



Review article

Rhamnose in plants - from biosynthesis to diverse functions

Nan Jiang, Francisco M. Dillon, Alexander Silva, Lina Gomez-Cano, Erich Grotewold *

Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, 48824, USA

ARTICLE INFO

Keywords:

Cell wall
Pectin
Rhamnogalacturonan I
Specialized metabolite
Flavonol
Flavone
Anthocyanin
Glycosyltransferase

ABSTRACT

In plants, the deoxy sugar L-rhamnose is widely present as rhamnose-containing polymers in cell walls and as part of the decoration of various specialized metabolites. Here, we review the current knowledge on the distribution of rhamnose, highlighting the differences between what is known in dicotyledoneous compared to commelinid monocotyledoneous (grasses) plants. We discuss the biosynthesis and transport of UDP-rhamnose, as well as the transfer of rhamnose from UDP-rhamnose to various primary and specialized metabolites. This is carried out by rhamnosyltransferases, enzymes that can use a large variety of substrates. Some unique characteristics of rhamnose synthases, the multifunctional enzymes responsible for the conversion of UDP-glucose into UDP-rhamnose, are considered, particularly from the perspective of their ability to convert glucose present in flavonoids. Finally, we discuss how little is still known with regards to how plants rescue rhamnose from the many compounds to which it is linked, or how rhamnose is catabolized.

1. Introduction

L-Rhamnose (Rha), a deoxy monosaccharide, is widely distributed in bacteria and plants but rare in animals. Over the past two decades, an increasing number of Rha-containing molecules have been demonstrated to play critical roles in diverse pathogenic bacteria as well as exhibit various biological functions in plants [1]. Rha is primarily found in plants as part of cell walls and conjugated to specialized metabolites. In plant cell walls, Rha is required as a building block for synthesizing pectic polymers and cell wall glycoproteins. Pectin polymers are a group of structurally-complex polysaccharides that form a gel-like matrix for embedding cellulose microfibrils and hemicelluloses [2]. Pectins are characterized by their rich D-galacturonic acid (GalA) content, and consist of three major groups: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) [3]. HG, the most abundant group of pectins, is a linear homopolymer of α -(1,4)-linked GalA residues [4]. The second most abundant pectin polymer is RG-I, which has a backbone of the repeating disaccharide unit (1,4)- α -GalA-(1,2)- α -Rha (Fig. 1). Rha residues of the RG-I backbone can be decorated with oligosaccharides, including arabinans and galactans [5]. RG-II polymers have a backbone of α -(1,4)-linked GalA residues similar

to HG, but are substituted with at least four types of oligosaccharides, including three different sidechains with highly conserved Rha decoration patterns across plant species (Fig. 1) [5]. Arabinogalactan proteins (AGPs), a group of cell wall glycoproteins, have a hydroxyproline-rich core protein branched with side chains that are abundant in arabinose and galactose residues, as well as a few Rha residues (Fig. 1) [6].

Rha is also conjugated to specialized metabolites, often through the formation of acid-labile ether bonds, such as found in the flavone maysin [2'-O-rhamnosyl-6-C-(6-deoxy-xylo-hexos-4-ulosyl) luteolin] [7], in the anthocyanin lobelinin A (3-O-p-coumaroylrutinosyl-5-O-malonylglucosyl-3'/5'-O-dihydroxycinnamoylglucosyl delphinidin) [8], in the flavonol rutin (3-O-rutinosyl quercetin) [9], in the steroidal alkaloid khasianine [1-O-(rhamnosyl-4-O-glucosyl) solasodine] [10], in the triterpenoid saponin α -hederin [1-O-(rhamnosyl-1-2-O-arabinosyl) hederagenin] [11], and in several glycosides of long-chain fatty alcohols, such as 1-O-(rhamnosyl-2-O-glucosyl-3-O-rhamnosyl-6-O-glucosyl) hexadecanol [12], and in glycosides of short-chain unsaturated alcohols, such as the volatile 1-O-(rhamnosyl-1-6-O-glucosyl) cis-3-hexenol [13]. However, Rha conjugation can also happen through the formation of carbon-carbon bonds, like those present in the C-glycosylflavone violanthin (6-C-glucosyl-8-C-rhamnosyl

Abbreviations: Rha, L-rhamnose; Glc, D-glucose; Fuc, L-fucose; Man, D-mannose; GalA, galacturonic acid; UDP, uridine diphosphate; GDP, guanine diphosphate; RHM, rhamnose synthase; GT, glycosyltransferase; RhaT, rhamnosyltransferase; UDP-Rha, UDP-L-rhamnose; UDP-Glc, UDP-D-glucose; GDP-Man, GDP-D-mannose; GDP-Fuc, GDP-L-fucose; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; AGP, arabinogalactan protein; dTDP-Rha, deoxythymidine diphosphate L-rhamnose; dTDP-Glc, deoxythymidine diphosphate D-glucose; UDP-4K6DG, UDP-4-keto-6-deoxy-glucose; RIO, rhamnosylisoorientin.

* Corresponding author.

E-mail address: grotewol@msu.edu (E. Grotewold).

<https://doi.org/10.1016/j.plantsci.2020.110687>

Received 26 June 2020; Received in revised form 15 September 2020; Accepted 16 September 2020

Available online 14 October 2020

0168-9452/© 2020 Elsevier B.V. All rights reserved.

apigenin) [14] (Fig. 1). The C-C bonds are much more stable and resistant to acid hydrolysis, compared to the more typical ether bonds.

The incorporation of Rha residues into Rha-containing metabolites is catalyzed by glycosyltransferases, specifically rhamnosyltransferases, using the nucleotide diphosphate-sugar UDP-rhamnose (UDP-Rha) as a substrate [15]. In *Arabidopsis*, the total amount of Rha in leaves is distributed between soluble cellular components (UDP-Rha and Rha-containing specialized metabolites), water-soluble polymers (AGPs), and cell wall matrix polysaccharides (RG-I and RG-II). The majority of Rha (~682 µg/g fresh weight corresponding to ~90 % of the total Rha) is incorporated into cell wall matrix polysaccharides [16]. The distribution of Rha in cell wall extracts from various grasses (commelinid monocotyledons) and dicotyledonous plant tissues is summarized in Table 1. Rha accumulation in the cell wall of grasses is significantly smaller than the amount of Rha in the cell wall of dicots. The amount of Rha in soluble cellular components was determined to be

~75 µg/g fresh weight in *Arabidopsis*, but less (~15 µg/g fresh weight) was found to be present as conjugated to water-soluble polymers (e.g., AGPs) [16].

This review provides an overview and recent updates on the biosynthesis and transport of UDP-Rha, and the incorporation of Rha residues onto Rha-containing metabolites in plants. The diverse functions of Rha-containing metabolites in primary and specialized metabolism are discussed, as suggested from recent advances in the analysis of mutants, biochemical characterizations, and metabolic analyses.

2. UDP-rhamnose biosynthesis

2.1. Formation of UDP-rhamnose by rhamnose synthase

In plants, UDP-Rha biosynthesis results from the catalytic action of rhamnose synthase (RHM) using UDP-Glc as a substrate. The RHM

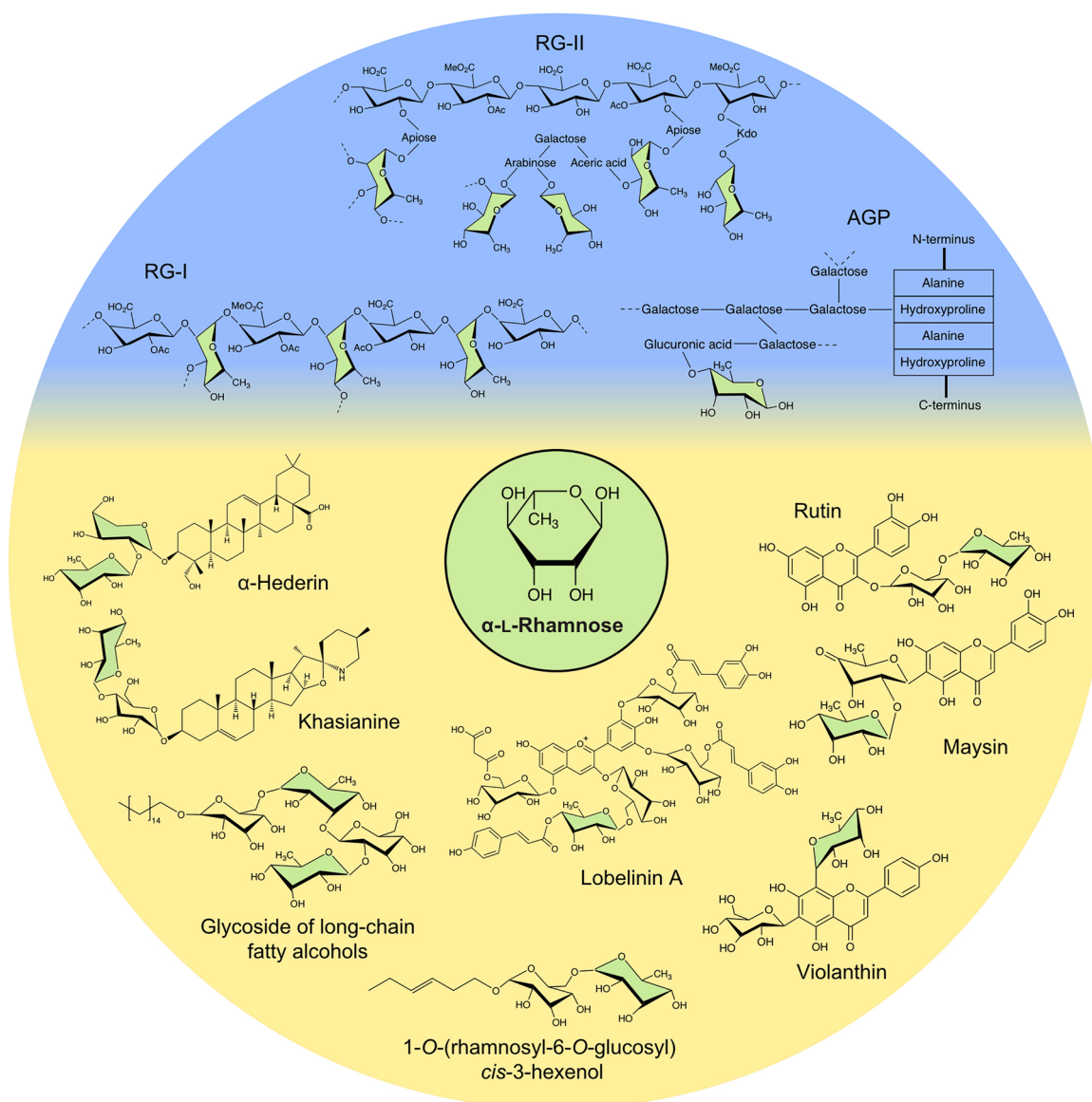


Fig. 1. Representative chemical structures of rhamnose-containing metabolites in plants. In primary metabolism (shaded blue), rhamnose residues are incorporated as part of the rhamnogalacturonan I (RG-I) backbone, or providing the sidechains decorations of rhamnogalacturonan II (RG-II) and arabinogalactan-proteins (AGPs). Rhamnose residues are also attached to various specialized metabolites (shaded yellow), including the C-glycosylflavone maysin and violanthin, the flavonol rutin, the anthocyanin lobelinin A, the triterpenoid saponin α-hederin, the steroidal alkaloid khasianine. α-L-Rhamnose and rhamnose residues are highlighted with light green color. Abbreviations: Kdo: 3-deoxy-d-manno-octulosonic acid; Ac: acetyl group; Me: methyl group.

Table 1

List of rhamnose composition in the cell walls from various grasses and dicotyledonous species.

Plant species and tissues	Rhamnose composition (per dry weight)		References
	µg/mg	Mol%	
Grasses (commelinid monocots)			
<i>Brachypodium distachyon</i> leaf, sheath, stem, and flowers/seed	~2.4-3.2 µg/mg		[17]
Rice (<i>Oryza sativa</i>) culm and internode	~2–3 µg/mg		[18,19]
Wheat (<i>Triticum aestivum</i>) straw	<1 µg/mg		[20]
Oat (<i>Avena sativa</i>) spelt	<1 µg/mg		[20]
Maize (<i>Zea mays</i>) mesophyll and epidermal tissues of coleoptiles	blank	~1.8-2.8 mol %	[21]
Switchgrass (<i>Panicum virgatum</i>) tiller		~0.3 mol%	[22]
Barley (<i>Hordeum vulgare</i>) coleoptiles		~0.7-1.4 mol %	[23]
Sorghum (<i>Sorghum bicolor</i>) leaf, leaf sheath, and stem	blank	~0.03-0.13 mol%	[24]
Dicots			
<i>Arabidopsis thaliana</i> leaf, stem, and root	~5–20 µg/mg		[18,25,26]
Hybrid poplar (<i>Populus deltoids</i> x <i>Populus nigra</i>) woody stems	~7–10 µg/mg		[18]
Alfalfa (<i>Medicago sativa</i>) leaves	~5.3–6.0 µg/mg		[27]
<i>Arabidopsis thaliana</i> petal, leaf, and embryo		~4.0–7.2 mol%	[28,29,30]
Soybean (<i>Glycine max</i>) hulls and cotyledons		~3.3–5.5 mol%	[31]

enzyme contains an N-terminal region with UDP-Glc 4,6-dehydratase activity to form the intermediate UDP-4-keto-6-deoxy-D-glucose (UDP-4K6DG) and a C-terminal domain with dual functional activities as UDP-4K6DG 3,5-epimerase and UDP-4-keto-L-rhamnose 4-keto-reductase, necessary to convert UDP-4K6DG into UDP-Rha [32]. In bacteria, the parallel reactions for deoxythymidine diphosphate rhamnose (dTDP-Rha) formation are catalyzed by three sequential enzymes, RmlB (or RfbB) with dTDP-Glc 4,6-dehydratase activity, RmlC (or RfbC) with dTDP-4K6DG 3,5-epimerase activity, and RmlD (or RfbD) with dTDP-4-keto-L-rhamnose 4-keto-reductase activity (Fig. 2A) [33]. An evolutionary analysis indicated that the plant RHM N-terminal region domain evolved from bacteria RmlB, while the RHM C-terminal region evolved from a fusion between RmlC and RmlD [34]. Therefore, the single plant RHM enzyme catalyzes reactions equivalent to the three bacterial enzymes (Fig. 2A).

In *Arabidopsis*, AtRHM1 (At1g78570), AtRHM2 (At1g53500), AtRHM3 (At3g14790), and AtUER1 (At1g63000) have been reported to be involved in UDP-Rha biosynthesis (Table 2) [35]. AtRHM1-3 showed UDP-Glc 4,6-dehydratase, UDP-4K6DG 3,5-epimerase, and UDP-4-keto-L-rhamnose 4-keto-reductase activities, when they were expressed in *Saccharomyces cerevisiae* [35]. In contrast, AtUER1 was shown to be a bifunctional enzyme that presents the dTDP/UDP-4K6DG 3,5-epimerase and dTDP/UDP-4-keto-L-rhamnose 4-keto-reductase activities, but lacks the UDP-Glc 4,6-dehydratase activity [36]. A functional domain analysis showed that the N-terminal region of AtRHM2 harbors the UDP-Glc 4,6-dehydratase activity, while the C-terminal region has the dual UDP-4K6DG 3,5-epimerase and UDP-4-keto-L-rhamnose 4-keto-reductase activities [35]. The Gly193 and Asp96 amino acids in AtRHM2 were identified as being important for the dehydratase activity, while Gly392 was shown to be critical for the epimerase/reductase activities [35]. The crystal structure of AtUER1 (PDB ID: 4QQR), along with site-directed mutagenesis experiments, demonstrated the importance of the Cys115 and Lys113 residues in the epimerase activity, as well as the significance of Thr113, Tyr144, and Lys148 residues in the reductase

activity [37].

The AtRHM2 mutants *mum4-1* and *mum4-2* (*MUCILAGE-MODIFIED4*) showed a ~50 % decrease in Rha and GalA content in *Arabidopsis* seed mucilage, which is a specialized cell wall polymer enriched in RG-I [38,39]. This defect was attributed to the absence of AtRHM2 activity as a result of amino acid mutations at Asp96 in *mum4-1* and Gly193 in *mum4-2* [35,39,40]. In contrast to the *mum4* mutants, AtRHM1 mutants exhibited several phenotypic defects, including short root hairs, hypostatic cotyledons, aberrant trichomes, and left handed helically-twisted petals and roots [41-43]. A genetic screening of suppressors of the *rhm1* mutant phenotype in *Arabidopsis* demonstrated that blocking flavonol biosynthesis by mutations of *CHS* (chalcone synthase), *FLS* (flavonol synthase), *MYB111* (positive regulator for flavonol accumulation), and *UGT89C1* (7-O flavonol rhamnosyltransferase) recovered the shoot phenotype of *rhm1* mutant [41-43]. These results were initially interpreted as related to the alleged effect of flavonols on auxin transport [42].

More recently, developmental defects of *Arabidopsis rhm1* mutants were identified in flowers as well. The defective growth of petals (helically twisted) was associated to the presence of reduced levels of RG-I in petal cell walls in the *rhm1* mutant [29]. Similar to what was found in seedlings, the phenotypes of *rhm1* flowers were suppressed by crossing the *tt4* (*CHS*) or *ugt89c1* mutants into *rhm1*. The fact that *rhm1 tt4* and *rhm1 ugt89c1* double mutants had increased Rha composition in cell walls of seedlings and flowers compared to the single *rhm1* mutant strongly supports the hypothesis that a common Rha pool is shared between cell wall components and flavonol rhamnosides [44]. Moreover, double mutants of *rhm1* and an enzyme involved in pectin biosynthesis (MUR1, a GDP-mannose dehydratase) enhanced the developmental defects in cotyledons of *rhm1* mutants [44]. Consequently, these two observations provide strong evidence that the suppression of the *rhm1* phenotypes was caused by diverting limiting UDP-Rha in *rhm1* mutants from the conjugation of flavonols to cell wall synthesis, specifically RG-I [44]. These findings highlight the importance of the quantitative aspects of Rha conjugation to flavonols and the link to potentially competitive processes between RG-I accumulation in the cell wall and flavonol rhamnoside biosynthesis. In *Arabidopsis* flowers, the amount of Rha conjugated to flavonols (~4.6 μg/mg dry weight) was found to be almost twice the amount of Rha involved in cell wall components (~2.5 μg/mg dry weight) [44]. One hypothesis put forward is that by directing UDP-Rha to flavonol conjugation, the plants are buffering fluctuations in UDP-Rha synthesis, while maintaining the proper rate of cell wall expansion [44]. However, no mechanism was proposed by which Rha conjugated to flavonols could be recycled for the incorporation into the cell walls in times of need for RG-I biosynthesis. It is also unclear whether this competition between conjugation of Rha to specialized metabolites and its utilization in cell wall formation is unique to *Arabidopsis* (and perhaps other dicots), or is present in monocots as well.

To date, no *Arabidopsis rhm3* single mutant has been described. Expression pattern analyses indicated that AtRHM3 is expressed in all tissues with a preponderance in roots and flowers [45]. It would be interesting to determine the phenotypes of *rhm1 mum4* double mutant and *rhm1 mum4 rhm3* triple mutant to understand the contributions of AtRHM3 to UDP-Rha biosynthesis.

The biosynthesis of UDP-Rha has also been reported in other plants, such as *Populus x canadensis* Moench (Carolina poplar) by a PRHM enzyme (an ortholog of AtRHM1 and AtRHM2) and *Camellia sinensis* (tea), plant in which three CsRHMa/b/c proteins have RHM activity (Table 2) [46,47]. However, UDP-Rha biosynthesis in monocots has not been extensively explored yet, with a few exceptions. *Ornithogalum caudatum* (False onion) has two genes, *OcrhS1* and *OcUER1*, encoding a trifunctional RHM and a bifunctional UDP-4K6DG 3, 5-epimerase/UDP-4-keto-L-rhamnose 4-keto-reductase, respectively (Table 2) [48]. *OcrhS1*, a multidomain protein with two sets of cofactor-binding motifs, was shown to convert UDP-Glc into UDP-Rha, similar as AtRHM1

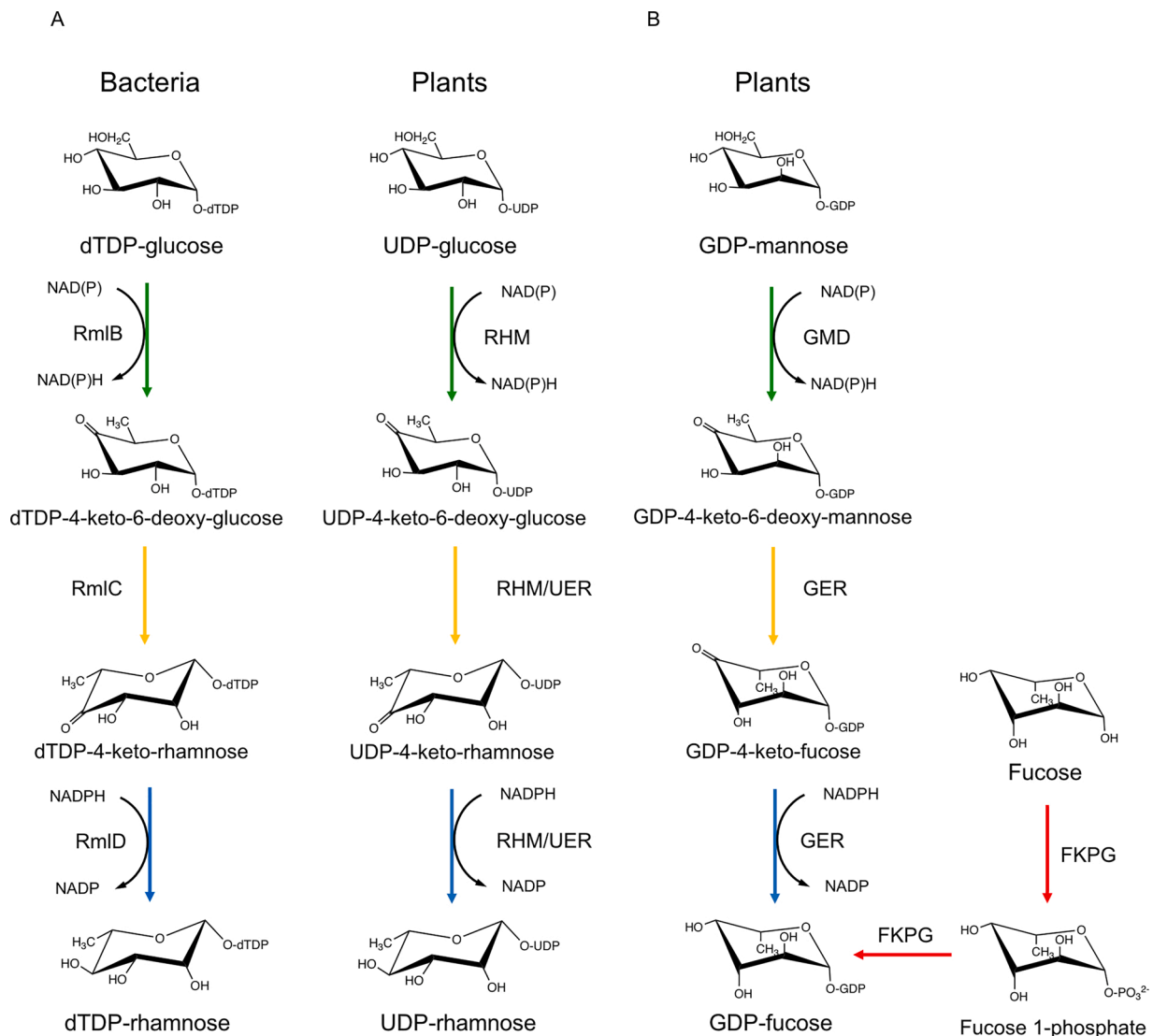


Fig. 2. Biosynthetic pathways for dTDP-rhamnose in bacteria and UDP-rhamnose in plants, and comparison with plant GDP-fucose biosynthesis. A) In bacteria, dTDP-rhamnose biosynthesis occurs from dTDP-glucose by three sequential enzymes: dTDP-Glc 4,6-dehydratase (RmlB), dTDP-4K6DG 3,5-epimerase (RmlC), and dTDP-4-keto-rhamnose 4-keto-reductase (RmlD). In plants, UDP-rhamnose derives from UDP-glucose by the action of a trifunctional rhamnose synthase (RHM). The last two steps can be conducted by a bifunctional UER. B) In plants, GDP-mannose is converted to GDP-fucose through dehydration by GMD (GDP-mannose dehydratase), and epimerization and reduction by GER (bifunctional epimerase-reductase). The alternative GDP-fucose biosynthetic pathway is catalyzed by FKGP (bifunctional L-fucokinase/GDP-L-Fuc pyrophosphorylase) with the intermediate fucose 1-phosphate. The process of dehydration, epimerization, reduction, and phosphorylation/pyrophosphorylation process is represented by green, yellow, blue, and red arrows, respectively.

and AtrHM2. *OcUER1* encodes a protein that shares high similarity with the C-terminal domain of *OcRhS1*, which has the ability to convert UDP-4K6DG into UDP-Rha, equivalent to *AtUER1* [48]. Thus, UDP-Rha production in False onion is mediated by either a trifunctional or bifunctional enzyme using two different substrates, UDP-Glc or UDP-4K6DG, respectively. However, the contribution of each substrate to UDP-Rha biosynthesis remains unknown.

In maize, at least one RHM is able to use specialized metabolites as substrates, while retaining the ability to convert UDP-Glc to UDP-Rha. *ZmRHM1* was shown to use the flavonoid-glucoside rhamnosylisoorientin (RIO) as a substrate, converting it into the C-glycosylflavone maysin, which confers natural resistance to *Helicoverpa zea* (corn earworm) (Fig. 1) [7]. The maize *salmon silk 1* (*sm1*) mutant is defective in *ZmRHM1*, resulting in the accumulation of 3-deoxyflavonoids and RIO in silks (stigma and style in the female flower). Interestingly, maysin biosynthesis involved only the dehydratase activity of *ZmRHM1*, converting the Glc group in RIO to 4K6DG without further epimerization

and reduction. It has not been established why the conversion of Glc stops after the dehydration step to produce maysin, even though *ZmRHM1* was able to convert UDP-Glc into UDP-Rha *in vitro* [7]. One hypothesis is that the flavone backbone of the substrate (RIO) blocks the epimerase/reductase activity through spatial interfering to prevent flipping the sugar ring of deoxy-glucose [37]. The additional Rha residue attached to position 2' in maysin has been proposed to inhibit the epimerase and reductase activities of *ZmRHM1*, suggesting a partial activity of RHM when the substrate is a C-glycosylflavone instead of a nucleotide sugar [7]. It is currently unknown whether this ability to use Glc attached to a flavonoid (flavone) as a substrate is a unique property of *ZmRHM1*, or whether other RHMs can do the conversion to 4K6DG as well. It will also be interesting to determine if *ZmRHM1* could be active on other specialized metabolites, given that Glc is one of the most common decorations. *ZmRHM1* provides one interesting case of an enzyme used in primary metabolism being recruited to perform a rather atypical reaction in specialized metabolism.

Table 2

Plant genes involved in UDP-Rha biosynthesis, transport, and conjugation. RHM: rhamnose synthase; RhaT: rhamnosyltransferase.

Protein	Accession No.	Species	Activity	Phenotype of Mutant	Reference
AtRHM1	At1g78570	<i>Arabidopsis thaliana</i>	RHM	Reduced rhamnosylated flavonols, RGI and RGII	[28,35]
AtRHM2/ MUM4	At1g53500	<i>Arabidopsis thaliana</i>	RHM	Reduced seed mucilage	[34]
AtRHM3	At3g14790	<i>Arabidopsis thaliana</i>	RHM	Not tested	[35]
AtUER1	At1g63000	<i>Arabidopsis thaliana</i>	dTDP/UDP-4K6DG 3,5-epimerase dTDP/UDP-4-keto-L-rhamnose 4-keto-reductase	Not tested	[36]
PRHM	TC158686	<i>Populus x canadensis</i> (Carolina poplar)	RHM	No mutant	[46]
CsRHMa	KY679572	<i>Camellia sinensis</i> (Tea)	RHM	No mutant	[47]
CsRHMb	KY679573	<i>Camellia sinensis</i> (Tea)	RHM	No mutant	[47]
CsRHMc	KY679574	<i>Camellia sinensis</i> (Tea)	RHM	No mutant	[47]
OcRhS1	ANK57460	<i>Ornithogalum caudatum</i> (False Onion)	RHM	No mutant	[48]
OcUER1	ANK57461	<i>Ornithogalum caudatum</i> (False Onion)	UDP-4K6DG 3,5-epimerase; UDP-4-keto-L-rhamnose 4-keto-reductase	No mutant	[48]
ZmRHM1/ SM1	AQK84802	<i>Zea mays</i> (Maize)	RHM	Salmon color in silks	[7]
AtURGT1	At1g76670	<i>Arabidopsis thaliana</i>	UDP-Rha transporter	Reduced seed mucilage	[67]
AtURGT2	At1g21070	<i>Arabidopsis thaliana</i>	UDP-Rha transporter	Reduced seed mucilage	[67]
AtURGT3	At5g42420	<i>Arabidopsis thaliana</i>		Not tested	[67]
AtURGT4	At4g39390	<i>Arabidopsis thaliana</i>		Not tested	[67]
AtURGT5	At4g09810	<i>Arabidopsis thaliana</i>	UDP-Rha transporter	Not tested	[67]
AtURGT6	At1g34020	<i>Arabidopsis thaliana</i>		Not tested	[67]
AtRRT1	At5g15740	<i>Arabidopsis thaliana</i>	RhaT	Slight reduced seed mucilage	[68]
AtUGT78D1	At1g30530	<i>Arabidopsis thaliana</i>	RhaT	Absence of 3-O flavonol rhamnosides	[72]
AtUGT89C1	At1g06000	<i>Arabidopsis thaliana</i>	RhaT	Absence of 7-O flavonol rhamnosides	[73]
AtUGT79B2	At4g27560	<i>Arabidopsis thaliana</i>	RhaT	Reduced anthocyanins in double mutant <i>ugt79b2/b3</i>	[80]
AtUGT79B3	At4g27570	<i>Arabidopsis thaliana</i>	RhaT		[80]
ZmUGT91L1/ SM2	ONM23883	<i>Zea mays</i> (Maize)	RhaT	Salmon color in silks	[7]
SoUGT79K1/ SOAP7	KNA23861.1	<i>Spinacia oleracea</i> (spinach)	RhaT	No mutant	[58]
LeABRT2	LC131336	<i>Lobelia erinus</i> (garden lobelia)	RhaT	Absence of lobelinins and flower mauve-colored	[8]
LeABRT4	LC131337	<i>Lobelia erinus</i> (garden lobelia)	RhaT	Absence of lobelinins and flower mauve-colored	[8]
UGT77B2	A0A2Z5CVA1	<i>Crocodylus x crocodymiflora</i> (montbretia)	RhaT	No mutant	[79]
Cm1,2RhaT	Q8GVE3	<i>Citrus maxima</i> (Pomelo)	RhaT	No mutant	[82]
Cs1,6RhaT	ABA18631	<i>Citrus sinensis</i> (Sweet orange)	RhaT	No mutant	[84]
StSGT3	ABB84472	<i>Solanum tuberosum</i> (Potato)	RhaT	No mutant	[86]
Ph3RT	Q43716	<i>Petunia hybrida</i> (Petunia)	RhaT	Reduced magenta or blue/purple coloured anthocyanins	[81]
GmUGT79A6	BAN91401	<i>Glycine max</i> (soybean)	RhaT	No mutant	[102]

2.2. Transcriptional regulation of rhamnose synthase genes

Although *in vitro* activity assays showed that both AtRHM1 and AtRHM2 catalyze the conversion of UDP-Glc to UDP-Rha, it remains to be explained why so different phenotypes are observed in the respective mutants. The variations on transcriptional regulation of different RHMs provide a possible explanation. Several transcription factors involved in seed mucilage formation and other developmental processes positively regulate *AtRHM2*, including AP2 (APETALA2), TTG1 (TRANSPARENT TESTA GLABRA1), and GL2 (GLABRA2) [39]. *AtRHM1* expression is higher in *Arabidopsis* seedlings and inflorescences, which is consistent with the elevated content of RG-I and RG-II in these tissues [49]. The expression pattern of *AtRHM1* correlated well with the expression of the flavonol rhamnosyltransferases (RhaT) *UGT89C1* and *UGT78D1*, while the correlation was much lower with the flavonol glucosyltransferase *UGT78D2* and *UGT78D3* [50]. Accordingly, the expression of *UGT89C1* was enhanced by UV radiation [51] and plants increased the accumulation of several di- or tri-glycosylated flavonols that include a shared 7-O-rhamnosylation during UV-B acclimation [52]. The preferential production of flavonol rhamnosides over flavonol glucosides during UV-B acclimation is something that has not yet been explained, given

that it is difficult to imagine that the different sugar decoration will provide different UV-shielding properties to the respective compounds. The tissue-specific and subcellular localization of flavonoids can affect their activity as antioxidants or sunscreens [53]. However, we are not aware of any research that has examined the differential flavonoid transport and/or stability depending on the presence/absence of Rha decorations, particularly when compared with other decorations such as glucose, galactose or arabinose. The R2R3-MYB transcriptional factors AtMYB11, AtMYB12, and AtMYB112 positively regulate most of the genes involved in the flavonol biosynthetic pathway in response to UV-B [50,54]. However, *AtRHM1* expression was not affected by any of these MYBs [50,54]. To our knowledge, no transcription factor has been yet associated with *AtRHM1* expression.

The C-glycosylflavone RIO has been shown to accumulate at high levels in leaves of maize landraces when exposed to UV-B [55]. *Pericarp Color1* (*P1*) encodes an R2R3-MYB TF that regulates the transcription of genes encoding enzymes involved in the C-glycosylflavone RIO and maysin biosynthesis, including *SM2* (*UGT91L1*) and *SM1* (*ZmRHM1*) [7]. Even though *P1* has been reported to be expressed exclusively in reproductive organs [56], several maize landraces adapted to grow in high altitudes also express *P1* in leaves when exposed to UV-B [57]. This

may suggest that during maize evolution/domestication, alleles of *P1* expressing primarily in floral organs might have been selected, with the UV-B induction in leaves remaining as a memory of a much broader *P1* expression pattern.

2.3. Comparison between UDP-rhamnose and GDP-fucose biosynthesis

Similar to Rha, Fucose (Fuc) is a deoxyhexose used to decorate sidechains of AGPs and RG-II in plant cell walls. Fuc is also present in xyloglucans and N-linked glycans, polysaccharides on which Rha has not been detected. In contrast to Rha, Fuc has been only rarely reported as attached to specialized metabolites, one example provided by the triterpenoid saponins (Yossoside I-V) [58].

The biosynthesis of GDP-fucose (GDP-Fuc) is conceptually analogous to the formation of UDP-Rha, and requires similar dehydration, epimerization, and reduction steps (Fig. 2B). Unlike UDP-Rha formation by the trifunctional RHM enzyme, GDP-Fuc biosynthesis is carried out by the MUR1/GMD2 or GMD1 enzymes (dehydration) and a second enzyme GER1 (epimerization and reduction) [59,60]. Different to Rha biosynthesis, there is a salvage pathway for cytosolic free Fuc, which consists of a phosphorylation and subsequent conjugation with GDP (Fig. 2B) [61]. Actually, UDP-Rha is one of the few UDP-sugars in plants for which no phosphorylation reaction has been reported (Fig. 2B) [62]. Another important difference between the biosynthesis of UDP-Rha and GDP-Fuc is that RHM uses UDP-Glc as substrate while GDP-Fuc is converted from GDP-mannose (GDP-Man). UDP-Glc is the precursor for UDP-Rha, UDP-Gal, and UDP-GalA (the precursor of UDP-pentoses), while GDP-Man, besides being the precursor for GDP-Fuc, is used for ascorbate biosynthesis [63]. Thus, RHM is able to use a much more common and abundant substrate, since the concentration of UDP-Glc is ~42 pmol/mg fresh weight in *Arabidopsis* leaf, compared with ~0.5 pmol/mg fresh weight of GDP-Man [64]. Given the findings with ZmRHM1 that was shown to use Glc coupled to a flavonoid as a substrate, it will be interesting to explore whether MUR1/GMD2 or GMD1 can similarly use Man residues attached to specialized metabolites as substrates to convert Man residues for example to 4-keto-6-deoxy-mannose.

3. UDP-rhamnose transporters

The nucleotide-sugar transporters (NST) play important roles in supplying sugar donors, primarily nucleotide sugars, to the glycosyltransferases for the biosynthesis of cell wall matrix and the formation of glycoconjugates. These nucleotide sugars are translocated by NSTs from the cytosol, where they are synthesized, across the Golgi apparatus membrane and into the Golgi lumen [65,66]. In *Arabidopsis*, a family of six bifunctional UDP-Rha/UDP-Gal transporters (AtURGT1-6, Table 2) belonging to NST-KT (highly conserved KT motif) subfamily was described [67]. All six URGTs were able to transport UDP-Rha with different preferences as well as UDP-Gal. URGT1 showed preference for UDP-Rha over UDP-Gal and, among all the six URGTs, presented the highest expression level during *Arabidopsis* development. The loss-of-function *urgt1* mutants showed a slight alteration in Gal content in leaf cell wall composition, but no change in the amount of Rha. Compared to wild type, the *urgt1* mutants did not show any obvious morphological differences [67]. AtURGT2 has a more specific expression in the seed coat during seed development. Consistent with this, *urgt2* mutants had reduced and altered seed mucilage patterns that were probably caused by the transport deficiency of UDP-Rha, impacting RG-I biosynthesis [67]. It would be interesting to investigate the roles of the remaining transporters. Given that the conjugation of Rha to specialized metabolites takes place in the cytoplasm, it will also be interesting to determine if such decorations take advantage of a transitory cytoplasmic UDP-Rha pool, or whether two distinct pools are available, one for the formation of polysaccharides in the Golgi, and another for specialized metabolite conjugation. However, the findings in *Arabidopsis* with the *rhm1* mutants [44], suggest that, most likely, the first mechanism is at

play (i.e., sharing a UDP-Rha pool).

4. Formation of rhamnose-conjugated metabolites by rhamnosyltransferases

4.1. Elongation of the RG-I backbone by RG-I-rhamnosyltransferase

The Golgi lumen-imported UDP-Rha is eventually incorporated into cell wall components through a process called glycosylation by the rhamnosyltransferase (RhaT) enzymes. In 2018, a novel plant-specific GT family (GT106) was identified and characterized in *Arabidopsis* corresponding to RG-I rhamnosyltransferases (AtRRTs), which catalyze a critical step of RG-I biosynthesis by extending the repeating disaccharide unit of the RG-I backbone in seed mucilage [68]. Except for the slight RG-I reduction in the mucilage content of *rrt1* mutant plants, none of the other growth or morphological defects characteristic of the *rhm1* or *rhm2* mutants were observed in *rrt1*. The results suggested that AtRRT1 is a seed coat mucilage-specific RhaT required for the backbone elongation of RG-I. Whether RhaT is involved in the initiation of RG-I backbone or in RG-I biosynthesis in other tissues remains to be identified. RRT activity has also been reported in azuki bean (*Vigna angularis* 'Erimo-wase'), and VaRRT activity was characterized in an *in vitro* assay using epicotyl extracts in which the enzyme was able to elongate RG-I concomitant with epicotyl growth, suggesting a role in plant growth [69].

4.2. Rhamnose conjugation to specialized metabolites

Glycosylation of small hydrophobic molecules (e.g., many specialized metabolites) in plant cells has multiple consequences, including an increase in hydrophilicity, stability, and vacuolar sequestration [70,71]. In addition to their involvement in the biosynthesis of cell wall components, several RhaTs have been reported in various plant species as targeting different types of specialized metabolites. As a consequence of the conjugation to specialized metabolites, Rha is commonly forming O-glycosidic bonds with flavonoids as well as some other type of compounds, including steroidal alkaloids [10] and triterpenoid saponins [11]. There are a few exceptions of Rha C-linked to flavones (C-C bonds) [14]. In *Arabidopsis*, the flavonol-3-O-RhaT UGT78D1 catalyzed the transfer of Rha to the 3-OH position of quercetin and kaempferol [72], and 7-O-RhaT UGT89C1 catalyzed the conversion of flavonols to flavonol 7-O-rhamnosides [73]. Flavonol rhamnosides play an important role in plant development, as described in a previous section.

Sugar decorations can affect the biological activity of flavonoid glycosides. For example, quercetin 3-O-glucoside has been reported as an antioxidant flavonol, whereas either quercetin 3-O-rhamnoside or 7-O-rhamnoside exhibited antiviral activity [74,75]. However, it has not been examined whether quercetin decorated with Rha has the same effect as quercetin glucoside. Kaempferol di-rhamnoside in *Arabidopsis* leaves confer resistance to a specialist lepidopteran larva [76], while kaempferol with other sugar decorations have not been so far reported as similarly bioactive. In addition, an increasing amount of kaempferol 3-O-rhamnoside-7-O-rhamnoside in *Arabidopsis* 3-O-glucosyltransferase *ugt78d2* mutant has been associated with reduced levels of endogenous auxin and basipetal auxin transport in shoots, which indicates that this kaempferol di-rhamnoside plays an important role in the inhibition of auxin transport [77]. Some flavonol glycosides have shown pharmacological activities, including Montbretin A (MbA), a flavonol glycoside used as a candidate to treat type-II diabetes due to its inhibition of human pancreatic α -amylase [78]. In the ornamental plant montbretia (*Crocsmia x crocosmiiflora*), a myricetin 3-O-rhamnosyltransferase UGT77B2 participates in the biosynthesis of MbA [79]. In *Arabidopsis*, the glycosyltransferases UGT79B2 and UGT79B3 are characterized as the anthocyanin RhaTs, which transfer Rha residue to cyanidin and cyanidin 3-O-glucoside. The RhaT UGT79B2 and UGT79B3 are involved in tolerance to low temperature and salt stress. The overexpression of

UGT79B2/B3 in *Arabidopsis* was shown to increase anthocyanin accumulation and enhance ROS scavenging [80].

Studies on other RhaTs have shown that the rhamnosylation of anthocyanins affects flower colors. For instance, in garden lobelia (*Lobelia erinus*), two RhaTs (ABRT2 and ABRT4) are involved in the rhamnosylation of delphinidin 3-O-glucoside to form the lobelinins, the anthocyanins responsible for the blue color in the Aqua Blue cultivar. In contrast, in the Aqua Lavender cultivar, truncated versions of ABRT2 and ABRT4 are responsible for the flower color changes from blue to mauve-colored [8]. In petunia (*Petunia hybrida*), the *rt* gene encodes a RhaT that is involved in the rhamnosylation of anthocyanin-3-O-glucosides to produce magenta or blue/purple anthocyanins [81].

Different flavanone glycoside compositions determine aspects of the flavor in citrus fruits. The biosynthesis of the bitter metabolites neohesperidin and naringin, which are mostly present in pomelo and grapefruit, requires 1,2-RhaT (Fig. 3) [82]. In contrast, the formation of the tasteless flavanone-7-O-rutinosides hesperidin and narirutin, characteristic of less bitter citrus fruits such as mandarins and oranges, is catalyzed by a 1,6-RhaT (Fig. 3) [83]. In citrus fruits, flavanone

glycosides play a role as antifungal agents, which is consistent with the high levels of these compounds in young tissues and lower concentrations in mature tissues [84]. Further works will be necessary to determine other roles that these compounds may play in citrus species.

In maize, SM2 has RhaT activity and catalyzes the conversion of the C-glycosylflavone isoorientin to RIO, the penultimate step in the biosynthesis of maysin in silks (Fig. 1) [7]. It is not clear whether the Rha residue in maysin plays a significant role on the corn earworm anti-feedant properties of this compound. Indeed, isoorientin appears to be equally effective against corn earworm, when compared to maysin [85], suggesting that the presence of Rha is not making maysin more biologically active. Rha is also critical for the biosynthesis of steroidal glycoalkaloids, which are involved in microbial and insect pests defense in some solanaceous species, such as potato and tomato. The RhaT SGT3 in potato (*Solanum tuberosum*) has been shown to catalyze the final step of α -chaconine and α -solanine biosynthesis [86]. A question that remains is why Rha is used as a decoration, instead of more abundantly distributed Glc groups.

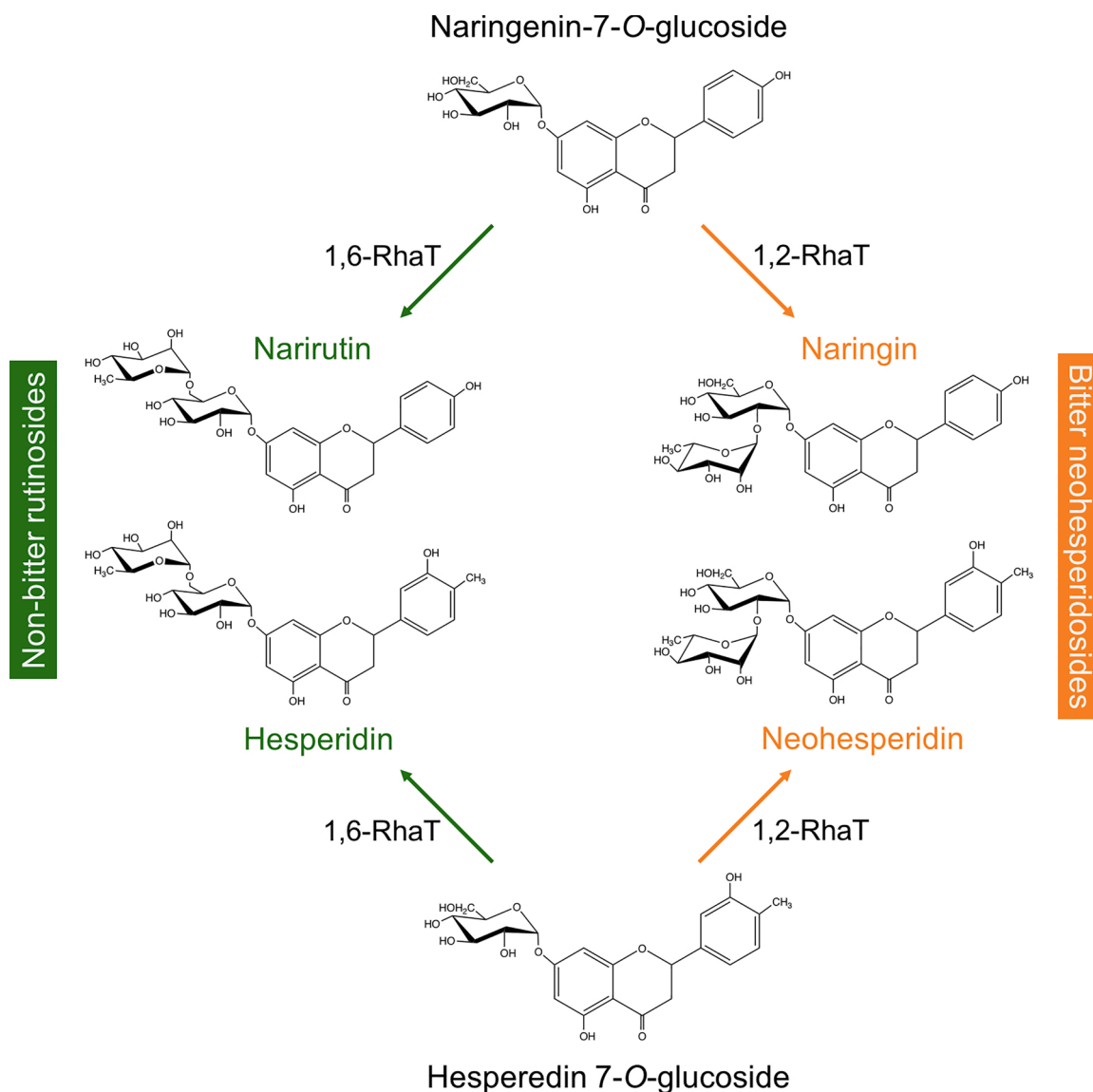


Fig. 3. Biosynthesis of *O*-rutinosides or *O*-neohesperidosides in citrus fruits. Neohesperidin and naringin biosynthesis are mediated by a 1,2-RhaT and these compounds are mostly present in bitter citrus plants such as pomelo. In contrast, the biosynthesis of hesperidin and narirutin is catalyzed by a 1,6-RhaT, which is mostly present in non-bitter species such as mandarin. RhaT, rhamnosyltransferase.

4.3. Sugar specificity of rhamnosyltransferases

The amino acid residues in RhaT determining the Rha-specific recognition have not been completely revealed. Conserved residues that interact with the UDP portion of the UDP-sugar substrate and the two phosphates in the sugar donor-binding pocket of GTs have been identified as important from crystal structures [71]. The recent crystallization of RhaT UGT89C1 showed that the amino acid residues Trp335, Asp356, and His357 located in the PSPG (Plant Secondary Product Glycosyltransferase) motif play a key role in determining sugar specificity through direct interactions with the Rha ring [87]. In contrast to the previous results, Asp356 is not unique to RhaT, but is also present in some xyloxytransferases, arabinosyltransferases, and galactosyltransferases [71]. To better understand whether the Trp335, Asp356, and His357 residues are conserved among RhaTs from different plants, the amino acid sequences of characterized RhaTs were compared. The alignment results indicated that Asp356 is conserved in all RhaTs except *Cm1,2RhaT*; Trp335 is conserved in six RhaTs, and His357 is only present in UGT89C1 and SM2 (Fig. 4). Other residues such as His332, Gly334, Glu340, and Pro352 in UGT89C1 are conserved in all RhaTs, but also in other glycosyltransferases. Currently, no residue is found to be specific for RhaT, suggesting that Rha specific recognition involves structural folds that go beyond just conserved residues. The crystallization of other RhaTs will allow us to get a better understanding of the sugar specificity.

5. Conclusions and future prospects

As we have seen in this review, Rha-containing metabolites resulting from the incorporation of Rha onto metabolites from primary and specialized metabolism, have diverse functions in plants. These include pectin polymers (RG-I and RG-II) that contribute to keeping the integrity of plant cell walls, anthocyanin rhamnosides that determine petal colors, or flavanone rhamnosides that affect organoleptic properties of citrus fruits. Despite the progress made in understanding the biosynthesis of Rha-containing metabolites, instances of Rha catabolism and derhamnosylation from Rha-containing metabolites in plants have been rarely described. In bacteria, Rha is isomerized, phosphorylated, and cleaved into a common triose phosphate plus L-lactaldehyde [88]. Given that L-lactaldehyde has not been reported in plants, it is likely that Rha breakdown in plants is different from Rha catabolism in bacteria [89]. For the derhamnosylation of Rha-containing natural products, α -L-rhamnosidase catalyzes the cleavage of terminal Rha residues, as determined for bacteria and fungi [90–95]. Until now, studies on plant α -L-rhamnosidase are limited to reports 45 years ago in buckthorns and buckwheat [96–98]. It will be interesting to determine how Rha residues are removed from Rha-containing metabolites in plants. Is the plant derhamnosylation process similar as in fungi and bacteria, involving α -L-rhamnosidases? Could the cleaved Rha be recycled and use for incorporation into other specialized metabolites or cell wall components?

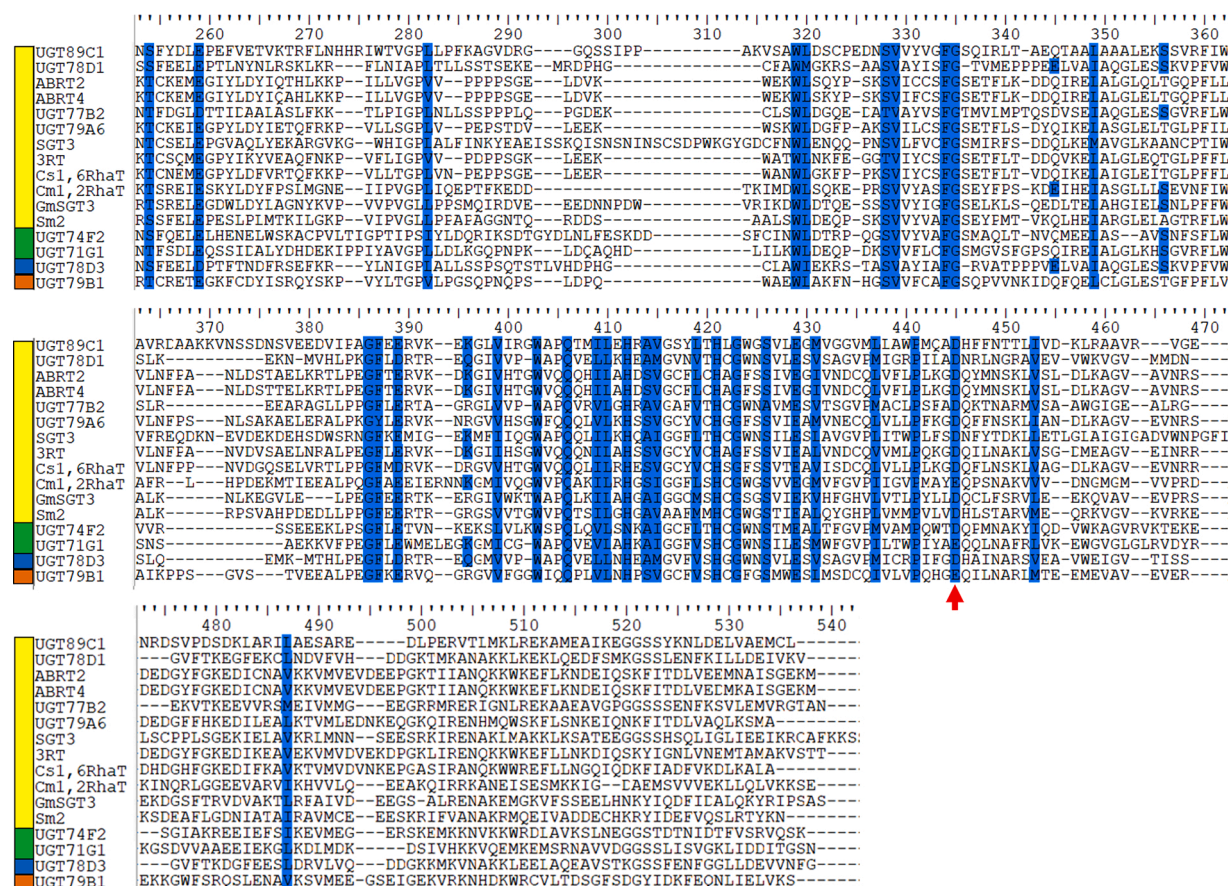


Fig. 4. Amino acid sequence alignment of glycosyltransferases from different plant species. From UGT89C1 to SM2 correspond to rhamnosyltransferases (RhaTs, indicated with the yellow bar on the left); UGT74F2 and UGT71G1 correspond to *Arabidopsis* and *Medicago truncatula* O-glucosyltransferases, respectively (indicated by the green bar); UGT78D3 corresponds to an *Arabidopsis* arabinosyltransferase (indicated with the blue bar); and UGT79B1 corresponds to an *Arabidopsis* xyloxytransferase (indicated with the orange bar). The amino acid residue Asp356 (red arrow) in UGT89C1 has been reported to play a key role for substrate specificity and is conserved in all the RhaTs except in 1,2RhaT from *Citrus maxima*, as well as in UGT74F2 and UGT78D3. Residues conserved in almost all the RhaTs and other glycosyltransferases are highlighted in blue.

Another interesting question pertains to how Rha flux into primary and specialized metabolites is controlled in commelinid monocots and dicots. As shown in Table 1, Rha quantities in cell walls of grasses are significantly less than in the cell walls of dicots, with the consequence that pectins in the cell walls of grasses are 4–7 times lower than in dicots [99]. However, recent transcriptomic and proteomic analyses showed no significant differences on gene transcript abundances and the translated levels of relevant enzymes for pectin biosynthesis between maize and *Arabidopsis* [100,101]. These results suggest that the presence of less pectins in maize cell walls compared with *Arabidopsis* is determined by mechanisms related to the metabolism or transport of pectin polymers, but probably not to pectin biosynthesis itself. In *Arabidopsis*, Rha could be partitioned for incorporation into pectin polymers and Rha-decorated flavonols [46]. Thus, Rha residues that would normally accumulate as part of pectin polymers that are not deposited into the cell walls could be recycled and then incorporated with flavonols as flavonol rhamnosides. It will be important to determine and compare the amounts of Rha in Rha-decorated flavonols and in cell walls between the grasses and the dicots.

Recently, a mechanism was revealed for the recruitment of an enzyme involved in cell wall biosynthesis to catalyze sugar decorations in the aglycone backbone of specialized metabolites. In spinach (*Spinacia oleracea*), SOAP5 belongs to the cellulose synthase-like G (CslG) clade in the cellulose synthase (CesA) superfamily that is related to cell wall assembly. Yet, SOAP5 was shown to have glucuronosyltransferase activity, with the ability to transfer glucuronic acid to the aglycone core of triterpenoid saponins, even though SOAP5 has less than 10 % amino acid similarity with other canonical glucuronosyltransferases [58]. Thus, it will be interesting to investigate the possibility that the capacity to conjugate Rha to specialized metabolites for enzymes annotated as participating in cell wall biosynthesis, particularly those in the cellulose synthase-like family.

Finally, the role of Rha at different plant developmental stages has yet to be fully elucidated. Many rhamnosylated compounds have protective effects against biotic (fungi and insects) and abiotic (e.g., UV radiation) stress conditions. The identification of the organs and stages where the genes implicated in the biosynthesis of Rha and related compounds are highly expressed will be useful to predict the more critical periods during plant development when Rha is required. For example, the soybean (*Glycine max*) RhaT UGT79A6 is expressed mostly in young leaves, and lower in flowers, pods and pod shells, but not in seeds and roots [102]. According to the Maize Genetics and Genomics Database (<https://www.maizegdb.org/>), the RhaT SM2 and the RHM SM1 are primarily expressed in silks participating in maysin biosynthesis, but also in the leaves of young maize plants, suggesting a yet-unknown function for Rha or Rha-derived metabolites in young maize plants. In *Arabidopsis*, the RhaT UGT78D1 is highly expressed in young leaves, mature flowers, and seeds. Taken together, these results indicate that compounds decorated with Rha could play a key role in early stages and in reproductive organs.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

The authors acknowledge financial support provided by the National Science Foundation grant IOS-1733633 and by the Agriculture and Food Research Initiative competitive USDA National Institute of Food and Agriculture grant #2018-67013-27424.

References

- [1] M.F. Giraud, J.H. Naismith, The rhamnose pathway, *Curr. Opin. Struct. Biol.* 10 (2000) 687–696.
- [2] N.C. Carpita, D.M. Gibeaut, Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth, *Plant J.* 3 (1993) 1–30.
- [3] D. Mohnen, Pectin structure and biosynthesis, *Curr. Opin. Plant Biol.* 11 (2008) 266–277.
- [4] K.H. Caffall, D. Mohnen, The structure, function, and biosynthesis of plant cell wall pectic polysaccharides, *Carbohydr. Res.* 344 (2009) 1879–1900.
- [5] M.A. Atmodjo, Z. Hao, D. Mohnen, Evolving views of pectin biosynthesis, *Annu. Rev. Plant Biol.* 64 (2013) 747–779.
- [6] G.J. Seifert, K. Roberts, The biology of arabinogalactan proteins, *Annu. Rev. Plant Biol.* 58 (2007) 137–161.
- [7] M.I. Casas, et al., Identification and characterization of maize *salmon silks* genes involved in insecticidal maysin biosynthesis, *Plant Cell* 28 (2016) 1297–1309.
- [8] Y.H. Hsu, et al., Functional characterization of UDP-rhamnose-dependent rhamnosyltransferase involved in anthocyanin modification, a key enzyme determining blue coloration in *Lobelia erinus*, *Plant J.* 89 (2017) 325–337.
- [9] K. Slámová, J. Kapešová, K. Valentová, "Sweet flavonoids": glycosidase-catalyzed modifications, *Int. J. Mol. Sci.* 19 (2018) 2126.
- [10] L.C. Chang, T.R. Tsai, J.J. Wang, C.N. Lin, K.W. Kuo, The rhamnose moiety of solamargine plays a crucial role in triggering cell death by apoptosis, *Biochem. Biophys. Res. Commun.* 242 (1998) 21–25.
- [11] M. Chwalek, N. Lalun, H. Bobichon, C. Plé, L. Voutquenne-Nazabadioko, Structure-activity relationships of some hederagenin diglycosides: haemolysis, cytotoxicity and apoptosis induction, *Biochim. Biophys. Acta* 1760 (2006) 1418–1427.
- [12] L. Voutquenne, C. Lavaud, G. Massiot, T. Sevenet, H.A. Hadi, Cytotoxic polyisoprenes and glycosides of long-chain fatty alcohols from *Dimocarpus fumatus*, *Phytochemistry* 50 (1999) 63–69.
- [13] C. Song, K. Härtl, K. McGraphery, T. Hoffmann, W. Schwab, Attractive but toxic: emerging roles of glycosidically bound volatiles and glycosyltransferases involved in their formation, *Mol. Plant* 11 (2018) 1225–1236.
- [14] V. Vukics, A. Kery, G.K. Bonn, A. Guttman, Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities, *Anal. Bioanal. Chem.* 390 (2008) 1917–1925.
- [15] L.L. Lairson, B. Henrissat, G.J. Davies, S.G. Withers, Glycosyltransferases: structures, functions, and mechanisms, *Annu. Rev. Biochem.* 77 (2008) 521–555.
- [16] W.D. Reiter, C. Chapple, C.R. Somerville, Mutants of *Arabidopsis thaliana* with altered cell wall polysaccharide composition, *Plant J.* 12 (1997) 335–345.
- [17] D.M. Rancour, J.M. Marita, R.D. Hatfield, Cell wall composition throughout development for the model grass *Brachypodium distachyon*, *Front. Plant Sci.* 3 (2012) 266.
- [18] S. Sakamoto, K. Yoshida, S. Sugihara, N. Mitsuda, Development of a new high-throughput method to determine the composition of ten monosaccharides including 4-O-methyl glucuronic acid from plant cell walls using ultra-performance liquid chromatography, *Plant Biotechnol.* 32 (2015) 55–63.
- [19] Z. Xu, et al., Genetic connection between cell-wall composition and grain yield via parallel QTL analysis in *indica* and *japonica* subspecies, *Sci. Rep.* 7 (2017) 12561.
- [20] S. Rovio, H. Simolin, K. Koljonen, H. Sirén, Determination of monosaccharide composition in plant fiber materials by capillary zone electrophoresis, *J. Chromatogr. A* 1185 (2008) 139–144.
- [21] N.C. Carpita, et al., Cell wall architecture of the elongating maize coleoptile, *Plant Physiol.* 127 (2001) 551–565.
- [22] I. Black, C. Heiss, P. Azadi, Comprehensive monosaccharide composition analysis of insoluble polysaccharides by permethylation to produce methyl aldol derivatives for gas chromatography/mass spectrometry, *Anal. Chem.* 91 (2019) 13787–13793.
- [23] D.M. Gibeaut, M. Pauly, A. Bacic, G.B. Fincher, Changes in cell wall polysaccharides in developing barley (*Hordeum vulgare*) coleoptiles, *Planta* 221 (2005) 729–738.
- [24] M. Goto, A.H. Gordon, A. Chesson, Changes in cell-wall composition and degradability of sorghum during growth and maturation, *J. Sci. Food Agric.* 54 (1990) 47–60.
- [25] H. Duruflé, C. Albenne, E. Jamet, C. Dunand, Phenotyping and cell wall polysaccharide composition dataset of five *Arabidopsis* ecotypes grown at optimal or sub-optimal temperatures, *Data Brief* 25 (2019), 104318.
- [26] L. Peng, C.H. Hocart, J.W. Redmond, R.E. Williamson, Fractionation of carbohydrates in *Arabidopsis* root cell walls shows that three radial swelling loci are specifically involved in cellulose production, *Planta* 211 (2000) 406–414.
- [27] K.A. Garleb, L.D. Bourquin, G.C. Fahey, Neutral monosaccharide composition of various fibrous substrates: a comparison of hydrolytic procedures and use of anion-exchange high-performance liquid chromatography with pulsed amperometric detection of monosaccharides, *J. Agric. Food Chem.* 37 (1989) 1287–1293.
- [28] A.M. Safer, N.C. Carpita, V.F. Irish, Rhamnose-containing cell wall polymers suppress helical plant growth independently of microtubule orientation, *Curr. Biol.* 27 (2017), 2248–2259.e4.

- [29] L.D. Gomez, C.G. Steele-King, L. Jones, J.M. Foster, S. Vuttipongchaikij, S. J. McQueen-Mason, Arabinan metabolism during seed development and germination in *Arabidopsis*, *Mol. Plant* 2 (2009) 966–976.
- [30] F.A. Pettolino, C. Walsh, G.B. Fincher, A. Bacic, Determining the polysaccharide composition of plant cell walls, *Nat. Protoc.* 7 (2012) 1590–1607.
- [31] I. Ouhida, J.F. Pérez, J. Gasá, Soybean (*Glycine max*) cell wall composition and availability to feed enzymes, *J. Agric. Food Chem.* 50 (2002) 1933–1938.
- [32] B. Yu, J. Sun, X. Yang, Assembly of naturally occurring glycosides, evolved tactics, and glycosylation methods, *Acc. Chem. Res.* 45 (2012) 1227–1236.
- [33] C. Dong, et al., A structural perspective on the enzymes that convert dTDP-D-glucose into dTDP-L-rhamnose, *Biochem. Soc. Trans.* 31 (2003) 532–536.
- [34] Y. Yin, J. Huang, X. Gu, M. Bar-Peled, Y. Xu, Evolution of plant nucleotide-sugar interconversion enzymes, *PLoS One* 6 (2011), e27995.
- [35] T. Oka, T. Nemoto, Y. Jigami, Functional analysis of *Arabidopsis thaliana* RHM2/MUM4, a multidomain protein involved in UDP-D-glucose to UDP-L-rhamnose conversion, *J. Biol. Chem.* 282 (2007) 5389–5403.
- [36] G. Watt, C. Leoff, A.D. Harper, M. Bar-Peled, A bifunctional 3,5-epimerase/4-keto reductase for nucleotide-rhamnose synthesis in *Arabidopsis*, *Plant Physiol.* 134 (2004) 1337–1346.
- [37] X. Han, L. Qian, L. Zhang, X. Liu, Structural and biochemical insights into nucleotide-rhamnose synthase/epimerase-reductase from *Arabidopsis thaliana*, *Biochim. Biophys. Acta* 1854 (2015) 1476–1486.
- [38] B. Usadel, A.M. Kuschinsky, M.G. Rosso, N. Eckermann, M. Pauly, RHM2 is involved in mucilage pectin synthesis and is required for the development of the seed coat in *Arabidopsis*, *Plant Physiol.* 134 (2004) 286–295.
- [39] T.L. Western, D.S. Young, G.H. Dean, W.L. Tan, A.L. Samuels, G.W. Haughn, *MUCILAGE-MODIFIED4* encodes a putative pectin biosynthetic enzyme developmentally regulated by APETALA2, TRANSPARENT TESTA GLABRA1, and GLABRA2 in the *Arabidopsis* seed coat, *Plant Physiol.* 134 (2004) 296–306.
- [40] G.J. Seifert, Nucleotide sugar interconversions and cell wall biosynthesis: how to bring the inside to the outside, *Curr. Opin. Plant Biol.* 7 (2004) 277–284.
- [41] C. Ringli, et al., The modified flavonol glycosylation profile in the *Arabidopsis rol1* mutants results in alterations in plant growth and cell shape formation, *Plant Cell* 20 (2008) 1470–1481.
- [42] B.M. Kuhn, M. Geisler, L. Bigler, C. Ringli, Flavonols accumulate asymmetrically and affect auxin transport in *Arabidopsis*, *Plant Physiol.* 156 (2011) 585–595.
- [43] B.M. Kuhn, et al., 7-Rhamnosylated flavonols modulate homeostasis of the plant hormone auxin and affect plant development, *J. Biol. Chem.* 291 (2016) 5385–5395.
- [44] A.M. Saffer, V.F. Irish, Flavonol rhamnosylation indirectly modifies the cell wall defects of *RHAMNASE BIOSYNTHESIS1* mutants by altering rhamnose flux, *Plant J.* 94 (2018) 649–660.
- [45] W.D. Reiter, G.F. Vanzin, Molecular genetics of nucleotide sugar interconversion pathways in plants, *Plant Mol. Biol.* 47 (2001) 95–113.
- [46] B. Kim, W.D. Jung, J. Ahn, Cloning and characterization of a putative UDP-rhamnose synthase 1 from *Populus euramericana* Guinier, *J. Plant Biol.* 56 (2013) 7–12.
- [47] X. Dai, et al., Involvement of three *CsRHM* genes from *Camellia sinensis* in UDP-rhamnose biosynthesis, *J. Agric. Food Chem.* 66 (2018) 7139–7149.
- [48] S. Yin, M. Liu, J.Q. Kong, Functional analyses of OcRHS1 and OcUER1 involved in UDP-L-rhamnose biosynthesis in *Ornithogalum caudatum*, *Plant Physiol. Biochem.* 109 (2016) 536–548.
- [49] J. Wang, Q. Ji, L. Jiang, S. Shen, Y. Fan, C. Zhang, Overexpression of a cytosol-localized rhamnose biosynthesis protein encoded by *Arabidopsis* *RHM1* gene increases rhamnose content in cell wall, *Plant Physiol. Biochem.* 47 (2009) 86–93.
- [50] K. Yonekura-Sakakibara, et al., Comprehensive flavonol profiling and transcriptome coexpression analysis leading to decoding gene-metabolite correlations in *Arabidopsis*, *Plant Cell* 20 (2008) 2160–2176.
- [51] A. Oravec, et al., CONSTITUTIVELY PHOTOMORPHOGENIC1 is required for the UV-B response in *Arabidopsis*, *Plant Cell* 18 (2006) 1975–1990.
- [52] K. Hectors, S. van Oevelen, J. Geuns, Y. Guisez, M.A. Jansen, E. Prinsen, Dynamic changes in plant secondary metabolites during UV acclimation in *Arabidopsis thaliana*, *Physiol. Plant.* 152 (2014) 219–230.
- [53] G. Agati, E. Azzarello, S. Pollastri, M. Tattini, Flavonoids as antioxidants in plants: location and functional significance, *Plant Sci.* 196 (2012) 67–76.
- [54] R. Stracke, et al., The *Arabidopsis* bZIP transcription factor HY5 regulates expression of the *PFG1/MYB12* gene in response to light and ultraviolet-B radiation, *Plant Cell Environ.* 33 (2010) 88–103.
- [55] P. Casati, V. Walbot, Differential accumulation of maysin and rhamnosylorientin in leaves of high altitude landraces of maize after UV-B exposure, *Plant Cell Environ.* 28 (2005) 788–799.
- [56] P. Zhang, S. Chopra, T. Peterson, A segmental gene duplication generated differentially expressed myb-homologous genes in maize, *Plant Cell* 12 (2000) 2311–2322.
- [57] S.P. Rius, E. Grotewold, P. Casati, Analysis of the P1 promoter in response to UV-B radiation in allelic variants of high-altitude maize, *BMC Plant Biol.* 12 (2012) 92.
- [58] A. Jozwiak, et al., Plant terpenoid metabolism co-opts a component of the cell wall biosynthesis machinery, *Nat. Chem. Biol.* 16 (2020) 740–748.
- [59] C.P. Bonin, W.D. Reiter, A bifunctional epimerase-reductase acts downstream of the *MUR1* gene product and completes the *de novo* synthesis of GDP-L-fucose in *Arabidopsis*, *Plant J.* 21 (2000) 445–454.
- [60] C.P. Bonin, I. Potter, G.F. Vanzin, W.D. Reiter, The *MUR1* gene of *Arabidopsis thaliana* encodes an isoform of GDP-D-mannose-4,6-dehydratase, catalyzing the first step in the *de novo* synthesis of GDP-L-fucose, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 2085–2090.
- [61] T. Kotake, S. Hojo, N. Tajima, K. Matsuoka, T. Koyama, Y. Tsumuraya, A bifunctional enzyme with L-fucokinase and GDP-L-fucose pyrophosphorylase activities salvages free L-fucose in *Arabidopsis*, *J. Biol. Chem.* 283 (2008) 8125–8135.
- [62] T. Kotake, S. Hojo, D. Yamaguchi, T. Aohara, T. Konishi, Y. Tsumuraya, Properties and physiological functions of UDP-sugar pyrophosphorylase in *Arabidopsis*, *Biosci. Biotechnol. Biochem.* 71 (2007) 761–771.
- [63] M. Bar-Peled, M.A. O'Neill, Plant nucleotide sugar formation, interconversion, and salvage by sugar recycling, *Annu. Rev. Plant Biol.* 62 (2011) 127–155.
- [64] J. Ito, et al., Analysis of plant nucleotide sugars by hydrophilic interaction liquid chromatography and tandem mass spectrometry, *Anal. Biochem.* 448 (2014) 14–22.
- [65] C.E. Caffaro, C.B. Hirschberg, Nucleotide sugar transporters of the Golgi apparatus: from basic science to diseases, *Acc. Chem. Res.* 39 (2006) 805–812.
- [66] M. Handford, C. Rodriguez-Furlán, A. Orellana, Nucleotide-sugar transporters: structure, function and roles *in vivo*, *Braz. J. Med. Biol. Res.* 39 (2006) 1149–1158.
- [67] C. Rautengarten, et al., The golgi localized bifunctional UDP-rhamnose/UDP-galactose transporter family of *Arabidopsis*, *Proc. Natl. Acad. Sci. U. S. A.* 111 (2014) 11563–11568.
- [68] Y. Takenaka, et al., Pectin RG-I rhamnosyltransferases represent a novel plant-specific glycosyltransferase family, *Nat. Plants* 4 (2018) 669–676.
- [69] Y. Uehara, et al., Biochemical characterization of rhamnosyltransferase involved in biosynthesis of pectic rhamnogalacturonan I in plant cell wall, *Biochem. Biophys. Res. Commun.* 486 (2017) 130–136.
- [70] J. Le Roy, B. Huss, A. Creach, S. Hawkins, G. Neutelings, Glycosylation is a major regulator of phenylpropanoid availability and biological activity in plants, *Front. Plant Sci.* 7 (2016) 735.
- [71] T. Louveau, A. Osbourn, The sweet side of plant-specialized metabolism, *Cold Spring Harb. Perspect. Biol.* 11 (2019), a034744.
- [72] P. Jones, B. Messner, J. Nakajima, A.R. Schäffner, K. Saito, UGT73C6 and UGT78D1, glycosyltransferases involved in flavonol glycoside biosynthesis in *Arabidopsis thaliana*, *J. Biol. Chem.* 278 (2003) 43910–43918.
- [73] K. Yonekura-Sakakibara, T. Tohge, R. Niida, K. Saito, Identification of a flavonol 7-O-rhamnosyltransferase gene determining flavonoid pattern in *Arabidopsis* by transcriptome coexpression analysis and reverse genetics, *J. Biol. Chem.* 282 (2007) 14932–14941.
- [74] F. De Bruyn, M. van Bremp, J. Maertens, W. van Bellegem, D. Duchi, M. De Mey, Metabolic engineering of *Escherichia coli* into a versatile glycosylation platform: production of bio-active quercetin glycosides, *Microb. Cell Fact.* 14 (2015) 138.
- [75] H.J. Choi, et al., Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus, *Antiviral Res.* 81 (2009) 77–81.
- [76] N. Onkokesung, M. Reichelt, A. van Doorn, K.C. Schuurink, J.J. van Loon, M. Dicke, Modulation of flavonoid metabolites in *Arabidopsis thaliana* through overexpression of the MYB75 transcription factor: role of kaempferol-3,7-dirhamnoside in resistance to the specialist insect herbivore *Pieris brassicae*, *J. Exp. Bot.* 65 (2014) 2203–2217.
- [77] R. Yin, K. Han, W. Heller, A. Albert, P.I. Dobrev, E. Zazimalová, A.R. Schäffner, Kaempferol 3-O-rhamnoside-7-O-rhamnoside is an endogenous flavonol inhibitor of polar auxin transport in *Arabidopsis* shoots, *New Phytol.* 201 (2014) 466–475.
- [78] S. Irmisch, S. Jancsik, M.M.S. Yuen, L.L. Madilao, J. Bohlmann, Biosynthesis of the anti-diabetic metabolite montbretin A: glucosylation of the central intermediate mini-MbA, *Plant J.* 100 (2019) 879–891.
- [79] S. Irmisch, et al., Discovery of UDP-glycosyltransferases and BAHD-acyltransferases involved in the biosynthesis of the antidiabetic plant metabolite montbretin A, *Plant Cell* 30 (2018) 1864–1886.
- [80] P. Li, Y. Li, F. Zhang, X. Jiang, H. Yu, B. Hou, The *Arabidopsis* UDP-glycosyltransferases UGT79B2 and UGT79B3, contribute to cold, salt and drought stress tolerance via modulating anthocyanin accumulation, *Plant J.* 89 (2017) 85–103.
- [81] J. Kroon, E. Souer, A. de Graaff, Y. Xue, J. Mol, R. Koes, Cloning and structural analysis of the anthocyanin pigmentation locus *Rt* of *Petunia hybrida*: characterization of insertion sequences in two mutant alleles, *Plant J.* 5 (1994) 69–80.
- [82] A. Frydman, et al., Citrus fruit bitter flavors: isolation and functional characterization of the gene *Cm1,2RhaT* encoding a 1,2 rhamnosyltransferase, a key enzyme in the biosynthesis of the bitter flavonoids of citrus, *Plant J.* 40 (2004) 188–100.
- [83] X. Liu, C. Lin, X. Ma, Y. Tan, J. Wang, M. Zeng, Functional characterization of a flavonoid glycosyltransferase in sweet orange (*Citrus sinensis*), *Front. Plant Sci.* 9 (2018) 166.
- [84] A. Frydman, et al., The molecular and enzymatic basis of bitter/non-bitter flavor of citrus fruit: evolution of branch-forming rhamnosyltransferases under domestication, *Plant J.* 73 (2013) 166–178.
- [85] N.W. Widstrom, M.E. Snook, A gene controlling biosynthesis of isoorientin, a compound in corn silks antibiotic to the corn earworm, *Entomol. Exp. Appl.* 89 (1998) 119–124.
- [86] K.F. McCue, et al., Potato glycosterol rhamnosyltransferase, the terminal step in triose side-chain biosynthesis, *Phytochemistry* 68 (2007) 327–334.
- [87] G. Zong, et al., Crystal structures of rhamnosyltransferase UGT89C1 from *Arabidopsis thaliana* reveal the molecular basis of sugar donor specificity for UDP-β-L-rhamnose and rhamnosylation mechanism, *Plant J.* 99 (2019) 257–269.
- [88] N.B. Schwartz, D. Abram, D.S. Feingold, L-Rhamnulose L-phosphate aldolase of *Escherichia coli*. The role of metal in enzyme structure, *Biochemistry* 13 (1974) 1726–1730.

- [89] M.T. Akhy, C.M. Brown, D.C. Old, L-Rhamnose utilisation in *Salmonella typhimurium*, J. Appl. Bacteriol. 56 (1984) 269–274.
- [90] K. Trummel, F. Effenberger, C. Syldatk, An integrated microbial/enzymatic process for production of rhamnolipids and L-(+)-rhamnose from rapeseed oil with *Pseudomonas* sp. DSM 2874, Eur. J. Lipid Sci. Technol. 105 (2003) 563–571.
- [91] H. Birgisson, G.O. Hreggvidsson, O.H. Fridjonsson, A. Mort, J.K. Kristjansson, B. Mattiasson, Two new thermostable alpha-L-rhamnosidases from a novel thermophilic bacterium, Enzyme Microb. Technol. 34 (2004) 561–571.
- [92] M. Ávila, et al., Physiological and biochemical characterization of the two alpha-L-rhamnosidases of *Lactobacillus plantarum* NCC245, Microbiology 155 (2009) 2739–2749.
- [93] D. Monti, A. Pisvejcová, V. Kren, M. Lama, S. Riva, Generation of an alpha-L-rhamnosidase library and its application for the selective derhamnosylation of natural products, Biotechnol. Bioeng. 87 (2004) 763–771.
- [94] T. Koseki, et al., Characterization of an alpha-L-rhamnosidase from *Aspergillus kawachii* and its gene, Appl. Microbiol. Biotechnol. 80 (2008) 1007–1013.
- [95] H.B. Hughes, J.P. Morrissey, A.E. Osbourn, Characterisation of the saponin hydrolysing enzyme avenacoside- α -L-rhamnosidase from the fungal pathogen of cereals, *Stagonospora avenae*, Eur. J. Plant Pathol. 110 (2004) 421–427.
- [96] H. Suzuki, Hydrolysis of flavonoid glycosides by enzymes (rhamnodiastase from *Rhamnus* and other sources), Arch. Biochem. Biophys. 99 (1962) 476–483.
- [97] R. Bourbouze, F. Percheron, J.E. Courtis, alpha-L-Rhamnosidase from *Fagopyrum esculentum*: purification and some properties, Eur. J. Biochem. 63 (1976) 331–337.
- [98] R. Bourbouze, F. Pratviel-Sosa, F. Percheron, Rhamnodiastase et α -L-rhamnosidase de *Fagopyrum esculentum*, Phytochemistry 14 (1975) 1279–1282.
- [99] J. Vogel, Unique aspects of the grass cell wall, Curr. Opin. Plant Biol. 11 (2008) 301–307.
- [100] B.W. Penning, M.C. McCann, N.C. Carpita, Evolution of the cell wall gene families of grasses, Front. Plant Sci. 10 (2019) 1205.
- [101] I.O. Okekeogbu, et al., Glycome and proteome components of golgi membranes are common between two angiosperms with distinct cell-wall structures, Plant Cell 31 (2019) 1094–1112.
- [102] F. Rojas Rodas, et al., Linkage mapping, molecular cloning and functional analysis of soybean gene *Fg2* encoding flavonol 3-O-glucoside (1 $\rightarrow$ 6) rhamnosyltransferase, Plant Mol. Biol. 84 (2014) 287–300.