

The *mur4* Mutant of Arabidopsis Is Partially Defective in the de Novo Synthesis of Uridine Diphospho L-Arabinose¹

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To obtain information on the synthesis and function of arabinosylated glycans, the *mur4* mutant of Arabidopsis was characterized. This mutation leads to a 50% reduction in the monosaccharide L-arabinose in most organs and affects arabinose-containing pectic cell wall polysaccharides and arabinogalactan proteins. Feeding L-arabinose to *mur4* plants restores the cell wall composition to wild-type levels, suggesting a partial defect in the de novo synthesis of UDP-L-arabinose, the activated sugar used by arabinosyltransferases. The defect was traced to the conversion of UDP-D-xylose to UDP-L-arabinose in the microsomal fraction of leaf material, indicating that *mur4* plants are defective in a membrane-bound UDP-D-xylose 4-epimerase.

L-Ara, the 4-epimer of D-Xyl, is a monosaccharide found primarily in plants. It is mainly present in the arabinogalactan side chains of pectic material, in glucuronoarabinoxylans, in Hyp-rich glycoproteins, and in arabinogalactan proteins (AGPs) (for review, see Carpita and Gibeaut, 1993). Arabinosylated glycans are believed to play important roles in plant development. The Ara-containing pectic material and the extensins have a structural role in determining wall porosity (Baron-Epel et al., 1988; McCann and Roberts, 1991, 1994) and in strengthening the cell wall (Showalter, 1993), respectively. AGPs are thought to be involved in embryogenesis (Kreuger and van Holst, 1995), cell-to-cell interactions (Knox, 1992), plant defense (Showalter and Varner, 1989), fertilization (Cheung et al., 1993, 1995; Wu et al., 1995; Roy et al., 1998), cell proliferation (Serpe and Nothnagel, 1994), and cell expansion (Schopfer, 1990; Zhu et al., 1993; Willats and Knox, 1996; Ding and Zhu, 1997).

In plants, Ara-containing polymers are derived from UDP-L-Ara, the activated sugar used by arabinosyltransferases (Feingold and Avigad, 1980). In most instances, transfer of Ara residues from the nucleotide sugar to acceptor molecules is accompanied by a ring contraction that converts the pyranose form of Ara to its furanose form (Fry and Northcote, 1983). UDP-Ara is synthesized de novo from UDP-Glc via UDP-GlcUA and 4-epimerization of UDP-Xyl (Fig. 1). UDP-Ara can also be synthesized through a salvage pathway from free Ara via the sequential action of the enzymes L-arabinokinase, for which a mutant has been described in Arabidopsis (Dolezal and Cobbett,

1991), and UDP-Ara pyrophosphorylase. No mutant has previously been described in the de novo synthesis of Ara.

To study the synthesis of arabinosylated glycans using a genetic approach, ethyl methanesulfonate-induced mutants with an altered Ara composition were isolated in Arabidopsis by a biochemical screening procedure (Reiter et al., 1997). One of these mutants, *mur4*, shows a 50% reduction in the Ara content of leaf-derived cell wall material. We demonstrate that the *mur4* mutation affects the final step in the de novo synthesis of Ara, causing reduced arabinosylation of cell wall polysaccharides and AGPs.

MATERIALS AND METHODS

Plant Material

Arabidopsis plants were grown in environmental chambers at 23°C and 70% RH under continuous fluorescent light (60–70 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Wild-type plants of the Columbia ecotype and mutant plants carrying the *mur4-1* allele were used for all biochemical assays. The *mur4-1* mutant was backcrossed six times to wild type to remove background mutations.

Cell Wall Fractionation

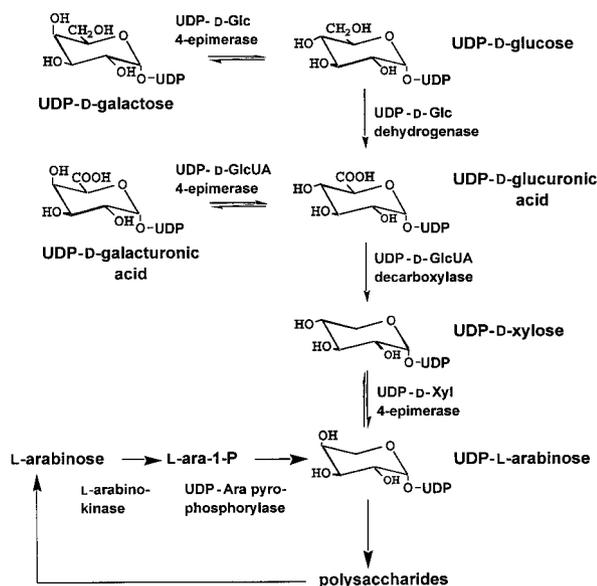
Three-week-old plants were starved for 24 h in the dark prior to harvesting to deplete starch reserves. Fifteen grams of fresh leaves were harvested, ground in the presence of dry ice and liquid N₂ to fracture the cell walls, and the leaf material was fractionated as described previously (Reiter et al., 1997). The cell wall material was resuspended in 25 mL of 90% (v/v) DMSO, spun at 10,000 rpm for 15 min in a rotor (model SS34, Sorvall, Newtown, CT), resuspended in another 25 mL of 90% (v/v) DMSO, and shaken overnight at room temperature to remove residual starch. The wall material was pelleted and washed six times with 30 mL each of water. Three aliquots of wall material were analyzed for monosaccharide composition by GC of alditol acetates (Blakeney et al., 1983; Reiter et al., 1993) and for total uronic acids by the *m*-hydroxybiphenyl method (Filisetti-Cozzi and Carpita, 1991).

AGP Extraction and Analysis

Soluble polymers were extracted from 15 g of leaf material as described previously (Reiter et al., 1997). The polymers from the supernatant were precipitated with one-tenth volume of 5 M ammonium acetate, 2.5 volumes of

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SALVAGE PATHWAY DE NOVO PATHWAY

Figure 1. Pathways involved in L-Ara biosynthesis and reutilization.

ethanol, and 2.5 volumes of acetone, and left in the cold room overnight. The precipitate was recovered by centrifugation at 10,000 rpm in the rotor for 15 min. The pellet was washed twice with 80% (v/v) ethanol and resuspended in a small volume of 4 M guanidinium chloride. The suspension was dialyzed against water in the cold room. The dialysate was spun at 5,000 rpm in the rotor for 10 min, and the supernatant containing the water-soluble polymers was neutralized to pH 7.0. This clear solution was redialyzed against water and then lyophilized. The lyophilized material was resuspended in water, spun, and the supernatant was saved as AGP extract. Total carbohydrates were measured by the phenol-sulfuric acid assay (Dubois et al., 1956), and total uronic acids were determined by the *m*-hydroxybiphenyl method (Filisetti-Cozzi and Carpita, 1991).

Electrophoresis of AGPs was performed in a 1% (w/v) agarose gel containing 90 mM Tris, 90 mM boric acid, and 2 mM EDTA (pH 8.3). Four percent (w/v) Suc and 0.025% (w/v) bromphenol blue (final concentrations) were added to the samples prior to loading. Gels were stained with 15 μ M Yariv reagent synthesized as described previously (Yariv et al., 1962, 1967) and destained in 1% (w/v) NaCl. AGPs were fractionated by gel permeation chromatography using Sepharose CL-4B in a 100- \times 1.5-cm i.d. column. The column was calibrated with gel filtration molecular mass markers from Sigma-Aldrich (St. Louis): 29 kD, carbonic anhydrase; 66 kD, BSA; 150 kD, alcohol dehydrogenase; 200 kD, β -amylase; blue dextran with an average molecular mass of 2,000 kD; and a 1% (w/v) Glc solution. The column was run in 50 mM ammonium acetate buffer, pH 7.0, at a flow rate of 0.5 mL min⁻¹. Eighty fractions of 2.2 mL each were collected and analyzed for their monosaccharide composition via GC of alditol acetates.

Biochemical Assays

Feeding Studies

Seeds were surface-sterilized by soaking in 30% bleach containing 0.1% Triton X-100 for 10 min, followed by several washes in sterile water. The seeds were transferred onto a nylon mesh on top of two layers of cheesecloth and placed on the surface of 0.7% agar plates containing nutrient medium (Haughn and Somerville, 1986) with different concentrations of Ara. Leaves were harvested after 16 d, extracted twice for 1 h each at 70°C with 1 mL of 70% (v/v) ethanol, once for 2 min at room temperature with 1 mL of acetone, and analyzed for their monosaccharide composition.

In Vivo Metabolism of L-Ara and D-Glc

Elongating inflorescence stems cut from soil-grown plants were added to 5 μ L of water containing 0.005% Silwet L-77 and either 18.5 kBq L-[1-¹⁴C]Ara (2.04 GBq/mmol, American Radiolabeled Chemicals, St. Louis) or 18.5 kBq D-[U-¹⁴C]Glc (11.1 GBq/mmol, American Radiolabeled Chemicals), vacuum infiltrated for 2 min at 2 torr, and incubated in the light for 90 min. After incubation, the samples were washed in water containing 0.1% Tween 20 and extracted five times for 20 min each with 2 mL of 80% (v/v) ethanol in a boiling water bath. The ethanol extracts were pooled and the stems were further extracted with acetone. The ethanol-insoluble fraction was hydrolyzed in 2 mL of 2 M TFA at 100°C for 3 h and then the TFA was removed in vacuo. The sugars were analyzed by TLC on 250- μ m cellulose plates (J.T. Baker, Phillipsburg, NJ) run in 1-butanol:acetic acid:water (12:3:5, v/v) followed by ethyl acetate:pyridine:water (8:2:1, v/v) in the same direction. The sugars were also analyzed by TLC on silica plates (Sigma) run in 1-propanol:30% (w/w) ammonium hydroxide:water (6:2:1, v/v) to separate the uronic acids from each other. Monosaccharides were identified by non-radiolabeled sugar standards stained with aniline-hydrogen phthalate (Fry, 1988). For the detection and quantitation of radiolabeled monosaccharides, the TLC plates were exposed to a phosphor-imaging screen (Bio-Rad Laboratories, Hercules, CA) and analyzed using Molecular Analyst software (Bio-Rad).

In Vitro Assay of the de Novo Synthesis of UDP-L-Ara

Membrane-bound and soluble proteins were extracted from 55 g of fresh Arabidopsis leaves by grinding with acid-washed sand in 2 volumes of ice-cold 100 mM Hepes-KOH (pH 7.4), 1 mM EDTA, 1 mM DTT, 0.4 M Suc, 0.1% (w/v) BSA, and 1% (w/v) polyvinylpolypyrrolidone. The homogenate was filtered through Miracloth (Calbiochem-Novabiochem, San Diego) and spun at 3,000g and 4°C in the rotor for 10 min to pellet cell debris. The pellet was discarded and the supernatant was spun at 105,000g in a swinging bucket rotor (model SW28, Beckman Instruments, Fullerton, CA) for 1 h at 4°C. The microsomal fraction was briefly washed and then resuspended in 210 mL of

homogenization buffer. After recentrifugation at 105,000g, the pellet was resuspended in 1.8 mL of 40 mM Tris-HCl (pH 7.6), 5 mM EDTA, 0.5 mM DTT, and 160 mM Suc.

Soluble proteins were precipitated from the ultracentrifugation supernatant by adding ammonium sulfate to 75% saturation at 0°C. The proteins were pelleted by centrifugation at 10,000 rpm for 45 min in the rotor, resuspended in 2 mL of 20 mM Tris-HCl (pH 7.6) and 0.5 mM DTT, and then dialyzed overnight at 4°C against 2 L of the same buffer. The protein extracts were assayed for protein concentration using the Dotmetric Kit (Geno Technology, St. Louis). Protein extracts (2 mg) were incubated with 1.85 kBq UDP-D-[U-¹⁴C]GlcUA (10.6 GBq/mmol, American Radiolabeled Chemicals) at ambient temperature for 15, 30, and 60 min in a final volume of 30 μ L. As a control, a boiled protein extract was incubated with 1.85 kBq UDP-D-[U-¹⁴C]GlcUA. The resulting UDP sugars were hydrolyzed by the addition of 300 μ L of 2 M TFA and incubation at 98°C for 20 min. The TFA was removed in vacuo. The samples were resuspended in 200 μ L of water and partitioned against 2 volumes of diethylether to remove most of the pigments associated with the microsomal fraction. The aqueous phase was dried, resuspended in 6 μ L of 80% (v/v) ethanol, and 2 μ L was analyzed by TLC on silica plates (Sigma) as described above.

RESULTS

Characterization of Ara Deficiency in *mur4* Plants

To investigate the effect of the *mur4* mutation, different tissues were analyzed for their cell wall composition. The Ara content of ethanol-insoluble glycans from wild-type and *mur4* plants varied substantially between organs, from an overall 50% reduction in cotyledons, leaves, and flowers to a 75% reduction in elongating inflorescence stems. The Ara content of *mur4* roots was only marginally reduced

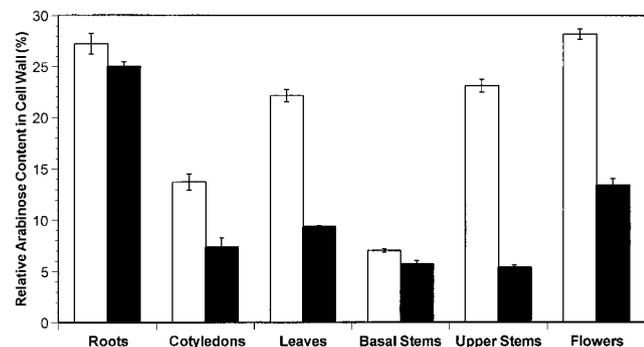


Figure 2. Relative Ara content in the cell wall of different organs of wild-type (white bars) and *mur4* (black bars) plants. The Ara content is given as a percentage of total neutral cell wall monosaccharides (except Glc). Cotyledons were collected from 200 plants, leaves from 40 plants, 2-cm basal and apical stem segments from 50 plants each, and flowers from 260 plants, and processed in four pools of samples for GC analysis. The bars are the mean of four samples \pm SD. For roots, plants were grown on plates and then transferred to hydroponic conditions for 10 d. Roots were collected from 20 plants and processed in three pools; bars are the means of three samples \pm SD.

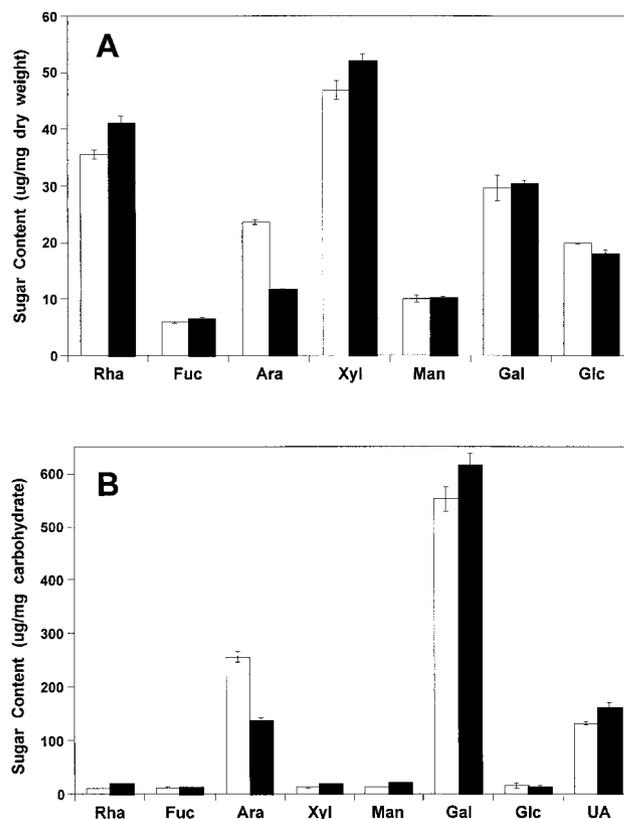


Figure 3. A, Monosaccharide content in leaf-derived cell wall matrix polysaccharides (pectins and hemicelluloses). Identical amounts of cell wall material were used for the wild type (white bars) and *mur4* (black bars). The bars represent the mean of three samples \pm SD. Total uronic acids determined via the *m*-hydroxybiphenyl assay were $293 \pm 38.7 \mu\text{g mg}^{-1}$ dry weight for the wild type ($n = 3$) and $275 \pm 23.0 \mu\text{g mg}^{-1}$ dry weight for *mur4* ($n = 3$). B, Glycosyl composition of Arabidopsis AGPs. Identical amounts of total carbohydrates as determined by the phenol-sulfuric acid colorimetric assay were used for wild-type (white bars) and *mur4* (black bars) AGP extracts. The bars represent the means of three samples \pm SD.

(Fig. 2). Leaf material was fractionated into cell wall polysaccharides and water-soluble polymers that include AGPs. The relative Ara content in these fractions (Fig. 3, A and B) was reduced by approximately 50% and 45%, respectively. Further fractionation of the cell wall into pectins, xylans, and xyloglucans revealed an approximately 2-fold reduction in Ara content in all of these fractions from *mur4* plants, indicating that the Ara deficiency was not specific to certain Ara-containing polymers (data not shown).

AGPs were further analyzed by agarose gel electrophoresis and gel permeation chromatography. For *mur4*, the population of AGP molecules showed a higher electrophoretic mobility than the wild type (Fig. 4), which could have been due to a difference in charge and/or size of *mur4* AGPs. As shown in Figure 3B, *mur4*-derived AGPs showed an increased uronic acid content, which is a possible explanation for the increased electrophoretic mobility. Gel permeation chromatography of wild-type and *mur4* AGPs yielded similar elution profiles (Fig. 5), suggesting that the



Figure 4. Agarose gel electrophoresis of AGPs from wild-type and *mur4* plants. AGPs were extracted from leaf material as soluble polymers from wild-type (A) and *mur4* (B) plants and from wild-type (C) and *mur4* (D) plants grown in the presence of 50 mM Ara. The AGPs were stained with the Yariv reagent.

mur4 mutation does not dramatically alter the size of AGP molecules. AGPs are known to be more heterogeneous in charge than in size, resulting in the formation of a smear of AGPs by gel electrophoresis, while AGPs elute in rather distinct peaks by gel filtration techniques (for review, see Kreuger and van Holst, 1996). The electrophoretic mobility

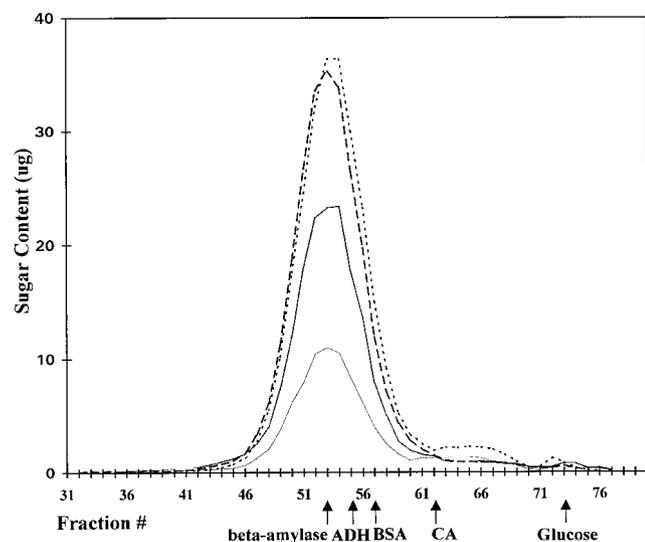


Figure 5. Sephacrose CL-4B elution profile of the Gal and Ara containing material from AGP extracts of wild-type and *mur4* leaves. Each fraction eluting from the Sephacrose column was analyzed by GC of alditol acetates. The elution positions of blue dextran (fraction 25, not shown), and the molecular mass markers β -amylase (200 kD), alcohol dehydrogenase (ADH, 150 kD), BSA (66 kD), carbonic anhydrase (CA, 29 kD), and Glc are shown by arrows. Thick solid line, Wild-type ARA; dashed line, wild-type Gal; thin solid line, *mur4* ARA; dotted line, *mur4* Gal.

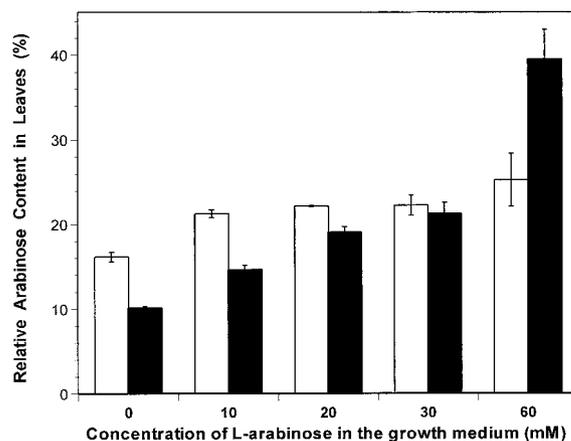


Figure 6. Response of wild-type (white bars) and *mur4* (black bars) plants to feeding with L-Ara. Plants were grown on agar plates with different concentrations of L-Ara in the medium. Bars represent the means of three samples \pm SD.

was similar between wild-type and *mur4* AGPs extracted from plants grown in the presence of 50 mM Ara in the medium (Fig. 4).

Inheritance of the *mur4* Mutation

To determine the inheritance of the *mur4* mutation, homozygous mutant plants were crossed to heterozygous plants and the F_1 progeny was analyzed by GC of alditol acetates, yielding 144 phenotypically *mur4* plants and 143 phenotypically wild-type plants. This indicated that the *mur4* mutation represents a single monogenic and recessive trait ($\chi^2 = 0.003$; $P > 0.8$). In a reciprocal cross using heterozygous plants as the maternal parent and homozygous *mur4* plants as the pollen donor, 143 phenotypically *mur4* plants and 148 phenotypically wild-type plants were obtained. This confirmed the above conclusion ($\chi^2 = 0.09$; $P > 0.8$).

Characterization of the Biochemical Defect

Feeding Studies Using L-Ara

L-Ara can be used directly by plants through the salvage pathway as a source of arabinosyl units for polymer synthesis (Fig. 1). To determine if the *mur4* mutant could be rescued by exogenous Ara, wild-type and *mur4* plants were grown on media containing different amounts of this monosaccharide. Both lines responded to Ara feeding by an increased incorporation of this sugar into cell wall material (Fig. 6), indicating that the salvage pathway for Ara is intact in *mur4* plants and that the availability of UDP-Ara represents a limiting factor for the arabinosylation of cell wall glycans. In the presence of 30 mM Ara in the medium, the Ara content in *mur4* cell walls increased to wild-type levels, while *mur4* plants grown in the presence of 60 mM Ara incorporated substantially more of this monosaccharide into cell wall polymers than wild-type plants grown under the same conditions. This difference could be explained by a reduced activity of the UDP-Ara/UDP-Xyl

interconversion reaction in the mutant plants leading to a higher accumulation of UDP-Ara in *mur4* than in the wild type.

In Vivo Labeling of Wild-Type and *mur4* Stems by L - $[^{14}C]$ Ara and D - $[^{14}C]$ Glc

To further test the incorporation of exogenous Ara into polysaccharides, elongating inflorescence stem segments from wild-type and *mur4* plants were incubated in the presence of radiolabeled Ara. The ethanol-insoluble material was then fractionated by TLC after hydrolysis of the radiolabeled polysaccharides. The results are presented in Table I. In the $[^{14}C]$ Ara feeding experiment, a higher amount of labeled Ara was found in the polysaccharides of *mur4* compared with the wild type. On the other hand, the amount of labeled Xyl in the polysaccharides was lower in *mur4* compared with the wild type, suggesting a possible defect in the conversion of UDP-Ara to UDP-Xyl. In *mur4*, less label appeared as Glc in the polysaccharides. The labeled Glc presumably results from the conversion of UDP-Xyl to UDP-Glc via the pentose phosphate pathway.

To test the de novo synthesis of UDP-Ara from UDP-Glc via UDP-GlcUA and 4-epimerization of UDP-Xyl, wild-type and *mur4* elongating inflorescence stem segments were incubated with radiolabeled Glc and the ethanol-insoluble material was hydrolyzed and fractionated by TLC. The results of the $[^{14}C]$ Glc labeling experiment are presented in Table II. A lower amount of labeled Ara was found in the polysaccharides of *mur4* compared with the wild type, while no difference in Xyl content was observed. The in vivo assays suggested a defect in the UDP-Xyl/UDP-Ara interconversion rather than in an arabinosyl transferase or an Ara transporter. Interestingly, even in the wild type, there was a low apparent UDP-Ara 4-epimerase activity in the $[^{14}C]$ Ara feeding experiment (Xyl/Xyl + Ara = 18% in wild-type polymers), while the UDP-Xyl/UDP-Ara interconversion appeared higher in the $[^{14}C]$ Glc feeding experiment (Xyl/Xyl + Ara = 52% in wild-type polymers), which is in agreement with the thermodynamic equilibrium value for the UDP-Xyl/UDP-Ara pair (Feingold and Avigad, 1980). This result suggests that part of the exogenously applied Ara is converted into UDP-Ara and utilized by arabinosyltransferases before it encounters a 4-epimerase activity.

Table I. Relative amounts of labeled monosaccharides fractionated by TLC following acid hydrolysis of the ethanol-insoluble material from wild-type and *mur4* elongating stem segments incubated in $[^{14}C]$ Ara

The data are the mean of two samples of four stem segments each \pm SD.

Monosaccharide	Wild Type	<i>mur4</i>
	%	
Xyl	17 \pm 3.3	9.2 \pm 2.1
Ara	75 \pm 3.8	88 \pm 2.1
Glc	7.9 \pm 0.5	3.3 \pm 0.1

Table II. Relative amounts of labeled monosaccharides fractionated by cellulose and silica TLCs following acid hydrolysis of the ethanol-insoluble material from wild-type and *mur4* elongating stem segments incubated in $[^{14}C]$ Glc

Data are the mean of three samples of four stem segments each \pm SD. The TLC system (b) was used to separate GlcUA from GalUA.

Monosaccharide	Wild Type	<i>mur4</i>
(a) Cellulose		
Xyl	13 \pm 0.4	13 \pm 0.5
Ara	12 \pm 0.8	5.4 \pm 0.1
Glc	22 \pm 4.1	21 \pm 4.6
Gal	40 \pm 1.6	48 \pm 3.9
Uronic acids	14 \pm 1.8	14 \pm 1.2
(b) Silica		
Xyl	13 \pm 0.6	14 \pm 0.7
Ara	13 \pm 0.5	5.7 \pm 0.4
Glc + Gal + GlcUA	57 \pm 1.9	64 \pm 0.9
GalUA	17 \pm 2.0	17 \pm 0.9

In Vitro Assay of the de Novo Synthesis of UDP-L-Ara

To determine the conversion rates of UDP-Xyl to UDP-Ara in vitro, wild-type and *mur4* microsomal protein fractions were incubated with radiolabeled UDP-GlcUA. The resulting UDP sugars (UDP-GlcUA, UDP-GalUA, UDP-Xyl, and UDP-Ara) were analyzed by TLC after hydrolysis to their respective sugars. The control, a boiled protein extract incubated with radiolabeled UDP-GlcUA, did not convert this nucleotide sugar. The amounts of Xyl and Ara at different time points are shown in Figure 7. A lower amount of Ara was obtained for *mur4* than for the wild type, while the amounts of Xyl were unaffected by the *mur4* mutation. No significant differences in UDP-Xyl 4-epimerase activities were observed when using wild-type and *mur4*

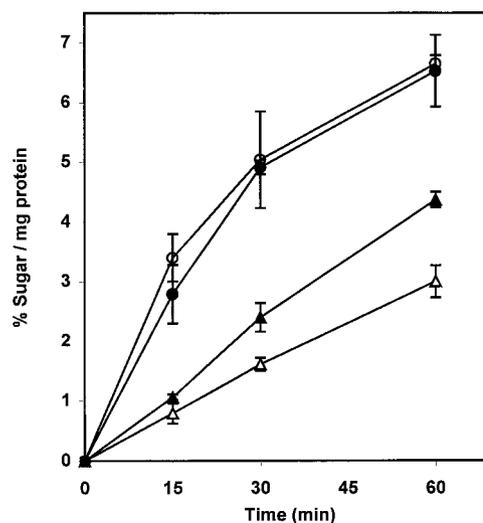


Figure 7. In vitro assay of the de novo synthesis of UDP-Ara from UDP-GlcUA via UDP-Xyl. Wild-type and *mur4* microsomal protein extracts were incubated with radiolabeled UDP-GlcUA. The UDP sugars produced at different time points were hydrolyzed and the sugars were separated and quantified by TLC. Data are mean values \pm SD with a sample size of two. ▲, Wild-type Ara; △, *mur4* Ara; ●, wild-type Xyl; ○, *mur4* Xyl.

soluble protein extracts incubated with radiolabeled UDP-GlcUA (data not shown).

DISCUSSION

The *mur4* mutant was isolated from ethyl methanesulfonate-mutagenized Arabidopsis plants screened for alterations in the monosaccharide composition of leaf-derived cell wall material. The mutant plants showed a 50% reduction in the monosaccharide L-Ara in the leaves, the highest reduction in Ara content observed during this mutant screen (Reiter et al., 1997). We have demonstrated that the *mur4* mutation also led to a 50% reduction in Ara in the cotyledons and flowers and an even higher reduction (75%) in elongating stem segments, but had little effect on basal stems and roots. The *mur4* mutation affected the cell wall polysaccharides as well as the AGPs. The Ara-containing cell wall polysaccharides are mainly the pectic component rhamnogalacturonan I, which shares some common carbohydrate side chains with the type II AGPs consisting of a $\beta(1\rightarrow3,1\rightarrow6)$ -galactan framework substituted with terminal Ara (Fincher et al., 1983; Bacic et al., 1988; Carpita and Gibeaut, 1993; Steffan et al., 1995; Zabackis et al., 1995). The wild-type and *mur4* AGPs were similar in size but differed in their uronic acid content, which may explain the higher electrophoretic mobility of *mur4* AGPs.

Growth of *mur4* plants in the presence of Ara restored the polysaccharide composition to wild-type levels, suggesting a partial defect in the de novo synthesis of UDP-Ara. Radiolabeled Ara was fed to *mur4* plants to analyze the incorporation of radiolabel into polysaccharides. The amount of radiolabeled Ara was slightly higher in *mur4* polysaccharides than in the wild type, while the amount of radiolabeled Xyl was reduced in *mur4* polysaccharides, suggesting a defect in the interconversion of the 4-epimers UDP-Ara and UDP-Xyl. The reversible 4-epimerization of the UDP sugars UDP-Glc/UDP-Gal, UDP-GlcUA/UDP-GalUA, and UDP-Xyl/UDP-Ara was studied in vivo by feeding radiolabeled Glc and analyzing the subsequent incorporation of radiolabel into polysaccharides. Only the amount of radiolabeled Ara was significantly affected in *mur4* polysaccharides, suggesting a partial defect in the final step of the de novo synthesis of UDP-Ara.

An in vitro assay confirmed that the *mur4* mutation affected the UDP-Xyl/UDP-Ara interconversion reaction in the microsomal fraction of mutant plants, while the activity of soluble UDP-Xyl 4-epimerase remained unchanged. Based on the partial Ara deficiency in all polysaccharides investigated, the rescue of the mutant phenotype by growth in the presence of Ara, and in vivo and in vitro labeling studies, we conclude that the *mur4* mutation affects the activity of a membrane-bound UDP-Xyl 4-epimerase. The *mur4* mutation has recently been mapped to a gene with sequence similarities to nucleotide sugar 4-epimerases, suggesting that the mutation affects the structural gene for an enzyme rather than a regulatory factor (E.G. Burget and W.-D. Reiter, unpublished results). Since four independent *mur4* alleles with similar reductions in Ara content are known, it appears likely that the 50% residual Ara content in most organs reflects genetic

redundancy in the de novo synthesis of this monosaccharide. We speculate that the *MUR4* gene encodes one of several isoforms of UDP-Xyl 4-epimerase, leading to a partial rather than a complete deficiency in the de novo synthesis of UDP-Ara. A completely Ara-deficient mutant may be lethal, since it would lead to major changes in the glycosylation pattern of rhamnogalacturonan I, AGPs, and the extensins that contain numerous arabinoside chains shown to stabilize their polyproline II structure (Stafstrom and Staehelin, 1986).

Growth of wild-type and *mur4* plants in the presence of Ara led to considerable increases in polysaccharide arabinosylation, suggesting that the availability of UDP-Ara represents a rate-limiting step in the arabinosylation of cell wall polymers in Arabidopsis. We hypothesize that the UDP-Xyl 4-epimerase affected by the *mur4* mutation represents a Golgi-resident enzyme providing UDP-Ara for direct utilization by arabinosyltransferases. In this scenario, UDP-Ara would not reach equilibrium with UDP-Xyl in vivo. When using in vitro assays for the quantitation of UDP-Xyl 4-epimerase activity in the absence of glycosyltransferases, an approximately 1:1 ratio between UDP-Xyl and UDP-Ara will eventually be established, which may explain why the in vitro data presented in Figure 7 do not fully reflect the substantial Ara-deficiency observed in vivo.

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