology 108, 223–235. |
| Bhargava A, Ahmad A, Wang S, Mansfield SD, Haughn GW, Douglas CJ, Ellis BE. | 2013. The interacting MYB75 and KNA17 transcription factors modulate secondary cell wall deposition both in stems and seed coat in Arabidopsis. | Planta 237, 1199–1211. |
| Dean GH, Zheng H, Tewari J, et al. | 2007. The Arabidopsis MUM2 gene encodes a β-galactosidase required for the production of seed coat mucilage with correct hydration properties. | The Plant Cell 19, 4007–4021. |
| Ezquer I, Mizzotti C, Ngueuma-Ona E, et al. | 2016. The developmental regulator SEEDSTICK controls structural and mechanical properties of the Arabidopsis seed coat. | The Plant Cell 28, 2478–2492. |
| Fabrissin I, Cuffe G, Berger A, Granier F, Sallé C, Poulain D, Ralet M-C, North HM. | 2019. Natural variation reveals a key role for rhamnogalacturonan I in seed outer mucilage and underlying genes. | Plant Physiology 181, 1498–1518. |
| Golz JF, Allen PJ, Li SF, Parish RW, Jayawardana NU, Bacic A, Doblin MS. | 2018. Layers of regulation—insights into the role of transcription factors controlling mucilage production in the Arabidopsis seed coat. | Plant Science 272, 179–192. |
| Huang J, DeBowles D, Estandiari E, Dean G, Carpita NC, Haughn GW. | 2011. The Arabidopsis transcription factor LUS/MUM1 is required for extrusion of seed coat mucilage. | Plant Physiology 156, 491–502. |
| Kong Y, Zhou G, Abdeen AA, et al. | 2013. GALACTURONOSYL-TRANSFERASE-LIKE5 is involved in the production of Arabidopsis seed coat mucilage. | Plant Physiology 163, 1203–1217. |
| Macquet A, Ralet MC, Kronenberger J, Marion-Poll A, North HM. | 2007a. In situ, chemical and macromolecular study of the composition of Arabidopsis thaliana seed coat mucilage. | Plant & Cell Physiology 48, 984–999. |
| Macquet A, Ralet MC, Loudet O, Kronenberger J, Mouillé G, Marion-Poll A, North HM. | 2007b. A naturally occurring mutation in an Arabidopsis accession affects a beta-o-galactosidase that increases the hydrophilic potential of rhamnogalacturonan I in seed mucilage. | The Plant Cell 19, 3990–4006. |
| Mohnen D. | 2008. Pectin structure and biosynthesis. Current Opinion in Plant Biology 11, 266–277. |
| Nascimento AM, Maria-Ferreira D, de Souza EF, de Souza LM, Sassaki GL, Lacomini M, Werner MFP, Cipriani TR. | 2017. Gastroprotective effect and chemical characterization of a polysaccharide fraction from leaves of Croton caucaca. | International Journal of Biological Macromolecules 95, 153–159. |
| Ndeh D, Gilbert HJ. | 2018. Biochemistry of complex glycan depolymerisation by the human gut microbiota. | FEMS Microbiology Reviews 42, 146–164. |
| Parra-Rojas JP, Largo-Gosens A, Carrasco T, et al. | 2019. New steps in mucilage biosynthesis revealed by analysis of the transcriptome of the UDP-rhamnose/UDP-galactose transporter 2 mutant. | Journal of Experimental Botany 70, 5071–5088. |
| Ralet MC, Crépeau MJ, Vigouroux J, Tran J, Berger A, Sallé C, Granier F, Botran L, North HM. | 2016. Xylans provide the structural driving force for mucilage adhesion to the Arabidopsis seed coat. | Plant Physiology 171, 165–78. |
| Rautengarten C, Ebert B, Herter T, Petzold CJ, Ishii T, Mukhopadhyay A, Usadel B, Scheller HV. | 2011. The interconversion of UDP-arabinopyranose and UDP-arabinofuranose is indispensable for plant development in Arabidopsis. | The Plant Cell 23, 1373–1390. |
| Saee-Aguayo S, Parra-Rojas JP, Sepúlveda-Orellana P, et al. | 2021. Transport of UDP-rhamnose by URGT2, URGT4, and URGT6 modulates rhamnogalacturonan-I length. | Plant Physiology 185, 914–933. |
| Saee-Aguayo S, Rautengarten C, Temple H, et al. | 2017. UUAT1 is a Golgi-localized UDP-uronic acid transporter that modulates the polysaccharide composition of Arabidopsis seed mucilage. | The Plant Cell 29, 129–143. |
| Saee-Aguayo S, Rondeau-Mouro C, Macquet A, et al. | 2014. Local evolution of seed flotation in Arabidopsis. | PLoS Genetics 10, e1004221. |
| Šola K, Dean GH, Haughn GW. | 2018. Arabidopsis seed mucilage: a specialised extracellular matrix that demonstrates the structure–function versatility of cell wall polysaccharides. | Annual Plant Reviews online 2, 1085–1116. |
| Takenaka Y, Kato K, Ogawa-Ohishi M, et al. | 2018. Pectin RG-I rhamnosyltransferases represent a novel plant-specific glycosyltransferase family. | Nature Plants 4, 669–676. |
| Tan L, Eberhard S, Pattathil S, et al. | 2013. An Arabidopsis cell wall proteoglycan consists of pectin and arabinxyloylan covalently linked to an arabinogalactan protein. | The Plant Cell 25, 270–287. |
| Thomson P, Medina DA, Ortúzar V, Gotteländ M, Garrido D. | 2018. Anti-inflammatory effect of microbial consortia during the utilization of dietary polysaccharides. | Food Research International 109, 14–23. |
Voiniciuc C, Engle KA, Günl M, Dieluweit S, Schmidt MHW, Yang JY, Moremen KW, Mohnen D, Usadel B. 2018. Identification of key enzymes for pectin synthesis in seed mucilage. Plant Physiology 178, 1045–1064.

Voiniciuc C, Günl M, Schmidt MHW, Usadel B. 2015b. Highly branched xylan made by IRREGULAR XYLEM14 and MUCILAGE-RELATED21 links mucilage to Arabidopsis seeds. Plant Physiology 169, 2481–2495.

Voiniciuc C, Yang B, Schmidt MHW, Günl M, Usadel B. 2015a. Starting to gel: how Arabidopsis seed coat epidermal cells produce specialized secondary cell walls. International Journal of Molecular Sciences 16, 3452–3473.

Western TL, Burn J, Tan WL, Skinner DJ, Martin-McCaffrey L, Moffatt BA, Haughn GW. 2001. Isolation and characterization of mutants defective in seed coat mucilage secretory cell development in Arabidopsis. Plant Physiology 127, 998–1011.

Western TL, Young DS, Dean GH, Tan WL, Samuels AL, Haughn GW. 2004. MUCILAGE-MODIFIED4 encodes a putative pectin biosynthetic enzyme developmentally regulated by APETALA2, TRANSPARENT TESTA GLABRA1, and GLABRA2 in the Arabidopsis seed coat. Plant Physiology 134, 296–306.

Williams MA, Cornuault V, Irani AH, Symonds VV, Malmström J, An Y, Sims IM, Carnachan SM, Salié C, North HM. 2020. Polysaccharide structures in the outer mucilage of Arabidopsis seeds visualized by AFM. Biomacromolecules 21, 1450–1459.

Xu Y, Wang Y, Du J, et al. 2022. A DE1 BINDING FACTOR–GLABRA2 module regulates rhamnogalacturonan I biosynthesis in Arabidopsis seed coat mucilage. The Plant Cell 17, doi: 10.1093/plcell/koac011.

Zhang Y, Yin Q, Qin W, Gao H, Du J, Chen J, Li H, Zhou G, Wu H, Wu A-M. 2022. The Class II KNOX family members KNAT3 and KNAT7 redundantly participate in Arabidopsis seed coat mucilage biosynthesis. Journal of Experimental Botany 73, 3477–3495.