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Plant Fucosyltransferases and the Emerging Biological Importance of Fucosylated Plant Structures

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1 Title: Plant Fucosyltransferases and the Emerging Biological Importance of  
2 Fucosylated Plant Structures

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25 **Title: Plant Fucosyltransferases and the Emerging Biological**  
26 **Importance of Fucosylated Plant Structures**

27

28 **Abstract**

29 Plants frequently incorporate the monosaccharide L-fucose (Fuc; 6-deoxy-L-  
30 galactose) into glycans and glycopolymers located in diverse cellular  
31 locations. The incorporation of Fuc onto these varied glycans is carried out  
32 by fucosyltransferases (FUTs), that make up a protein superfamily with  
33 equally varied and diverse functions. The structures wherein Fuc is found  
34 have numerous proposed and validated functions, ranging from plant growth  
35 and development, cell expansion, adhesion and signaling, to energy  
36 metabolism, among others. FUTs from several different plant species have  
37 been identified and described; however, very few of them have been  
38 extensively characterized biochemically and biologically. In this review, we  
39 summarize plant FUTs that have been biochemically characterized and  
40 biologically investigated for associated phenotypes, offering greater insight  
41 and understanding into the physiological importance of Fuc in plants and in  
42 plant cell wall structures, glycans, and proteins.

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45 **Keywords:** Fucosyltransferase, xyloglucan, arabinogalactan proteins,  
46 rhamnogalacturonan, *N*-glycan, *O*-fucosylation

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## 51 **Introduction**

52 Fucose (Fuc; 6-deoxy-L-galactose) is a deoxyhexose sugar that is found in  
53 the glycans of diverse macromolecules in numerous species of plants,  
54 bacteria, fungi, mammals, and invertebrates. Fuc and other sugars are  
55 incorporated into macromolecules via the action of glycosyltransferases  
56 (GTs), which are enzymes that catalyze the transfer of a sugar from an  
57 activated sugar donor containing a phosphate leaving group. The  
58 incorporation of Fuc into these varied structures is carried out by specific  
59 enzymes, fucosyltransferases (FUTs), which are Leloir glycosyltransferases  
60 that catalyze the transfer of Fuc from guanine 5'-diphosphate- $\beta$ -L-Fucose  
61 (GDP-Fuc) to a suitable acceptor substrate, often an *N*-glycan,  
62 polysaccharide, or protein. FUTs belong to an enzyme superfamily that is  
63 sub-categorized based on the linkage in which Fuc is added onto the  
64 acceptor substrate, as follows:  $\alpha$ -(1,2) FUT,  $\alpha$ -(1,3) FUT,  $\alpha$ -(1,4) FUT,  $\alpha$ -(1,6)  
65 FUT, protein *O*-fucosyltransferase family 1 (POFUT1) and protein *O*-  
66 fucosyltransferase family 2 (POFUT2) (Martinez-Duncker, et al., 2003).  
67 Furthermore, these enzymes are classified into GT families, including; GT10,  
68 GT11, GT23, GT37, GT41, GT65, and GT68 in the Carbohydrate-Active  
69 enZYmes Database (CAZy, [www.cazy.org](http://www.cazy.org)); however, only GT10, GT37, and  
70 GT41 FUTs have been found in plants thus far (Both et al., 2011; Cantarel et  
71 al., 2009; Coutinho et al., 2003; Lombard et al., 2014).

72 In plants, Fuc has been found in the hemicellulosic polysaccharide,  
73 xyloglucan (XyG) in an  $\alpha$ -(1,2)-linkage (Pauly and Keegstra, 2016); in the

74 pectic polysaccharides, rhamnogalacturonan I (RG-I) in an  $\alpha$ -(1,2)-linkage,  
75 and rhamnogalacturonan II (RG-II) in both  $\alpha$ -(1,2) and  $\alpha$ -(1,4) linkages  
76 (Atmodjo et al., 2013); and on the extracellular, arabinogalactan proteins  
77 (AGPs) in an  $\alpha$ -(1,2)-linkage (Tan et al., 2012), all of which are cell wall  
78 glycans. In addition, Fuc can also be present attached to proteins, either on  
79 *N*-glycans in an  $\alpha$ -1,3 linkage to the proximal *N*-acetyl glucosamine (GlcNAc)  
80 of the core, in an  $\alpha$ -1,4 linkage to the terminal GlcNAc residue of complex-  
81 type *N*-glycans (Staudacher et al., 1999); or directly attached to  
82 serine/threonine residues of proteins, in an *O*-linkage (Hallgren et al., 1975;  
83 Figure 1).

84 A common feature of FUTs is that they all use the activated sugar  
85 nucleotide, GDP-Fuc as a donor. GDP-Fuc is synthesized from GDP-Mannose  
86 (GDP-Man), in a pathway consisting of three steps: 4,6-dehydration, 3,5-  
87 epimerization, and 4-reduction (Reiter and Vanzin, 2001). In the model plant  
88 species, *Arabidopsis thaliana* (*A. thaliana*) these three reactions are carried  
89 out by two separate types of enzymes. The first are GDP-D-Man-4,6-  
90 dehydratases encoded by isoforms *GMD1* and *GMD2*. *GMD2* was first  
91 identified in a mutagenesis screen as *AtMUR1*, taken from the Latin word,  
92 murus, or wall (Reiter et al., 1993, 1997). The second enzyme is a GDP-4-  
93 keto-6-deoxy-D-Man (GDP-KDM) 3,5-epimerase-4-reductase, encoded by  
94 *GER1* (Bonin et al. 1997; Bonin and Reiter 2000). Interestingly, the *GMD2*  
95 (*AtMUR1*) gene contributes to the *de novo* biosynthesis of GDP-Fuc in most  
96 tissues, while *GMD1* contributes to its synthesis in a limited number of cell

97 types (Bonin et al., 2003). GDP-Fuc can also be synthesized from a salvage  
98 pathway that involves the direct phosphorylation of free Fuc followed by the  
99 attachment of guanosine monophosphate (GMP) (Feingold and Avigad,  
100 1980).

101 In this review, we will offer an overview of plant FUTs that have been  
102 biochemically and biologically investigated and characterized. Although the  
103 number of plant FUTs that have been extensively characterized are few, the  
104 FUTs responsible for the addition of Fuc to many known fucosylated plant cell  
105 wall polysaccharides and other glycan structures have been identified, with  
106 the exception of the FUTs specific for RG-I and RG-II. Though relatively few in  
107 number, the plant FUTs included in this review offer valuable insight into the  
108 wide diversity of activities and specificities of these plant enzymes.

109

### 110 **Xyloglucan-specific FUTs**

111 XyGs are a family of hemicellulosic polysaccharides that have a  $\beta$ -(1,4)-  
112 linked-Glucose (Glc) backbone with sidechains that are initiated at the O-6  
113 position with  $\alpha$ -D-xylose (Xyl) (Pauly and Keegstra, 2016). XyGs are thought  
114 to contribute to cell wall strengthening in dicots and non-graminaceous  
115 monocots by binding to the hydrophobic surfaces of cellulose fibrils  
116 (Cosgrove, 2014; Darvill et al., 1985). To date, 19 different XyG sidechains  
117 have been identified from various plant species, and are described using an  
118 accepted single-letter nomenclature (Fry *et al.*, 2006; Tuomivaara *et al.*,  
119 2015; Figure 1A). For example, an unsubstituted Glc is denoted by the letter

120 **G**, a backbone residue appended with  $\alpha$ -D-Xyl is termed **X**, and when this  
121 xylosyl residue is further substituted by  $\beta$ -D-Gal it is called **L**. The **F**  
122 sidechain, characteristic of fucogalactoxyloglucan, consists of a backbone  
123 Glc residue that is substituted with  $\alpha$ -L-Fuc-(1,2)- $\beta$ -D-Gal-(1,2)- $\alpha$ -D-Xyl (Fry et  
124 al., 1993; Tuomivaara et al., 2015).

125 Three XyG-specific FUTs have been identified and characterized in plants,  
126 all of which are classified in the plant-specific GT37 family. The first XyG-  
127 specific FUT to be identified was isolated and purified from microsomal  
128 fractions of etiolated pea, *Pisum sativum*, stems (Farkas and Maclachlan,  
129 1988). The enzyme, called *PsFUT1*, was demonstrated to catalyze the *in vitro*  
130 transfer of radiolabeled Fuc from GDP-Fuc onto a Gal residue of exogenously  
131 available XyG acceptors. *PsFUT1* was shown to prefer tamarind XyG, where  
132 almost all Gal residues are not fucosylated, over XyG isolated from wildtype  
133 (WT) pea cell walls, where most Gal residues are already fucosylated. In the  
134 process of characterizing *PsFUT1* and its corresponding gene in pea, *AtFUT1*  
135 in *A. thaliana* was identified, based on sequence similarity to the pea gene.  
136 The corresponding gene in *A. thaliana* is also listed as *MUR2*, and was  
137 initially identified by screening chemically mutagenized *A. thaliana* plants for  
138 changes in neutral monosaccharide content of their walls (Reiter et al.,  
139 1997). The mutation responsible for the *mur2* chemotype was eventually  
140 shown to be in the gene *AtFUT1* (Faik et al., 1997; Perrin et al., 1999).  
141 Heterologous expression of *AtFUT1* in a mammalian COS cell line yielded 41  
142 times higher fucosyltransferase activity for tamarind XyG relative to a control



143 COS cell line expressing an empty vector, confirming that AtFUT1, like  
144 PsFUT1, is a XyG-specific FUT (Perrin et al., 1999). Interestingly, *in planta*  
145 AtFUT1 has also been shown to fucosylate galacturonic acid (GalA) in certain  
146 types of XyGs, demonstrating that AtFUT1 is capable of recognizing at least  
147 two XyG acceptor residues, Gal and GalA (Peña et al., 2012).

148 In *A. thaliana*, XyG is produced by a Golgi-localized multi-protein complex  
149 that consists, at a minimum, of three xylosyltransferases (XXTs), XXT1,  
150 XXT2, and XXT5 as well as one  $\beta$ -(1,4)-glucan synthase, Cellulose Synthase-  
151 Like C4 (CSLC4) in the trans-Golgi network (TGN) (Chou et al., 2012). AtFUT1  
152 can simultaneously form homo-complexes through disulfide bonds or  
153 heterocomplexes via two interaction surfaces on the protein. Two separate  
154 heterocomplexes formed by AtFUT1 have been documented, one with the  
155 galactosyltransferases (MUR3 and XLT2), another with XXT2 and XXT5 (Chou  
156 et al., 2015; Lund et al., 2015). Together these results suggest that AtFUT1  
157 along with the galactosyltransferases MUR3 and XLT2 also form part of the  
158 multi-protein complex involved in XyG biosynthesis (Chou et al., 2012, 2015;  
159 Lund et al., 2015).

160 In addition to the biochemical research done to determine the activity and  
161 specificity of AtFUT1, structural studies have led to its successful  
162 crystallization (Rocha et al., 2016; Urbanowicz et al., 2017) and detailed  
163 analysis of its mechanism of activity (Urbanowicz et al., 2017). Subsequent  
164 analyses of the enzyme structure determined that it adopts the  
165 glycosyltransferase B (GT-B) fold and is metallo-independent, like all other

166 FUT proteins that have been structurally characterized to date. A third XyG-  
167 specific FUT was identified in rice, *Oryza sativa*, by phylogenetic and  
168 coexpression analyses, and was subsequently named *OsMUR2*. Although the  
169 *OsMUR2* protein has yet to be biochemically or biologically characterized in  
170 rice, the XyG Fuc deficiency in the *mur2 A. thaliana* mutant was successfully  
171 rescued when this mutant line was transformed with *OsMUR2*, indicating that  
172 *AtFUT1* and *OsMUR2* are functionally equivalent *in planta* (Liu et al., 2015;  
173 Vanzin et al., 2002).

174 In addition to being implicated in cell wall strengthening, fucosylated XyG  
175 has long been postulated to be involved in several plant growth responses  
176 (Pauly and Keegstra, 2016). To characterize the function of *PsFUT1 in planta*,  
177 pea hairy root lines expressing full-length *PsFUT1* antisense mRNA were  
178 constructed (Wen et al., 2008). Hairy root lines expressing the *PsFUT1*  
179 antisense mRNA had 40-50% of the WT levels of *PsFUT1* mRNA. Emerging  
180 root tips appeared WT in morphology; however, elongating cells developed  
181 bulges that progressed into undifferentiated calluses within 2-4 weeks (Wen  
182 et al., 2008). Additionally, antisense hairy root tips surface labeled with the  
183 CCRC-M1 monoclonal antibody, that specifically recognizes  $\alpha$ -L-fucosylated  
184 XyG (Puhlmann et al., 1994), displayed labeling patterns that differed from  
185 those observed in WT hairy root cells. This was due to cells being collapsed  
186 and wrinkled, which inhibited recognition and binding by CCRC-M1, as was  
187 discovered upon visualization with scanning electron microscopy (SEM) (Wen  
188 et al., 2008).

189 Similar disruptions to morphology have been reported for the trichomes of  
190 *mur2 A. thaliana* mutants, which have less than 2% of WT levels of  
191 fucosylated XyG (Vanzin et al., 2002). Accordingly, *mur2* mutants lack  
192 fucosylated XyG in all major plant organs, indicating that AtFUT1 is solely  
193 responsible for the fucosylation of XyG. Despite the severe reduction of  
194 fucosylated XyG throughout the entire plant, *mur2* mutant plants grow  
195 indistinguishably from WT plants when grown under normal conditions, as  
196 well as under cold, heat, and salt stress, with the only detectable phenotype  
197 being the previously mentioned disruptions to trichomes (Vanzin et al.,  
198 2002).

### 199 **AGP-Specific FUTs**

200 AGPs are an abundant and diverse family of cell wall glycoproteins, with  
201 numerous and varied functions in plants, including cellular growth and stress  
202 responses. AGPs contain abundant amounts of hydroxyproline (Hyp), Ala,  
203 Ser, and Thr residues, and are extensively glycosylated on non-contiguous  
204 Hyp residues. Polysaccharide chains on the glycan portions of AGPs consist  
205 of  $\beta$ -(1,3) linked galactose (Gal) backbones decorated with  $\beta$ -(1,6) linked Gal  
206 side-chains that are further modified with  $\alpha$ -linked arabinose (Ara) residues,  
207 as well as  $\alpha$ -(1,2) linked Fuc,  $\alpha$ -linked rhamnose (Rha),  $\alpha$ -linked glucuronic  
208 acid (GlcA), and other sugars to a lesser extent (Showalter and Basu, 2016).

209 One AGP-specific FUT,  $\alpha$ -L-FTase, from radish (*Raphanus sativus* L.), and  
210 two AGP-specific FUTs from *A. thaliana*, AtFUT4 and AtFUT6, have been  
211 identified and studied (Liang et al., 2013; Misawa et al., 1996; Tryfona et al.,

212 2012, 2014; Wu et al., 2010).  $\alpha$ -L-FTase was identified in microsomal  
213 preparations from roots of 6-day old radish seedlings. Enzyme activity for  $\alpha$ -  
214 L-FTase was measured fluorimetrically, and it was found that the enzyme  
215 successfully fucosylated a pyridylaminated (PA) trisaccharide consisting of L-  
216 Araf- $\alpha$ -(1,3)-D-Galp- $\beta$ -(1,6)-D-Galp (AraGalGal-PA). Subsequent chemical and  
217 enzymatic analyses of the fucosylated reaction product, (FucAraGalGal-PA),  
218 confirmed that fucosylation occurred on the O-2 of L-Araf attached to  $\beta$ -(1,6)-  
219 linked D-Gal (Misawa et al., 1996). AtFUT4 and AtFUT6 are members of the  
220 plant-specific GT37 family and were initially postulated to be putative FUTs  
221 based on their sequence similarity to AtFUT1 (Sarria et al., 2001). Early  
222 studies conducted on AtFUT4 and AtFUT6 were done using tobacco Bright  
223 Yellow-2 (BY-2) suspension-cultured cells that make non-fucosylated AGPs.  
224 Transient overexpression of AtFUT4 and AtFUT6 in BY-2 cells resulted in the  
225 production of AGPs with a Fuc moiety appended to O-2 of L-Araf (Wu et al.,  
226 2010). However, AtFUT4 and AtFUT6 were unable to add Fuc to other  
227 glycopolymers such as RG-I and XyG *in vitro*, demonstrating the specificity of  
228 these two FUTs for AGPs. While AtFUT4 and AtFUT6 appear to have similar  
229 activities *in vitro*, their expression patterns *in planta* differ. AtFUT6 is only  
230 expressed in the root, while AtFUT4 is expressed in both the leaf and root  
231 (Sarria et al., 2001). Due to differences in their expression patterns, studies  
232 have demonstrated that AtFUT4 is solely responsible for the fucosylation of  
233 leaf AGPs, while AtFUT4 and AtFUT6 are both required for the fucosylation of  
234 root AGPs (Liang et al., 2013; Tryfona et al., 2012, 2014).

235 Characterization of *fut4*, *fut6*, and *fut4/fut6* single and double mutants in  
236 *A. thaliana* revealed that the loss of these genes does not seriously impact  
237 plant growth. More specifically, when grown under normal physiological  
238 conditions *fut4*, *fut6*, and *fut4/fut6* grew comparably to WT plants when  
239 evaluated for phenotypes such as rosette size, height, branch number, dry  
240 weight, and flowering time (Tryfona et al., 2014). Interestingly, the *fut4/fut6*  
241 double mutant displayed an observable phenotype that was detected when  
242 mutant plants were subjected to stressful growth conditions, particularly salt  
243 stress. Under salt-stress conditions, ranging from 100-150 mM NaCl,  
244 *fut4/fut6* double mutants had significantly shorter roots relative to WT  
245 control plants also grown under salt stress (Liang et al., 2013; Tryfona et al.,  
246 2014). This observation supports the hypothesis that fucosylated AGPs are  
247 involved in some aspect of cell expansion in elongating root cells.  
248 Furthermore, these results suggest that the presence or absence of Fuc on  
249 AGP glycan structures may be a key determinant for proper cell growth  
250 under osmotic, or potentially other extracellular/environmental stresses.

251 This finding is in support of previous studies on *mur1* mutants of *A.*  
252 *thaliana*, which are impaired in Fuc biosynthesis. Accordingly, the AGPs  
253 isolated from *mur1* mutants are not substituted with Fuc in leaves and roots.  
254 Furthermore, these mutants also exhibited decreased root growth resulting  
255 from concurrent regions of normal and abnormal cell elongation. Despite  
256 phenotypic similarities, *mur1* mutants lack Fuc in all analyzed fucosylated  
257 glycopolymers, including AGPs, XyG, *N*-glycans, RG-I, and RG-II. Thus, the

258 root growth phenotype of *mur1* plants cannot be solely ascribed to the lack  
259 of fucosylated AGPs, but rather an overall reduction of Fuc in plant structures  
260 (Bonin et al., 1997). Regardless, the decreased root growth of *fut4/fut6* and  
261 *mur1* mutants appear to be related to the under-fucosylation of AGPs and,  
262 possibly other structures, suggesting the importance of Fuc attached on  
263 oligosaccharides and/or glycoproteins for proper cell expansion and  
264 elongation in plants.

265 More recent findings on the *AtFUT4* and *AtFUT6* proteins suggest that they  
266 are functionally equivalent *in vitro*, as both are able to fucosylate various  
267 arabinogalactan (AG)-related oligosaccharide structures (unpublished results  
268 of the authors). Furthermore, the differences in expression patterns of the  
269 *AtFUT4* and *AtFUT6* genes at the cellular level, suggest that *AtFUT4* is  
270 responsible for the majority of AGP fucosylation throughout the plant body,  
271 while both *AtFUT4* and *AtFUT6* work concurrently in the root, albeit in  
272 different locations. *AtFUT4* expression localizes only to the basal regions of  
273 the tap root and emerging lateral roots, while *AtFUT6* is expressed only in  
274 the tips of the tap root and emerging lateral roots (unpublished results of the  
275 authors).

## 276 **Pectic Polysaccharides**

277 In addition to XyG and AGPs, RG-I and RG-II are two other major classes of  
278 cell wall polysaccharides that contain Fuc. The pectic polysaccharides RG-I  
279 and RG-II are among the most structurally complex cell wall polysaccharides  
280 in plants. RG-I has a backbone of repeating  $[\alpha\text{-}(1,4)\text{-D-GalA-}\alpha\text{-}(1,2)\text{-L-Rha}]_n$

281 units, with sidechain modifications of variously linked arabinose and  
282 galactose residues that also contain Fuc and GlcA to a lesser extent (Ridley  
283 et al., 2001). RG-II consists of an  $\alpha$ -(1,4)-linked galacturonic acid (GalA)  
284 backbone, modified with sidechains A-F that consist of 12 different  
285 monosaccharides, including Fuc and 2-O-methyl-L-Fuc (MeFuc) present in  
286 sidechains A and B, respectively (Ndeh et al., 2017). RG-I and RG-II are  
287 implicated in various plant functions, ranging from cellular growth and  
288 expansion to wall porosity (Darvill et al., 1985; Mohnen, 2008; Ridley et al.,  
289 2001; Willats et al., 2001).

290 While Fuc has long been known to be present on RG-I and RG-II, the FUTs  
291 specific for adding Fuc to these polysaccharides remain unidentified. The Fuc  
292 found on RG-I is  $\alpha$ -(1,2)-linked, and as such, the FUT responsible for this  
293 fucosylation is potentially one of the 7 uncharacterized members of GT37,  
294 which are predicted to be  $\alpha$ -(1,2) FUTs in *A. thaliana* (Sarría et al., 2001). RG-  
295 II also has two well characterized L-Fuc residues and a terminal L-Gal, which  
296 only differs from L-Fuc by having a hydroxymethyl group at C-6. There is a  
297 terminal non-reducing 2-O-Me- $\alpha$ -L-Fuc residue that is  $\alpha$ -(1,2)-linked to D-Gal  
298 in sidechain B that is often acetylated. The Gal-Fuc disaccharide structure in  
299 sidechain B of RG-II is identical to that found in XyG; therefore, we  
300 hypothesize that the FUT responsible for catalyzing the transfer of Fuc to this  
301 Gal is related to the XyG-specific AtFUT1 and also is a member of GT37. The  
302 second Fuc in RG-II is a 3,4-linked  $\alpha$ -L-Fuc residue in the core oligosaccharide  
303 structure of sidechain A. This Fuc is more likely added by a FUT from an

304 entirely different GT family, possibly a member of the GT10 family that  
305 include  $\alpha$ -(1,3)- and  $\alpha$ -(1,4)-specific FUTs (Martinez-Duncker et al., 2003).  
306 However, three FUTs from this family, one in *A. thaliana* (Wilson et al., 2001),  
307 one in mung bean (*Vigna radiata*) (Leiter et al., 1999), and one from tomato  
308 (*Solanum lycopersicum*) (Wilson, 2001), have been characterized, and all  
309 three are involved in *N*-glycosylation. Interestingly, there is also a terminal L-  
310 Gal present in sidechain A that is  $\alpha$ -(1,2)-linked to D-GalA. Prior work on the  
311 *mur1* mutant of *A. thaliana*, which encodes GMD2, results in plants that lack  
312 L-Fuc and substitute L-Fuc with L-Gal (O'Neill et al., 2001; Reuhs et al., 2004),  
313 indicating that the FUTs catalyzing the synthesis of these glycans can also  
314 utilize GDP-L-Gal as a donor. Taken together, we hypothesize that the  
315 enzyme responsible for catalyzing the addition of the non-reducing terminal  
316 L-Gal on side chain A is also a member of GT37. The identification and  
317 detailed characterization of these additional FUTs would provide a more  
318 complete view on the fucosylation of cell wall polysaccharides, providing  
319 additional comparative insight into the specific activities of the GT37 FUTs,  
320 as well as the possible GT10 FUT involved in the synthesis of RG-II sidechain  
321 A.

### 322 ***N*-glycan Specific FUTs**

323 *N*-glycosylation is a highly conserved modification in plants and animals  
324 and is one of the most important post-translational modifications of proteins.  
325 *N*-glycosylation involves the attachment of oligosaccharides to asparagine  
326 residues with an Asn-X-Ser/Thr consensus sequence, termed a sequon, with



327 X being any amino acid other than proline (Staudacher et al., 1999). Unlike,  
328 mammalian *N*-glycans, plants often incorporate an  $\alpha$ -(1,3)-linked Fuc onto  
329 the proximal *N*-acetylglucosamine (GlcNAc) of the core oligosaccharide  
330 attached to the protein (Strasser et al., 2004). This fucosyl residue is the key  
331 element that makes plant *N*-glycans antigenic to mammals, and has  
332 hindered the use of plants for the production of recombinant glycoproteins  
333 for medical applications (Bardor et al., 2003; Harmoko et al., 2016). The  $\alpha$ -  
334 (1,3) and  $\alpha$ -(1,4) FUTs required for *N*-linked glycan biosynthesis are more  
335 closely related to each other than to the  $\alpha$ -(1,2) FUTs of GT37, such as those  
336 responsible for the fucosylation of XyGs and AGPs, and are therefore  
337 separately classified in GT10 in the CAZy database (Martinez-Dunker et al.,  
338 2003). The first FUT with *N*-glycan core  $\alpha$ -(1,3)-fucosyltransferase activity  
339 was identified and purified from mung bean (*Vigna radiate*) seedlings (Leiter  
340 et al., 1999; Staudacher et al., 1995). The enzyme was demonstrated to  
341 transfer Fuc from GDP-Fuc onto the Asn-linked GlcNAc core residue of *N*-  
342 glycans, as well as onto *N*-glycopeptides and oligosaccharides with the  
343 GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> glycan structure. The enzyme was unable to transfer  
344 onto *N*-glycans without terminal GlcNAc residues or onto *N*-  
345 acetyllactosamine, lacto-*N*-biose and *N*-acetylchito-oligosaccharides (Leiter  
346 et al., 1999; Staudacher et al., 1995). Following the characterization of the  $\alpha$ -  
347 (1,3) FUT from mung bean, three genes related to the mung bean gene  
348 sequence were identified in *A. thaliana*; *AtFucTA* (*AtFUT11*), *AtFucTB*  
349 (*AtFUT12*), and *AtFucTC* (*AtFUT13*) (Wilson et al., 2001). Of the three, only

350 *AtFucTA* (*AtFUT11*) was successfully expressed in *Pichia pastoris*, and was  
351 demonstrated to catalyze the same reaction as the FUT from mung bean  
352 (Wilson *et al.*, 2001). Finally, an  $\alpha$ -(1,4) FUT from tomato, expressed in *Pichia*  
353 *pastoris*, was demonstrated to have Lewis-a activity on the *N*-glycans of  
354 tomato, catalyzing the transfer of Fuc from GDP-Fuc to lacto-*N*-tetraose as  
355 well as  $\beta$ -(1,3) and  $\beta$ -(1,4)-galactosylated *N*-glycans (Wilson, 2001). Although  
356 *N*-glycan specific FUTs have been identified and biochemically studied in  
357 other plant species (Table 1), no follow up studies have been conducted for  
358 phenotypes associated with their mutations in those plant species, and as  
359 such, they will not be discussed in the scope of this review.

360 Few studies have been carried out to understand what, if any, impact the  
361 loss of  $\alpha$ -(1,3) and  $\alpha$ -(1,4) FUTs would have in plants. The *A. thaliana fucTA*,  
362 *fucTB*, and *fucTC* mutants have yet to be characterized. However, *A. thaliana*  
363 mutants that are otherwise impaired in the plant *N*-glycosylation pathway  
364 are generally embryo lethal or developmentally impaired and are, therefore,  
365 unable to be bred (Boisson *et al.*, 2001; Lukowitz *et al.*, 2000; von Schaewen,  
366 *et al.*, 1993). Studies to elucidate the physiological significance of  $\alpha$ -(1,3)  
367 and  $\alpha$ -(1,4) *N*-glycan fucosylation have been successfully carried out in other  
368 model plant species, like rice and tobacco (Harmoko *et al.*, 2016; Joly *et al.*,  
369 2002; Sim *et al.*, 2018). Two independent studies conducted on T-DNA  
370 insertion lines for an  $\alpha$ -(1,3)-fucosyltransferase gene in rice, *Os08g36840*,  
371 found that mutants are impaired in a number of features, including shoot  
372 growth, root elongation, flowering time, and plant height. Furthermore, these

373 plants are also impaired in their ability to respond to stresses such as high  
374 salinity, and the rice pathogen *Magnaporthe oryzae* (Harmoko et al., 2016;  
375 Sim et al., 2018). Mutants were also found to have lower levels of auxin-  
376 related transcription factors relative to their progenitor lines, and were  
377 accordingly determined to be impaired in polar auxin transport, the primary  
378 mechanism for the transport of auxin in the vascular meristem (Harmoko et  
379 al., 2016; Helen & Goldsmith, 1977). Studies on an  $\alpha$ -(1,4) FUT protein in  
380 tobacco flowers showed that a constant, but relatively low level of  
381 expression ( $\sim 20$  pmol Fuc  $\text{h}^{-1}$   $\text{mg}^{-1}$  protein) could be detected in different  
382 parts of the tobacco flower, and a 3-fold increase in activity was detected in  
383 both the stamen during anthesis and in pollinated pistils, with the highest  
384 levels of activity ( $\sim 120$  pmol Fuc  $\text{h}^{-1}$   $\text{mg}^{-1}$  protein) being measured in mature  
385 pollen grains. The basal FUT activity detected in tobacco flowers suggest that  
386  $\alpha$ -1,4 fucosylation of *N*-glycans is a basic requirement during tobacco flower  
387 maturation, while the peaks in activity during pollen maturation could be  
388 ascribed to microgametogenesis and pollen tube elongation; no analyses on  
389 mutations in tobacco FUT proteins or genes have been conducted (Čapková  
390 et al., 1997; Joly et al., 2002).

### 391 **POFUTs**

392 As with *N*-glycan fucosylation, protein *O*-fucosylation is conserved between  
393 plants and other organisms, and entails the transfer of Fuc from GDP-Fuc  
394 directly onto a serine/threonine residue of proteins, an activity that was first  
395 identified in human urine (Hallgren et al., 1975). Protein *O*-fucosylation in

396 mammals and invertebrates is found on folded Epidermal Growth Factor-like  
397 (EGF) repeats and Thrombospondin Type 1 repeats (TSRs) and occurs in the  
398 endoplasmic reticulum (ER), where it is catalyzed with strict specificity by  
399 POFUT1 and POFUT2, respectively (Luo et al., 2006; Wang et al., 2001).  
400 About 100 potential human proteins have EGF repeats that POFUT1 could  
401 target, with the Notch receptor family being the most prevalent protein  
402 family to contain this motif (Okajima and Irvine, 2002; Shi and Stanley,  
403 2003). The Notch signaling pathway is widely conserved evolutionarily and  
404 has been implicated in neurogenesis and embryonic development (Imayoshi  
405 and Kageyama, 2011). About 49 proteins in humans contain the TSR  
406 sequence targeted by POFUT2, most of which are secreted factors destined  
407 for the extracellular matrix, or are cell surface proteins that are involved with  
408 modulating cell signaling (Schneider et al., 2017).

409 The putative POFUTs in plants are unrelated to the POFUT1 and POFUT2  
410 families found in other organisms and were classified by the presence of a  
411 domain of unknown function (DUF) 246 (PF03138/IPR024709) and are  
412 distantly related to CaZY family GT65, sometimes termed plant GT65R  
413 proteins (Hansen et al., 2012). They appear to be prevalent in plant  
414 genomes, with *A. thaliana* having 39 predicted POFUT-like genes (Hansen et  
415 al., 2012; Smith et al., 2018a) and are involved in growth and reproduction  
416 (Smith et al., 2018b). Despite their predicted prevalence, this family of GTs is  
417 by far the most understudied, with studies on members of this family having  
418 only been published within the last decade. Those that have been identified,

419 though, have not been biochemically characterized until recently, as  
420 described below.

421 Plants carrying mutations in proteins with a DUF246 domain have been  
422 investigated due to the variety of interesting phenotypes exhibited by plants  
423 when these genes are lost or disrupted, including the effects on diverse cell  
424 wall polymers. A Golgi-localized DUF246 containing protein, FRIABLE1  
425 (FRB1), was found to affect cell adhesion and organ fusion in *A. thaliana*, and  
426 was the first member of this family to be identified in plants (Neumetzler et  
427 al., 2012). Loss of the *FRB1* gene product resulted in pleiotropic effects on  
428 cell wall architecture, particularly cell adhesion. This was due to alterations  
429 in both extensins and pectins that resulted in changes to the structure of the  
430 cell wall and middle lamella and consequently affected cell adhesion  
431 (Neumetzler et al., 2012). Interestingly another member of this family,  
432 ESMERALDA1 (ESMD1) did not exhibit any associated phenotype when the  
433 *esmd1* single mutant plant were generated. However, *frb1-2/esmd1-1* double  
434 mutants showed a rescue of the cell adhesion defect associated with *frb1*  
435 (Verger et al., 2016). In another suppressor screen, *quasimodo* mutants,  
436 defective in the putative pectin methyltransferase gene *QUASIMODO2* (*TSD2*,  
437 *OSU1*) similarly show a cell-detachment phenotype (Verger et al., 2016) that  
438 was rescued in the *qua2-1/esmd1-1* double mutant. Furthermore, a  
439 *qua2-1/frb1-2/esmd1-1* triple mutant also showed rescue of the cell-  
440 detachment phenotype, indicating that knocking out *ESMD1* rescues the cell  
441 adhesion defects caused by single mutations in *QUA2* and *FRB1* (Verger et

442 *al.*, 2016). Recently, four members of the DUF246 family were biochemically  
443 characterized for the first time and shown to be UDP- $\beta$ -L-Rha dependent 4- $\alpha$ -  
444 rhamnosyltransferases (RRTs) involved in the synthesis of the repeating  
445 disaccharide unit [2)- $\alpha$ -L-Rha-(1,4)- $\alpha$ -D-GalA-(1] of the RG-I backbone  
446 (Takenaka et al., 2018). This family is now classified as a new plant-specific  
447 GT family, GT106. The functional characterization of these enzymes calls into  
448 question the original bioinformatics predictions that this family is involved in  
449 protein fucosylation; however, more members will need to be biochemically  
450 characterized to elucidate the role of this protein family in plants (Takenaka  
451 et al., 2018).

452 The putative POFUT, SPINDLY (SPY) is classified as a GT41 enzyme and  
453 was recently shown to *O*-fucosylate DELLA proteins. DELLA proteins are  
454 negative transcriptional regulators of gibberellin (GA) signaling (Zentella et  
455 al., 2017). In *A. thaliana*, *O*-fucosylation activates DELLA proteins, so that  
456 they are then able to interact with other transcription factors involved in, for  
457 example, brassinosteroid and light signaling pathways (Zentella et al., 2017).  
458 Finally, the most recently studied putative plant POFUT, is *A. thaliana* *O*-  
459 FUCOSYLTRANSFERASE1 (*AtOFUT1*). Mutant analyses showed that this  
460 protein is involved in pollen-pistil interactions, where a pollen tube physically  
461 penetrates specialized tissues during fertilization and germination (Smith et  
462 al., 2018a). Phylogenetic analysis indicated that *AtOFUT1* is more similar to  
463 metazoan POFUT1s, which are GDP-Fuc dependent FUTs that fucosylate  
464 specific Ser or Thr residues in CXXXX(S/T)C consensus sequences within EGF

465 repeat or TSR domains (Smith et al., 2018). In contrast to other putative or  
466 known plant POFUTs, *AtOFUT1* is categorized as a non-classified  
467 glycosyltransferase (GTnc) in the CAZy database. *Atoft1* mutants were  
468 significantly impaired in the ability of their pollen tubes to penetrate the  
469 stigma-style interface, resulting in an almost 2,000-fold decrease in pollen  
470 transmission efficiency, and consequently displayed 5 to 10-fold decreased  
471 seed set (Smith et al., 2018). However, more data will be needed to confirm  
472 the biochemical function of *AtOFUT1*.

### 473 **Plant FUT phylogeny**

474 Although the activities that plant FUTs catalyze are broad and diverse due  
475 to the innate complexities of plant cell wall polysaccharides, proteins and  
476 associated glycans, the plant FUTs are also distinguishable in how they relate  
477 phylogenetically to each other and to FUTs from the other kingdoms of life.  
478 Unlike vertebrate FUTs that form clades based on predicted specificity and  
479 function (Martinez-Duncker et al., 2003), the few and limited trees that have  
480 been published on plant FUTs exhibit an unusual relationship, with clades  
481 largely forming by species rather than predicted function (Sarria et al., 2001;  
482 Liu, Paulitz and Pauly, 2015).

483 A much larger phylogenetic analysis, generated for this review, of 206  
484 plant FUTs sequences from 33 species corroborates this unique phylogenetic  
485 relationship among plant FUTs, with terminal clades generally comprising  
486 single-, or closely related species (Figure 2). This unique phylogenetic  
487 relationship, overall, suggests that sequence homology alone cannot be used

488 to deduce functional homology of FUTs from one plant species to another.  
489 This is exemplified by the case of the rice XyG FUT, *OsMUR2*, that is  
490 phylogenetically distinct from its functional homolog in *A. thaliana*, *AtFUT1*  
491 (Liu et al., 2015) (Figure 2).

492 It is interesting to note that while the FUTs from monocot grasses cluster  
493 within one sector of the phylogenetic tree distinct from other plant FUTs  
494 (Figure 2), the FUTs from any single monocot grass species are dispersed  
495 among the various terminal clades within the monocot grass domain of the  
496 tree. This pattern suggests that there might be functional orthologies among  
497 FUTs from different grass species, but this awaits experimental verification.  
498 It is also interesting that monocot grasses have large FUT families (>10) in  
499 spite of the fact that two commonly fucosylated cell wall glycans, XyGs and  
500 rhamnogalacturonans, are significantly less abundant in monocot grass walls  
501 than in walls from dicots and monocots outside of the Poales.

502 The unusual phylogenetic tree structure for plant FUTs also suggests that  
503 these proteins have very species-specific functions, perhaps even down to  
504 the cellular level. The three GT37 FUTs biochemically characterized thus far  
505 in *A. thaliana*, *AtFUT1*, *AtFUT4*, and *AtFUT6*, exemplify this, as *AtFUT1* is XyG-  
506 specific, while *AtFUT4* and *AtFUT6* are both AGP-specific, but sub-localize to  
507 two distinct regions of the developing root (Sarria et al., 2001; unpublished  
508 results of the authors). As we have alluded to throughout this review, a  
509 greater number of plant FUTs need to be functionally characterized to see if  
510 this hypothesis is valid. Unfortunately, the unusual phylogenetic relationship



511 exhibited by known and putative plant FUTs will complicate the functional  
512 characterization of additional FUTs in diverse plant species.

### 513 **Conclusions**

514 The carbohydrate-active enzymes involved in the biosynthesis of the plant  
515 cell wall are varied and unique in their activities and functions, and are  
516 typically encoded by large gene families, with the various known and  
517 putative FUTs being no exception to this pattern. While the activities of plant  
518 FUTs and the fucosylation of diverse glycans and proteins have been studied  
519 readily across many organisms, the biological importance of fucosylation *in*  
520 *planta* is just starting to be understood. With suggested and proven functions  
521 ranging from cellular communication and growth to cellular adhesion, the  
522 presence or absence of Fuc on various plant structures appears to have  
523 serious implications for proper plant development and response to diverse  
524 stimuli and stress. The FUTs specific for XyG fucosylation are by far the most  
525 thoroughly studied and well-understood. However, recent progress on the  
526 activities of the AGP-specific FUTs has offered additional insights into the  
527 activities and specificities of the plant-specific GT37 family. *N*-glycan  
528 fucosylation and the recent identification of the downstream targets of  
529 POFUTs, offer insight into the involvement of Fuc modifications in structures  
530 beyond the cell wall, as well as into the differences between conserved  
531 pathways in plants and vertebrates.

532 Characterization of FUTs in plant species other than *A. thaliana* has proven  
533 difficult, but not impossible. The continued research into the identification

534 and characterization of functional homologs from additional plant species, as  
535 well as the identification of the FUTs specific for RG-I and RG-II fucosylation  
536 promise to extend our understanding of the physiological role and  
537 importance of Fuc in plant cell wall polysaccharides. In addition, the  
538 characterization of more FUTs from other plant species would aid in  
539 understanding the unique evolutionary diversification pattern exhibited by  
540 this important family of biosynthetic enzymes in plants.

541

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555 M.J.S, B.R.U., and M.G.H. wrote the manuscript.

## 556 **Competing interests:**

557 The authors declare no competing interests.

558 **Data and materials availability:**

559 The data that support the findings of this study are present in the paper and  
560 any data are available from M.G.H. and B.R.U. upon reasonable request.

561

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862 **Tables**

FUT Type	Plant Species	Citation
$\alpha$ -(1,3) FUT	<i>Zea mays</i>	Bondili et al., 2006
$\alpha$ -(1,4) FUT	<i>Silene alba</i>	Léonard et al., 2005
$\alpha$ -(1,4) FUT	<i>Vaccinium myrtillus L.</i>	Palma et al., 2001
$\alpha$ -(1,4) FUT	<i>Mangifera indica L.</i>	Okada et al., 2017
Undetermined	<i>Ricinus communis</i>	Roberts, Mellor and Lord, 1980

863

864 Table 1. Plant FUTs from additional plant species. These FUTs have been  
 865 biochemically characterized to varying extents, but no mutational studies  
 866 have been conducted for associated phenotypes.

867

868 **Figure Legends**

869 **Figure 1.** Fucosylated cell wall poly- and oligosaccharides. (A) Xyloglucan,  
 870 (B) Arabinogalactan proteins, (C) *N*-Glycans, (D) Rhamnogalacturonan II, and  
 871 (E) Rhamnogalacturonan I. Glc, glucose; Araf, arabinofuranose; Arap,  
 872 arabinopyranose; GlcA, glucuronic acid; Gal, galactose; GalA, galacturonic  
 873 acid; Kdo, 3-deoxy-*D*-manno-2-octulosonic acid; GlcNAc, *N*-  
 874 acetylglucosamine; Dha, 3-deoxy-*D*-lyxo-2-heptulosonic acid; Xyl, xylose;  
 875 Man, mannose; Rha, rhamnose; Fuc, fucose; Hyp, hydroxyproline; Ser,  
 876 serine; Thr, threonine.

877

878 **Figure 2.** Phylogenetic tree of 206 plant FUTs from 33 species. A multiple  
 879 sequence alignment of the amino acid sequences of these genes was  
 880 truncated from position 1-340 and from positions 1,156-1,178 to omit large,  
 881 poorly resolved gaps in the alignment. The truncated alignment was then  
 882 used to make a phylogenetic tree by Neighbor-Joining with 200 bootstraps  
 883 and rooted with a *Physcomitrella* clade consisting of the genes  
 884 *Physcomitrella|Pp3c6\_13740V3.1* and *Physcomitrella|Pp3c6\_13730V3.1*; both  
 885 the alignment and tree were made in Geneious. Highlighted in red are the  
 886 ten *A. thaliana* genes, nine of which form a terminal clade. The  
 887 phylogenetically distinct, yet functional homolog to *AtFUT1* in rice, *OsmUR2*,  
 888 is highlighted in purple. Finally, three more species are highlighted: banana  
 889 in green, in which 12 out of 15 genes form a terminal clade; clubmoss in  
 890 blue, in which five out of seven genes form a terminal clade; and *Populus* in  
 891 orange, in which seven out of eight genes form terminal clades. These three  
 892 additional clades are highlighted as further examples of the unusual,  
 893 species-specific phylogenetic grouping of the plant FUTs.

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895