Plant-Type N-Glycans Containing Fucose and Xylose in Bryophyta (Mosses) and Tracheophyta (Ferns)

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The presence of typical plant-type N-glycans (eg, M3FX, Gn2M3FX, and Le\(^{a}\)2M3FX) in mosses, ferns, and other organisms was examined to determine which plant initially acquired glycosyltransferases to produce plant-type N-glycans during organic evolution. No M3FX-type N-glycan was detected in lichens (Cladonia humilis) or in any one of the three preland plants Enteromorpha prolifera, Ulva pertusa Kjellman, and Chara braunii Gmelin. In Bryophyta, M3FX-type N-glycan was detected at trace amounts in Anthocerotopsida (hornworts) and at certain amounts in Bryopsida (mosses), but not in Hepaticopsida (liverworts). Le\(^{a}\)2M3FX was detected in some Bryopsida of relatively high M3FX content. Most Tracheophyta (ferns and higher plants) contained the three typical M3FX-type glycans as the main N-glycans in different ratios. These results suggest that organisms acquired xylosyltransferase and fucosyltransferase during the development of mosses from liverworts, and that later all plants retained both enzymes. Bryopsida have also obtained galactosyltransferase and fucosyltransferase to synthesize the Le\(^{a}\) antigen.

Key words: N-glycan; fern; moss; M3FX Gn2M3FX and Le\(^{a}\)M3FX; Le\(^{a}\) antigen

Since the structure of the oligosaccharide of pineapple stem bromelain was elucidated,\(^{1}\) the determined fucose- and xylose-containing core structure has been found universally in plant glycoproteins. M3FX, consisting of three Man (M), two GlcNAc (Gn), and one each of Fuc (F) and Xyl (X) residues is the most common structure in plants. The oligosaccharides GnM3FX and Gn2M3FX, composed of one and two GlcNAc residues respectively attached to M3FX, are also found universally in plants. The oligosaccharide with the Lewis a antigen, Le\(^{a}\)2M3FX, in which galactose and fucose residues are attached to both GlcNAc residues of Gn2M3FX, is also a main N-glycan universally found in plants\(^{2–5}\) except for Brassicaceae plants such as Arabidopsis thaliana and Raphanus sativus (kaiware raddish). Accordingly, the processing of plant N-glycans in the Golgi apparatus as shown in Fig. 1 is assumed to be almost universal.\(^{5,6}\) N-Glycan processing by \(\alpha\)-glucosidases I and II in ER is important in plants,\(^{7–10}\) whereas N-glycan processing in the Golgi apparatus has little effect on plant growth, as determined in the varous studies: Arabidopsis mutants that lack N-acetylglucosaminyltransferase I\(^{11}\) or both fucosyltransferase and xylosyltransferase activities\(^{12}\) grow normally and produce normal seeds. Fucosyltransferase and xylosyltransferase knockout plants of Bryopsida (Physcomitrella patens) grow normally.\(^{13}\) In my study using glycosidase inhibitors on germination, the seedling and leaf growth of R. sativus showed that ER-glucosidase inhibition inhibited the growth of the plant, although the inhibition of N-glycan processing in Golgi by deoxy-mannojirimycin, swainsonine, and 1,2-dideoxy-2-acetamido-nojirimysin had no eminent effect except for a change in the N-glycan structure.\(^{10,14}\) These results indicate that the final N-glycan structure is not directly associated with plant growth or differentiation. However, M3FX-type N-glycans are supposed to have important unknown functions, because almost all the plants have retained M3FX-type glycans (M3FX, Gn2M3FX, and Le\(^{a}\)2M3FX) as the main N-glycans during evolution. Hence, in an attempt to search for the function of oligosaccharides, I searched for boundary organisms that have acquired the M3FX-type structure in the evolutionary lineage. The moss P. patens has 19 N-glycans, including M3FX-type oligosaccharides as main components.\(^{15}\) Its fucosyltransferase and xylosyltransferase genes and their promoters have been studied.\(^{16}\)

In this study, the presence of typical plant-type N-glycans was examined in pre- and some post-land plants (shown in the photographs in Fig. 2), and some other plants. These analyses showed that M3FX-type oligo-
saccharides not detected in preland plants appeared in mosses and became major components in vascular plants except for *Psilotum* and *Selaginella* species.

**Materials and Methods**

**Materials.** *Dawsonia longifolia* (a tall Bryopsida), *Spiridens reinwardtii* (a kind of moss parasitic to tree ferns), and *Anthoceros punctatus* (a kind of hornwort) were kindly donated by Dr. H. Akiyama (Museum of Nature and Human Activities, Hyogo, Japan). *Physcomitrella patens* Bruch & Schimp subsp. *patens* was kindly donated by Professor M. Hasebe (National Institute for Basic Biology, Okazaki, Japan). *Chara braunii Gmelin* (a kind of Charophyceae) was kindly donated by Dr. S. Takagi (Osaka University). Ferns such as *Psilotum nudum*, *Angiopteris lygodifolia*, *Lycopodium clavatum*, *Salvinia natans* (L.) All., *Microsorium pteropus*, *Selaginella tamariscina*, *Selaginella uncinata*, and *Nephrolepis auriculata* were obtained commercially. The other plants were obtained in a field at Osaka University, in some temple gardens in Kyoto, and in some other places. Both jack bean α-mannosidase and β-N-acetylgalactosaminidase were purchased from Seikagaku Kogyo Co., Ltd. (Tokyo). *Streptomyces* sp.142 1-3/4 fucosidase was purchased from Takara-Bio (Ohtu), β-galactosidase from *A. oryzae* (St. Louis, MO), and β1,3-specific galactosidase from Calbiochem-Merck (Darmstadt).

**Preparation of PA-oligosaccharides from plants.** The plants were lyophilized after they were washed with water. Dried samples, mainly leaves of the plants (2–6 mg), were heated at 100 °C for 10 h in 0.4 ml of hydrazine in a tube. Oligosaccharides released in hydrazine were N-acetylated, pyridylaminated, and analyzed by HPLC after purification by phenol-chloroform extraction and Dowex 50 NH⁴⁺ filtration with H₂O according to the conventional method used in Hase’s laboratory, and as also described in my previous papers. SF-HPLC was performed with an Asahipak NH2P-50 column (2.0 × 150 mm) at a flow rate of 75 μl/min. RP-HPLC was performed with a Cosmosil SC18-P column (1.5 × 250 mm) at a flow rate of 150 μl/min.

**Analysis of PA-oligosaccharides.** PA-oligosaccharide mixtures obtained from the samples were dissolved in water, usually at a concentration of 1 mg dry plants/10 μl, and 1 or 2 μl of each sample was analyzed by SF-HPLC (Figs. 3A-1, 4A-2 and A-3). Peaks that eluted between 45–90 min, corresponding to 5–13 glucose units (PA-isomalto-oligosaccharides), were fractionated usually using 4 μl of the sample, and each fraction was analyzed by RP-HPLC. The results of RP-HPLC are summarized in B-1, B-2, and B-3 in Figs. 3 and 4. The above HPLCs were performed for all samples. PA-oligosaccharides were detected by HPLC by measuring fluorescence. The excitation and emission wavelengths were 310 and 380 nm respectively.

The structures of M3FX and Gn2M3FX were confirmed with jack bean α-mannosidase (Figs. 3C-1, 4C-2, 7A inset, 8, and 9C) and jack bean β-N-acetylgalactosaminidase (Figs. 3D-1, 4D-2, and 5E) digestion. Glycosidase digestion of PA-oligosaccharides was performed by 0.2 u of α-mannosidase in 8 μl of 0.08 M acetate buffer (pH 4.5) or with 0.1 u of β-N-acetylgalactosaminidase in 8 μl of 0.08 M acetate buffer (pH 5.0) at 37 °C overnight. The digest was analyzed by SF-HPLC. A mixture of N-glycans is sometimes used without fractionation for some samples of high M3FX-type oligosaccharide content, such as most vascular plants. Isolated fractions were also used in some cases to confirm the oligosaccharide structure.

The Le²M3FX structure was confirmed by 1 μu of α1-3/4 fucosidase (*Streptomyces*) digestion in 0.05 M acetate buffer (pH 6.0) at 37 °C overnight, followed by β1,3-specific galactosidase (0.01 u) digestion in the

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**Fig. 1.** Post Endoplasmic Reticulum N-Glycan Processing in Plant, and Abbreviations of Oligosaccharides.
Fig. 2. Photographs of Samples Used.
same buffer at 37 °C overnight. These enzymatic analyses were performed for the 11 kinds of plant that showed peaks at the Lea2M3FX position.

The amounts injected in SF- and RP-HPLCs usually corresponded to the amount from 0.1 and 0.02 mg of lyophilized plants respectively. When the detected peaks were small, twofold or fourfold the amount of the sample was injected. M3FX, Gn2M3FX, and Lea2M3FX contents were basically calculated from the area of the peak in the RP-HPLC profile without compensation depending on the loss of the HPLC repeat and reaction yield. When the peak was a main clearly separated peak on SF-HPLC and found to be pure on RP-HPLC, the peak area on SF-HPLC was used. Total oligosaccharide content was estimated from the total area of all peaks that appeared between 5 and 13 glucose units in the SF-HPLC profile. The amounts of plant type N-glycans detected in ferns are shown in Table 1. The amount of each plant-type oligosaccharide is summarized in Table 2 with percent ratio to the total oligosaccharide content. In the table, the M3FX-type oligosaccharide contents are shown as +++ for plants with oligosaccharide contents higher than 15% of total oligosaccharides. Those with contents higher than 5%, 0.5%, and 0.1% are shown as ++, +, and ± respectively. Those with no oligosaccharide are shown as −. A blank means that no eminent peak of the oligosaccharide was detected.

**Results**

N-Glycans in lyophilized leaves of plants (Fig. 2) were analyzed according to the method described above. All the samples presented several peaks that might have been derived from N-glycans in the SF- and RP-HPLC profiles. Of these peaks, only those that might contain M3FX-type N-glycans were selected and examined. Representative HPLC profiles are shown to facilitate understanding. Figure 3 shows the result for *E. arvense* L. (photo 23) as an example of a plant with a high M3FX-type oligosaccharide content. Figure 4 shows those for *S. tamariscina* (photo 20) and *P. nudam* (photo 19) as examples of plants with low and trace M3FX oligosaccharide contents. Figure 5 shows the result for *Cinamomum* (photo 30) as evidence of the identification of Lea2M3FX, which was used as the standard compound. Figure 6 shows chromatograms that confirmed the presence of Lea2M3FX in some plants. As Fig. 7 shows, no M3FX was detected in Hepaticopsida (liverwort, photos 4–7). Figure 8 shows chromatograms confirming the presence of M3FX in some mosses. As Fig. 9 shows, a tall moss had a high M3FX content. Figure 10 shows the result of *P. patens* as a control-experiment moss, because its N-glycan structures have been reported (15). These figures are explained briefly below.
Example results for some ferns

Figure 3A-1 is a size-fractionation chromatogram of the PA-oligosaccharide mixture from *E. arvense* (pho-to 23). Peaks that eluted between 5 and 13 glucose units were separated into 11 fractions, as shown in the figure. Peaks corresponding to M3FX (a), Gn2M3FX (b), and Le\(^a\)2M3FX (c) were detected in Fr-2, Fr-6, and Fr-11 respectively in the RP-HPLC profile (B-1). The SF-HPLC profile of the \(\alpha\)-mannosidase digest of Fr-3 isolated from chromatogram A-2 is shown in C-2. That of \(\beta\)-N-acetylglucosaminidase digest of A-2 (PA-oligosaccharide mixture) is shown in D-2. The top chromatogram in D-2 shows the enzyme digest; the bottom chromatogram shows no digest. Arrowheads are the same as in Fig. 3.

**Fig. 4.** Results for *S. tamariscina* (photo 20) and *P. nudum* (photo 19) as Representative Plants Containing Small and Trace Amounts of M3FX-Type Oligosaccharide Respectively.

The SF-HPLC profiles of PA-oligosaccharide mixture isolated from *S. tamariscina* and *P. nudum* are A-2 and A-3, and the RP-HPLC profiles of the separated fractions are B-2 and B-3 respectively. SF-HPLC profile of \(\alpha\)-mannosidase digest of Fr-3 isolated from chromatogram A-2 is shown in C-2. That of \(\beta\)-N-acetylglucosaminidase digest of A-2 (PA-oligosaccharide mixture) is shown in D-2. The top chromatogram in D-2 shows the enzyme digest; the bottom chromatogram shows no digest. Arrowheads are the same as in Fig. 3.

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digest (Fig. 3, D-1) shows the presence of Gn2M3FX on the basis of an increase in the size of M3FX peak a and a decrease in that of peak b at 63 min after enzyme digestion. The presence of Le\(^2\)M3FX in Fr-11 was confirmed by \(\alpha\)1-3/4 fucosidase and \(\beta\)1,3-specific galactosidase digestion (data not shown). Most ferns such as *E. arvense* contained M3FX-type oligosaccharides as main sugars, and hence the assignment and determination of M3FX-type oligosaccharides in these plants was easy, except in those with low oligosaccharide content, as explained below.

The HPLC profiles of the PA-oligosaccharides of a Glossopsida species (*S. tamariscina*, photo 20) and
P. nudum (photo 19) are shown in Fig. 4 (A-2 and B-2) and Fig. 4 (A-3 and B-3) respectively. A small peak was detected at the elution position of M3FX by RP-HPLC in Fr-3 (B-2) and trace amounts or nothing in Fr-1 (B-3). α-Mannosidase digestion of Fr-3 gave peak a’ at 37 min, corresponding to MFX (Fig. 4, C-2). The β-N-acetylglucosaminidase digest of the PA-oligosaccharide mixture of the Glossopsida species (Fig. 4, D-2) and the RP-HPLC profiles of Fr-6, Fr-7, and Fr-8 (B-2) showed no presence of Gn2M3FX. No Leα2M3FX was detected in the RP-HPLC profile of Fr-12 or Fr-13 (B-2).

Fr-1, Fr-5, and Fr-8 in (A-3), corresponding to the elution positions of M3FX, Gn2M3FX, and LeαM3FX, gave no peaks corresponding to the oligosaccharides in the RP-HPLC profile (B-3), except for a trace peak corresponding to M3FX.

Identification of Leα structure in cinnamomum leaf (photo 30) and other plants

As in Fig. 5, the cinnamomum leaf gave a peak at the estimated elution position of Leα2M3FX, corresponding to 11.6 glucose units on SF-HPLC (A-4), and the peak was fractionated as Fr-9, which gave a peak at 25 min on RP-HPLC (B-4). The results of sequential exo-glycosidase digestion of Fr-9 with α1-3/4 fucosidase, β1,3-specific galactosidase, A. oryzae β-galactosidase, and β-N-acetylglucosaminidase are shown in (E). They indicate the presence of Leα2M3FX. A. oryzae β-galactosidase did not hydrolyze the de-Fuc-oligosaccharide (c’), although β1,3-specific galactosidase effectively released two galactose residues, producing Gn2M3FX (b) (Fig. 5, E and F), because the A. oryzae enzyme was inactive to β1,3-linked-galactoside, like diplococcal β-galactosidase.4)

A peak corresponding to Leα2M3FX was detected in 11 kinds of plant. These peaks were confirmed by α-fucosidase digestion followed by β1,3-specific galactosidase digestion. The elution profile of the α-fucosidase digests of some of these samples (Fig. 6A) shows that peak at Leα2M3FX(c) decreased in area, indicating the production of de-Fuc-oligosaccharide (c’) by the digestion, except in the case of P. dozyanum (photo 15). Sequential digestion with β1,3-specific galactosidase (Fig. 6B) produced Gn2M3FX (b) in P. multifida (photo 28), S. natans (photo 27), N. auriculata (photo 26), D. fuscipes (photo 25), and M. flagellaris (photo 9), except for P. dozyanum (photo 15).

M3FX identification in some mosses

Nondetection of M3FX in Hepaticopsida (liverworts)

The RP-HPLC profiles of M3FX fractions separated on SF-HPLC of four Hepaticopsida species are shown in Fig. 7. Two fractions (Fr-1 and Fr-2) around M3FX for each sample were examined. No peak was detected around M3FX except for Fr-1 of A, but the fraction gave no MFX in α-mannosidase digests (Fig. 7A inset). These analyses suggest that Hepaticopsida species contain small amounts or none of M3FX, although
Fig. 6. Identification of Leα in Certain Plants by Sequential Exo-Glycosidase Digestion with α1-3/4 Fucosidase (A) Followed by Digestion with β1,3-Galactosidase (B).

Samples are the fractions on SF-HPLC those presumably contain Leα2M3FX. 1, P. multifida (photo 28); 2, S. natans (photo 27); 3, N. auriculata (photo 26); 4, D. fuscipes (photo 25); 5, F. dozyanum (photo 15); 6, M. flagellaris (photo 9). Arrowheads show the elution positions of standard PA-oligosaccharides of Leα2M3FX (c), Gal2Gn2M3FX (c'), and Gn2M3FX (b).

Fig. 7. RP-HPLC Profiles of Two Fractions (Fr-1 and Fr-2) for Each Hepaticopsida Species That Might Contain M3FX, to Show That Hepaticopsida Does Not Contain the Oligosaccharide.

A, M. polymorpha (photo 5). Inset A is the SF-HPLC profile of α-mannosidase digest of Fr-1 (top) and that of before digestion (bottom). B, P. endiviifolia (photo 7). C, R. hemisphaerica subsp. orientalis (photo 4). D, C. japonicum (photo 6). Arrowhead shows the elution position of M3FX.
Vieitor et al. immunologically detected both core fucose and core xylose in some Hepaticopsida species.\textsuperscript{15}

Detection of M3FX in Anthocerotopsida (hornworts) and Bryopsida (mosses)

All Anthocerotopsida and many species of Bryopsida presented small peaks around M3FX together with large peaks among glucose units 5–13 on SF-HPLC. The corresponding M3FX fractions were reanalyzed to confirm the structure by SF-HPLC after α-mannosidase digestion. A peak corresponding to MFX (a’) was detected (Fig. 8) in these mosses. This analysis revealed the presence of M3FX in trace amounts in Anthocerotopsida species (hornworts: A and D) and small amounts in almost all Bryopsida species (mosses: B, C, and E). The amount in D. longifolia (photo 18), which grows up to 80 cm tall, was the highest (Fig. 9) among the mosses, and that in P. commune (photo 17, about 10 cm tall) was higher than those in other mosses. The structure of the peak in Fr-1 was confirmed to be M3FX using α-mannosidase (Fig. 9C). A small peak corresponding to \( \text{Le}^\text{a2M3FX} \) was also detected in Fr-10 (Fig. 9B). The M3FX content of the parasitic moss S. reinwardtii (photo 16), which lives on Cyateaceae (a kind of fern) and grows up to 30 cm in length, was low. The M3FX contents of creeping mosses, such as B. novae-angliae (photo 10) and P. proligera, were relatively low among mosses (data not shown). The M3FX content of the relatively tall moss P. dozyanum (photo 15, about 10 cm tall) was slightly high. P. patens is a model plant, and its N-glycan structure has been elucidated. Then the N-glycan structure was examined to verify the analyses as a control (Fig. 10). The elution profiles of the N-glycans on SF-HPLC and its α-mannosidase digest manifested the presence of M3FX,

Fig. 9. Results of D. longifolia (photo 18) Showing High M3FX-Type N-Glycan Content Although It Is a Moss.
A, SF-HPLC profile of N-glycans of D. longifolia. B, RP-HPLC profile of Fr-1, Fr-5 and Fr-10. C, SF-HPLC profile of α-mannosidase digest of Fr-1. D, β-N-acetylglucosaminidase digest of oligosaccharide mixture (top) and no digest (bottom). Arrowheads are as in Fig. 3.

Fig. 8. SF-HPLC Profile of α-Mannosidase Digest of Certain Moss Fractions That Might Contain M3FX.
A, A. punctatus (photo 8); B, P. proligera; C, T. cymbifolium (photo 12); D, M. flagellaris (photo 9); E, P. dozyanum (photo 15). a’ is the elution position of MFX which is a product of M3FX by α-mannosidase digestion.
Gn2M3FX, and Leα2M3FX, and this corresponds to the results previously reported. The relative M3FX content of *P. patens* was high though it is a small moss, but the M3FX content was not very different from those of other small mosses.

The amounts of M3FX-type N-glycans detected in vascular plants are summarized in Table 1. The M3FX, Gn2M3FX, and Leα2M3FX amounts were calculated from the area of the peak on RP-HPLC or SF-HPLC. Total oligosaccharide amounts were estimated from the total area of the all peaks that appeared between glucose units 5 and 13 in the SF-HPLC profile, because most of the peaks that appeared in these areas were found to be derived from N-glycans in the previous analyzes of *R. sativus* (10, 14). The yield of the analysis varied greatly (roughly 20–60%), although the relative area of each peak was constant. Hence the M3FX-type oligosaccharide contents were expressed as the amount (%) relative to the total oligosaccharide content. They summarized in Table 2 together with the results for all the other samples. The values indicate minimum contents, because the total oligosaccharide contents contained impurities derived from nonglycans and free oligosaccharides, and because the M3FX-type oligosaccharide content was not corrected, as described. The results for *Pinaceae Cedrus* (Gymnospermophyta), *Cinnamomum* (Anthophyta), and *R. sativus* (Anthophyta) are shown in Table 2.

**Discussion**

*M3FX-type N-glycans in pre-land plants, mosses, and ferns*

Bryophyta are supposed to be the first land plants, and the species in this division are classified into three classes: Hepaticopsida, Anthocerotopsida, and Bryopsida. The present analysis of M3FX-type N-glycans in mosses showed different results for each class (Table 2). M3FX was not detected in Hepaticopsida (Fig. 7), was detected in trace amounts in Anthocerotopsida (Fig. 8), and was detected in various amounts in Bryopsida, depending on species (Figs. 8–10). The oligosaccharide was not detected in lichens, green algae, or chara (Table 2), which are ancestors of Bryophyta during lineage or organic evolution. These results suggest that the formation of M3FX-type glycans was an event in the evolution of Bryophyta.

From the results for the five classes of Tracheophyta, namely, Psilotophyta, Lycopodsya (Glossopsida), Sphenopsida, Marratiopsida, and Pteropsida (Letosporangiopsida), M3FX was detected in all these plants, except for a Psilotophyta, *P. nudum* (photo 19). *P. nudum* is a primitive fern consisting of a stem that does not develop leaves or roots. Both Selaginella, *S. tamariscina* (photo 20) and *S. uncinata* (photo 21), resembling mosses morphologically in spite of having vascular bundle, contained a low M3FX content, similarly to many Bryophyta species. *Lycopodium clavatum* (photo 22) contained M3FX as the main oligosaccharide, differently from Selaginella species, although both belong to the same class on a classification. There remains a little

![Fig. 10. SF-HPLC Profile of N-Glycan from *P. patens* (Bottom) and Its α-Mannosidase Digest (Top). Arrowheads are as in Fig. 3.](image)

Table 1. Detected Amounts of M3FX-Type Oligosaccharides in Certain Trachephtophyta Species

<table>
<thead>
<tr>
<th>Tracheophyta</th>
<th>M3FX p mol/mg</th>
<th>Gn2M3FX p mol/mg</th>
<th>Leα2Gn2M3FX p mol/mg</th>
<th>Total oligosaccharides p mol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psilotum nudum</em></td>
<td>(1.8)</td>
<td>—</td>
<td>—</td>
<td>510</td>
</tr>
<tr>
<td><em>Selaginella tamariscina</em></td>
<td>4.7</td>
<td>—</td>
<td>—</td>
<td>430</td>
</tr>
<tr>
<td><em>Selaginella uncinata</em></td>
<td>2.3</td>
<td>—</td>
<td>—</td>
<td>390</td>
</tr>
<tr>
<td><em>Lycopodium clavatum L.</em></td>
<td>44</td>
<td>6.2</td>
<td>60</td>
<td>230</td>
</tr>
<tr>
<td><em>Equisetum arvense L.</em></td>
<td>14</td>
<td>21</td>
<td>16</td>
<td>240</td>
</tr>
<tr>
<td><em>Aglisteris lygodifolia</em></td>
<td>170</td>
<td>54</td>
<td>nc</td>
<td>720</td>
</tr>
<tr>
<td><em>Dryopteris fuscipes</em></td>
<td>22</td>
<td>39</td>
<td>43</td>
<td>270</td>
</tr>
<tr>
<td><em>Nephrolepis auriculata</em></td>
<td>1</td>
<td>8.7</td>
<td>4.4</td>
<td>68</td>
</tr>
<tr>
<td><em>Salvinia natans</em></td>
<td>2.5</td>
<td>28</td>
<td>5.1</td>
<td>170</td>
</tr>
<tr>
<td><em>Pteris multifida</em></td>
<td>56</td>
<td>67</td>
<td>24</td>
<td>540</td>
</tr>
</tbody>
</table>

Total amount of oligosaccharides was calculated from the total peak area of all peaks eluted between glucose units 5 and 13 on SF-HPLC. Amounts of M3FX, Gn2M3FX, and Leα2M3FX were calculated from the peak area on RP-HPLC or SF-HPLC.

—, trace amount if present
nc, small peak detected but no structure confirmed
( ), not confirmed by α-mannosidase digestion
null
hydrophilic nature of N-glycans contributes to growth, but this speculation conflicts with the finding that Arabidopsis mutants that lack both fucosyltransferase and xylosyltransferase\textsuperscript{12} or N-acetylgalcosaminyltransferase-I\textsuperscript{11} grow normally. This contradiction might be explained by the fact that oligomannose-type N-glycans also have a similar hydrophilic property, and accordingly can replace M3FX-type oligosaccharides. The report that the STT3 subunit isoform of Arabidopsis oligosaccharyltransferase is associated with salt/osmotic regulation, because the decreased amount of hydrophilic N-glycan in subunit isoforms might be directly associated with osmosis.

In recent years, evolution has been studied, in most cases using molecular biology, involving a comparison of gene sequences, amino acid sequences, or 16S-rRNA sequences. Present study indicates that PA-analysis of N-glycan structure\textsuperscript{17–19} is an effective method of studying evolution, because the presence of M3FX-type N-glycans means the presence of many enzymes that participate in the biosynthesis of N-glycans (Fig. 1). That is, the presence of Le\textsuperscript{2}M3FX means at least the presence of β1,3-galactosyltransferase, both α1,4 and α1,3-fucosyltransferases, β1,2-xylosyltransferase, β1,2-N-acetylgalcosaminyltransferase, and α-mannosidase in the Golgi apparatus. Le\textsuperscript{2}M3FX was detected first in a Bryophyte, P. patens,\textsuperscript{15} and was confirmed in D. longifolida and in P. patens in this study. Lewis a has been detected immunologically in certain Hepaticopsida species.\textsuperscript{15} This probably means that both β1,3-galactosyltransferase and α1,4-fucosyltransferases were evolved before Bryopsida. Land plants are supposed to have appeared about 400 million years ago. Ferns appeared in the Devonian Period (350–300 million years ago) and have survived until today. All the land plants tested, except for liverworts, retained considerable amounts of M3FX-type N-glycans. These results correspond to the genomics of mosses, that is, P. patens has a high homology to that of the higher plant A. thaliiana.\textsuperscript{21} N-Glycan analyses yielded more precise information on the expression of related genes in the organism, and on efficiencies of the expressed enzymes, because the amounts of N-glycans depend mostly on biosynthesis and degradation in the organism, and biosynthesis depends on the efficiency, amount, stability, and specificity of the enzymes that participate in processing (Fig. 1).

An Arabidopsis mutant that lacks both fucosyltransferase and xylosyltransferase can grow normally,\textsuperscript{12} and an N-acetylgalcosaminyltransferase I mutant that has oligomannose-type N-glycans not of the complex type due to lack of the initial processing enzyme in the Golgi apparatus can also grow normally.\textsuperscript{11} Inhibition of glycosidases in the Golgi by low-molecular-weight inhibitors had little effect on R. sativus growth.\textsuperscript{10,14} In spite of these facts, M3FX-type N-glycans considered to have important unknown functions in plants, because all plants retained M3FX-type N-glycans for millions of years. The following are candidate functions of N-glycans that do not contradict the above results: N-glycans in plants contribute (a) the stabilization of enzymes that participate in cell wall synthesis, (b) protection of such enzymes from attack by proteases, (c) reinforcement of the cell wall, (d) protection of plants against low and high temperatures, and (e) protection of plants against drying. These functions all depend on the physical properties of N-glycans.

Analyses of N-glycans in more than 30 species of plants showed that plant-type N-glycans appeared during the evolution of mosses in small amounts and became major N-glycans in a few specieis of moss. Ferns containing M3FX-type oligosaccharides as main N-glycans grew larger and prospered in the Carboniferous Period. Since then, almost all plants have retained the oligosaccharide as major N-glycans. N-Glycans must have some advantages for plants, although some mutants without M3FX-type N-glycans grow well.

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