Developmental expression and perturbation of arabinogalactan-proteins during seed germination and seedling growth in tomato

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Arabinogalactan-proteins (AGPs) are a family of highly glycosylated hydroxyproline-rich glycoproteins present throughout the plant kingdom. A synthetic chemical reagent, (β-D-Gal), Yariv reagent, specifically binds AGPs and can be used for histochemical staining, isolating and probing the function of AGPs. Here, the role of AGPs in tomato (Lycopersicon esculentum Mill. cv. UC82B) seed germination and seedling growth was examined by following expression of AGPs during these events and by treatment with (β-D-Gal), Yariv to perturb AGP function. AGP expression changed during germination and seedling development both quantitatively and qualitatively as revealed by analysis of total AGP content, crossed electrophoresis patterns, RNA blots using LeAGP-1 probe, and western blots with LeAGP-1, JIM13, and MAC207 antibodies. (β-D-Gal), Yariv treatment of seeds and developing seedlings did not affect percent seed germination, but markedly inhibited seedling growth in roots and to a lesser degree in shoots. Root growth inhibition encompassed reductions in overall root length, epidermal root cell elongation, root cell numbers and root hair formation. This growth inhibition was reversible following removal of (β-D-Gal), Yariv. In a related experiment, water uptake by tomato seedlings was greatly inhibited by (β-D-Gal), Yariv treatment. Based on these experiments, AGPs are clearly associated with tomato seedling development and likely to function in root growth, more specifically in cell elongation, cell proliferation, root hair formation and water uptake.

Introduction

Seed germination and seedling growth are important stages in a plant’s life cycle. Germination is marked by emergence of a radicle from the micropyle of a seed. Completion of germination is usually followed by seedling growth (Bewley and Black 1994, Weithoff et al. 1998). Developing an autotrophic plantlet from a heterotrophic seed is accompanied by major changes in plant size, morphology and metabolism. At the physiological level, increases in water uptake, nutrient mobilization and respiration provide the necessary building blocks and energy for germination and growth. At the molecular level, enhanced expression of numerous genes implements the mobilization of these resources. Protein synthesis can be initiated from pre-existing seed mRNAs or with newly transcribed mRNAs. There is overlapping expression of many genes in seedlings and in adult plants, suggesting an important role(s) for them in maintaining basic plant growth (Bewley and Black 1994, Bewley 1997).

Arabinogalactan-proteins (AGPs) are a diverse family of hydroxyproline-rich glycoproteins with more than 90% carbohydrate and 1–10% protein by weight (Clarke et al. 1979, Showalter and Varner 1989, Nothnagel 1997). The protein cores of AGPs are usually rich in Hyp, Ala, Ser and Thr, as shown by amino acid analysis, protein sequence analysis, and cDNA cloning (Nothnagel 1997). In addition, Ifis-rich (Kieliszewski et al. 1992) and Hyp-deficient AGPs (Baldwin et al. 1993, Mollard and Joseleau 1994) are known. Sugar analysis indicates that arabinose and galactose are the two main components in the carbohydrate side chains of AGPs. The galactose residues form a (1–3)-β-D-galactan backbone with (1–6)-β-D-galactan side chains that in turn are modified by arabinose and other lesser abundant monosaccharides, such as L-rhamnose and glucuronic acid (Fincher et al. 1983, Bacic et al. 1987, Komalavilas et al. 1991). In addition to the complex polysaccharide side chains, short

Abbreviations – AGP, arabinogalactan-protein; DPI, days post-imbibition; DW, dry weight; (β-D-Gal), Yariv or β, (β-D-galactosyl), Yariv phenylglycoside; (α-D-Gal), Yariv or α, (α-D-galactosyl), Yariv phenylglycoside.
AGPs are distributed throughout the plant kingdom and are found in all organs, tissues and cell types. At the subcellular level, AGPs are localized mainly at the cell surface, including plasma membranes, cell walls and extracellular secretions (Nothnagel 1997). Several functions are proposed for AGPs in plants. Monoclonal antibodies against carbohydrate epitopes and polyclonal antibodies against protein backbones have detected the temporal and spatial expression of AGPs in various plant tissues and organs (Schindler et al. 1995, Casero et al. 1998, Samaj et al. 1998). The appearance and disappearance of specific AGP epitopes over time have led to the suggestion that AGPs function as developmental markers during plant growth. Additional reports indicate that AGPs are involved in cell differentiation. In a leafy-liverwort, treatment with biosynthetic inhibitors of AGPs and other hydroxyproline-rich glycoproteins causes desuppression of leaf primordial differentiation (Basile 1990, Basile and Basile 1993). Furthermore, manipulation of total AGPs by exogenously added AGPs or perturbation of AGPs by (β-D-D-glucosyl)₃ Yariv or monoclonal antibodies against AGP carbohydrate epitopes can inhibit or stimulate somatic embryogenesis, depending on the particular AGPs, cell type composition, cell developmental stage and culture condition (McCabe et al. 1997, Toonen et al. 1997, Thompson and Knox 1998, Butowt et al. 1999).

Yariv reagents are a class of synthetic phenoglycosides that selectively bind AGPs and can be used for histochemical staining, isolation, and probing the function of AGPs. With the addition of Yariv reagents to living systems, researchers have shown that AGPs are involved directly or indirectly in cell proliferation (Serpe and Nothnagel 1994), cell expansion (Willats and Knox 1996, Ding and Zhu 1997), pollen tube growth (Jauh and Lord 1996, Roy et al. 1998) and programmed cell death (Gao and Showalter 1999). As part of our ongoing research aimed at defining the function of AGPs, we report here on the developmental expression of AGPs and their perturbation by (β-D-D-Gal)₃ Yariv during seed germination and seedling development in tomato.

Materials and methods

Plant materials and synthesis of Yariv reagents

Tomato (Lycopersicon esculentum Mill. cv. UC82B) seeds were surface-sterilized with 20% bleach for 15 min followed by thorough rinsing with water. Seeds were sown on Murashige and Skoog medium with 0.8% agar (Murashige and Skoog 1962) in single file in the middle of 150 × 15 mm Petri dishes (Fisher Scientific Co., Hanover Park, IL, USA). Seeds were germinated and grown in a vertical position at 24°C under a 16 h light/8 h dark cycle in a growth chamber.

Yariv reagents were synthesized according to the method of Yariv et al. (1962). To study the effects of Yariv reagents on germination and growth, MS medium supplemented with (α-D-Gal)₃ or (β-D-D-Gal)₃ Yariv reagent was used. Seedlings were collected at different days post-imbibition (DPI) for further analysis.

Determination of percent germination and water content

Germination was defined by the visible emergence of a radicle from the micropyle of a seed (Bewley and Black 1994, Westhoff et al. 1998). Percent germination was defined as the number of germinated seeds/total number of seeds × 100. At least 300 seeds were used to determine percent germination. To measure water content of tomato seedlings at different ages, fresh weight and dry weight were measured before and after freeze-drying, respectively. Water content was defined as (fresh weight – dry weight)/fresh weight × 100. At least 30 seedlings were used for each data point.

AGP extraction, quantitation and crossed electrophoresis

Total AGPs were extracted according to Gao et al. (1999). To measure their concentration, a small aliquot of AGPs was mixed with an equal volume of 1 M (β-D-Gal) Yariv reagent in 2% (w/v) NaCl for 1 h. After centrifugation at 13000 g for 2 min, the AGP/(β-D-Gal) Yariv precipitate was rinsed twice with 1% NaCl (w/v) and dissolved in 1 ml 0.02 M NaOH before measuring A₂₅₀ in a spectrophotometer. A standard curve made from gum arabic (Sigma, St Louis, MO, USA) was used to determine the concentration of each AGP sample.

CROSSED ELECTROPHORESIS OF AGPS WAS PERFORMED AS DESCRIBED BY VAN HOLST AND CLARKE (1986). BRIEFLY, FOR THE FIRST DIMENSION, A 10 × 7 × 0.3 cm (length × width × thickness) 1% (w/v) agarose gel was used to separate 30 µg of total AGPs extracted from 2-, 5-, or 8-day-old seedlings. The gel was run at 80 V for 40 min. For the second dimension, lanes containing AGPs were cut from the first gel as 7 × 0.8 × 0.3 cm strips and placed horizontally in a 10 × 20 cm gel tray. Then 35 ml of 1% (w/v) molten agarose containing 30 µM (β-D-Gal) Yariv was poured into the gel tray. After solidification, the gel was electrophoresed at 40 V for 12 h. Gel buffer and running buffer for both dimensions were identical: 25 mM Tris-HCl, 200 mM glycine (pH 8.3). After electrophoresis, gels were washed with 1% (w/v) NaCl, rinsed with distilled water, and air-dried on 3MM Whatman paper (Whatman International Ltd, Maidstone, UK). Gel images were recorded by using a HP ScanJet 4c scanner with DeskScan II software, version 2.3 (Hewlett-Packard Co., Palo Alto, CA, USA).

Microscopy and photography

Seedling roots were prepared for microscopy by rinsing with distilled water to remove excess medium. A region between 1 and 2 cm above the root tip was used. The length of root epidermal cells was measured under a light microscope with an ocular micrometer calibrated with an objective micrometer. At least 100 individual cells were used for each data point. Free hand, root cross sections were made with a razor blade and observed with a Nikon EFO-3 light microscope (Fryer Company, Inc., Huntley, IL, USA). Kodak Gold 400 film was used for light microscopy photography. Tomato seedlings were photographed with a MicroLumina digital camera (Electro-image Inc., Great Neck, NY, USA).
Cell number determination

Cell numbers in roots were determined as described by Brown and Broadbent (1950). The whole root was cut from a seedling, sliced into 5-mm sections, and incubated in 5% (w/v) chromic acid (Sigma) until the tissue began to separate (about 8 h). Cell aggregates were forced to separate by repeated passage through a fine tip glass pipette. Suspended cells were counted in a 0.1 mm deep haemocytometer with a Fuchs-Rosenthal grid (American Optical Corporation, Buffalo, NY, USA) with a light microscope. The number of cells was calculated as the average number of cells per unit volume of the haemocytometer × total volume of each sample. Cell number per root was determined with the average cell number of 5 roots from each treatment.

Northern and western analysis

Total RNA was isolated from seedlings with a plant RNA extraction kit (Qiagen Inc., Valencia, CA, USA) and quantified by measuring A_{260}. RNA samples (15 μg lane^{-1}) were separated on a 1.2% formaldehyde agarose gel by electrophoresis, and then transferred to a Zeta-Probe nylon membrane (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer’s instructions. Hybridization was performed overnight at 60°C in a hybridization solution (7% SDS, 250 mM phosphate buffer, pH 7.4). A 600 bp EcoRI fragment from the LeAGP-1 cDNA clone was used as a probe to detect LeAGP-1 mRNA (Li and Showalter 1996). An H1 gene, a constitutively expressed gene, was used as a probe to show equal loading of each sample (Lawton and Lamb 1987). Blots were washed at 60°C with 2 × SSC (0.3 M NaCl, 0.03 M Na citrate, pH 7.0), 0.1% SDS 3 times (20 min wash^{-1}), and exposed to X-ray film at – 80°C.

For western blot analysis, 40 μg of total AGPs from seedlings of different ages was subjected to 7.5% SDS-PAGE and electroblotted onto a PVDF membrane (Bio-Rad Laboratories). Blots were blocked with 4% BSA in TTBS buffer (20 mM Tris-HCl, 500 mM NaCl, 0.05% Tween 20, pH 7.5) for 1 h and then incubated at room temperature overnight with anti-LeAGP-1 antiserum (also called PAP) at a 1:2000 dilution, JIM13 antiserum at a 1:100 dilution, or MAC207 antiserum at a 1:100 dilution. Alkaline phosphatase conjugated goat anti-rabbit IgG (for PAP) and rabbit anti-rat IgG (for JIM13 and MAC207) served as secondary antibodies and were used at a 1:3000 dilution. Sigma Fast™ 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium tablets (Sigma) were used for color development in the alkaline phosphatase reaction.

Results

Water uptake, percent germination and AGP accumulation in tomato seedlings

Water uptake is the initial physiological event required for seed germination. The water absorbing capability of a seed is related to many intrinsic seed characteristics (Bewley and Black 1994). Water content was measured in germinating tomato seeds and seedlings following imbibition (Fig. 1). Water content increased rapidly to 60% within 2 DPI and continued to increase up to 8 DPI when seedlings contained 95% water. Germination, defined as the emergence of a radicle from the micropyle, reached 20% by 3 DPI, following initial, rapid water uptake and 85% by 5 DPI. Thereafter, the rate of germination slowed with nearly 100% germination achieved by 8 DPI.

Total AGPs were extracted and quantified from tomato seeds and seedlings (Fig. 1). AGPs were present in dry tomato seeds at 0 DPI (8.1 μg mg^{-1} dry weight) and increased following imbibition on a DW basis up to 5 DPI (18 μg mg^{-1} dry weight) before decreasing at 6–8 DPI to amounts comparable to those of dry seeds. Crossed electrophoresis was performed to analyze total AGP profiles. With this method, AGPs with different sizes and/or charges were separated in a two dimensional agarose gel and visualized by (β-d-Gal) Yariv precipitation. Distinct AGP patterns were found in different aged tomato seedlings (Fig. 2). AGPs extracted from 2-day-old seedlings had a single, asymmetrical peak. Two peaks were discerned in AGP extracts from 5- and 8-day-old seedlings. In addition, the relative abundance of the peaks also changed as seedlings grew. Taken together, these data indicated that not only the amount, but also the composition of total AGPs changed during tomato seedling development.

Expression of LeAGP-1 and other AGPs during seedling development

Expression of LeAGP-1, a major AGP present in tomato cell suspension cultures and plants, can be monitored at the RNA level with a DNA probe (Li and Showalter 1996) and at the glycoprotein level with a polyclonal antibody probe, called PAP, designed to recognize the Lys-rich subdomain in LeAGP-1 (Gao et al. 1999). These two probes were used to examine LeAGP-1 expression during tomato seed germination and seedling growth.

Fig. 1. Time course of water absorption, seed germination, and AGP accumulation during tomato seedling development. Vertically cultured tomato seedlings were collected at different days post-imbibition (DPI) and analyzed for water content (▲), seed germination (●), and total AGP content (□). Bars indicate SE. Each experiment was repeated at least 3 times.
RNA blot analysis showed that \textit{LeAGP-1} mRNA began to accumulate 3 DPI and accumulated rapidly to a high steady state level by 4 DPI that continued up to 8 DPI (Fig. 3A). The H1 probe was used to verify approximately equal loading of RNA samples (Fig. 3A). Accumulation of \textit{LeAGP-1} mRNA was accompanied by a gradual increase of \textit{LeAGP-1} at the protein level, as detected by western blot analysis (Fig. 3B). Heterogeneity displayed by \textit{LeAGP-1} in this western blot, is characteristic of AGPs and reflects carbohydrate microheterogeneity. As the intensity of the detected bands increased, the range of the molecular mass displayed for the \textit{LeAGP-1} glycoforms also increased. By 6 DPI, the \textit{LeAGP-1} glycoprotein reached a stable and relatively high level and ranged in mass from 50 to 115 kDa.

\textit{JIM13} and \textit{MAC207} are rat monoclonal antibodies that recognize different carbohydrate epitopes found on certain AGPs (Knox et al. 1989, Pennell et al. 1989). To examine expression of these AGPs during tomato seed germination and seedling growth and for comparison to \textit{LeAGP-1} western blots, identical blots were probed with \textit{JIM13} and \textit{MAC207}. \textit{JIM13} detected high molecular mass smears from 0 through 8 DPI. The amount of AGPs detected with the \textit{JIM13} antibody increased at 6–8 DPI and corresponded to a molecular mass range of 80–208 kDa (Fig. 3C). In contrast, \textit{MAC207}-labeled AGPs ranging in size from 115 to about 300 kDa (Fig. 3D). Strong, moderate and no binding of \textit{MAC207} to AGPs were found, respectively, in 5–8, 3–4 and 0–2 DPI seedlings (Fig. 3D).
Table 1. Effect of \((\beta\text{-D-Gal})_3\) Yariv reagent on seed germination and growth in 8-day-old tomato seedlings. The tomato seeds were germinated and the seedlings were grown continuously on MS, MS/40 \(\mu\text{M} (\alpha\text{-D-Gal})_3\), or MS/40 \(\mu\text{M} (\beta\text{-D-Gal})_3\), Yariv medium. After 8 days imbibition, the percentage of germination, root length, and shoot length were determined. Data are means \(\pm\) SE. In each column, same superscript indicates no significant difference at \(P<0.05\) with Fisher’s PLSD test.

<table>
<thead>
<tr>
<th></th>
<th>Germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
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<tbody>
<tr>
<td>MS</td>
<td>97.00 (\pm) 3.25(^a)</td>
<td>6.54 (\pm) 0.47(^a)</td>
<td>3.28 (\pm) 0.32(^a)</td>
</tr>
<tr>
<td>((\alpha\text{-D-Gal})_3)</td>
<td>98.75 (\pm) 1.77(^a)</td>
<td>6.60 (\pm) 0.46(^a)</td>
<td>3.68 (\pm) 0.37(^b)</td>
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<tr>
<td>((\beta\text{-D-Gal})_3)</td>
<td>96.68 (\pm) 1.11(^a)</td>
<td>1.35 (\pm) 0.37(^a)</td>
<td>1.67 (\pm) 0.31(^a)</td>
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\((\beta\text{-D-Gal})_3\) Yariv treatment affects seedling growth but not seed germination

\((\beta\text{-D-Gal})_3\) Yariv, a synthetic chemical reagent that selectively binds and aggregates AGPs, was used to probe the function of AGPs in seed germination and seedling growth. Two control treatments were used in these experiments: (1) (\(\alpha\text{-D-Gal})_3\) Yariv, a structural analog of \((\beta\text{-D-Gal})_3\) Yariv that does not bind AGPs and (2) no supplementation with Yariv reagent. Tomato seeds were germinated and grown vertically on media containing various concentrations of \((\beta\text{-D-Gal})_3\) Yariv (10, 20, 40, 60 and 80 \(\mu\text{M}\)). Seed germination was not affected by \((\beta\text{-D-Gal})_3\) or \((\alpha\text{-D-Gal})_3\) Yariv treatment at any of the above concentrations. However, seedling growth was inhibited by 10–80 \(\mu\text{M} (\beta\text{-D-Gal})_3\) Yariv treatment in a dose-dependent manner (data not shown). Further experiments utilized the 40 \(\mu\text{M} (\beta\text{-D-Gal})_3\) Yariv concentration unless indicated otherwise. Compared with controls, seedlings grown in media supplemented with \((\beta\text{-D-Gal})_3\) Yariv were reduced in size. Both roots and shoots were reduced in length, but root length was inhibited to a greater degree (Table 1 and Fig. 4A).

Further examination of tomato roots showing growth inhibition in the presence of \((\beta\text{-D-Gal})_3\) Yariv revealed that root cells were reduced in length by approximately 50% compared with \((\alpha\text{-D-Gal})_3\) Yariv and untreated controls (Figs 4B and 5B). Root cross sections showed a red staining ring in the presence of \((\beta\text{-D-Gal})_3\) Yariv; this staining was most intense in the outer two cell layers, epidermal cells and underlying cortical parenchyma cells (Fig. 4C). Despite strong binding of \((\beta\text{-D-Gal})_3\) Yariv on the root surface, the cross-sectional anatomy and diameter of \((\beta\text{-D-Gal})_3\) Yariv-treated roots were similar to those of controls, showing a complete cortex and vascular cylinder (Fig. 4C). In addition, a striking difference was found at the root surface. Namely, few root hairs were present after \((\beta\text{-D-Gal})_3\) Yariv treatment compared with control treatments (Fig. 4D).
Inhibitory effects of (β-D-Gal)_3 Yariv on seedling growth are reversible. (A) Root lengths of seedlings grown continuously on MS, MS supplemented with (β-D-Gal)_3 Yariv [β], and of seedlings transferred from MS/(β-D-Gal)_3 Yariv to MS at 5 DPI [β → MS] or vice versa [MS → β]. (B) Cell length within a region between 1 and 2 cm above root tip 8-day-old seedlings subjected to these treatments. (C) Cell numbers per root of 8-day-old seedlings subjected to these treatments.

**Inhibitory effect of (β-D-Gal)_3 Yariv on growth is reversible**

Although growth inhibition in tomato seedlings was observed after (β-D-Gal)_3 Yariv treatment, this effect was reversible. When 5-day-old seedlings grown in MS medium or MS supplemented with (α-D-Gal)_3 Yariv were transferred to (β-D-Gal)_3 Yariv medium, there was no appreciable further increase in root length, indicating growth inhibition by (β-D-Gal)_3 Yariv reagent (Figs 4A and 5A). When (β-D-Gal)_3 Yariv-treated 5-day-old seedlings were switched to control media, a resumption of growth was observed within hours and the growth rate thereafter was essentially identical to that obtained from control seedlings grown continuously on MS media (Figs 4A and 5A). This growth was accompanied by an increase in root epidermal cell length and root hairs. Indeed, 3 days after transferring (β-D-Gal)_3 Yariv-treated seedlings to MS media, epidermal cells in the elongation zone were almost equal in length to those of control cells (Fig. 5B). In contrast, no change in cell length was detected in the elongation zone after 3 days in the reciprocal transfer experiment (Fig. 5B).

To test whether altered cell division contributes to reduced root length in these experiments, cell numbers per root were measured in the various treatments (Fig. 5C). The number of root cells in (β-D-Gal)_3 Yariv-treated seedlings was only 30% of that from seedlings grown on MS or MS supplemented with (α-D-Gal)_3 Yariv. A switch from (β-D-Gal)_3 Yariv treatment to MS for 3 days increased root cell numbers toward, but not as much as, control values. In the reciprocal transfer, cell numbers were about 50% of the controls (Fig. 5A).

(β-D-Gal)_3 Yariv treatment blocks water uptake

Ten-day-old tomato seedlings with two fully expanded true leaves and a well-developed root system with branching roots and root hairs, were transferred to MS media containing (β-D-Gal)_3 Yariv, (α-D-Gal)_3 Yariv, or no Yariv supplementation. Six days after transfer, water uptake by seedlings treated with (β-D-Gal)_3 Yariv was 60% of that exhibited by the two controls (Fig. 6). During this experiment, seedling size remained relatively unchanged in the 3 treatment groups, although a slight chlorosis of leaves in (β-D-Gal)_3 Yariv-treated seedlings was observed.

**Discussion**

**Accumulation of AGPs is developmentally regulated during tomato seedling growth**

AGPs are present in both tomato seeds and seedlings. Amounts of total AGPs as well as AGP profiles change following imbibition over the course of an 8-day growth period (Figs 1 and 2). These changes, at least in part, are related to the specific developmental expression patterns for LeAGP-1 and JIM13-/MAC207-reactive AGPs displayed over this time course (Fig. 3). Based on western blot analysis, LeAGP-1, JIM13-reactive AGPs, and MAC207-reactive AGPs accumulate with time; however, only JIM13-reactive AGPs are detected in the dry seed. In contrast, LeAGP-1 and MAC207-reactive AGPs are first detected around the time when germination is completed. Interestingly, LeAGP-1 protein is detected just prior to detection of LeAGP-1 mRNA and may reflect greater sensitivity of western blot analysis in this instance. In any event, such developmentally correlated expression of AGPs during seed germination and seedling growth suggests that different sets of AGPs are
involved in particular aspects of seedling development. Indeed, it would now be useful to determine the immunolocation patterns produced with these antibodies during germination to provide further insight, as done for LeAGP-1 using more mature tomato organs and tissues (Gao et al. 1999, Gao and Showalter 2000).

Yariv reagent implicates AGPs in seedling growth and development

Yariv reagent serves as a useful probe to examine possible roles of AGPs in growth and development. This reagent generally binds all AGP family members as opposed to a specific AGP family member but is unable to penetrate multiple cell layers in intact, living tissue. In this study, (β-D-Gal)₃ Yariv treatment did not affect seed germination, most likely because this reagent does not penetrate into the seed and reach the embryo. This treatment, however, significantly inhibited growth of tomato seedlings following germination (Figs 4A and 5A and Table 1). Growth inhibition is manifested primarily in roots and to a lesser extent in shoots. Growth inhibition in shoots, however, may be a secondary effect, as a result of reduced water and nutrient uptake by roots, as suggested previously by Willats and Knox (1996). (β-D-Gal)₃ Yariv directly inhibits root cell elongation and proliferation, and root hair production (Figs 4B,D and 5B,C); all of these responses can contribute to the overall reduction in root growth. Previous studies using 3,4-dehydro-L-proline, a selective inhibitor of prolyl hydroxylase, similarly implicate the involvement of hydroxyproline-rich glycoproteins in cell wall assembly, cell division, and root hair formation (Cooper et al. 1994, Bucher et al. 1997).

Apparently, the effect of (β-D-Gal)₃ Yariv reagent on root growth inhibition occurs soon after seedlings come into contact with the reagent (i.e., either immediately upon germination or when the reagent is added at a time such as 5 DPI); however, whether a time window for responsiveness exists is unknown. Upon removal of (β-D-Gal)₃ Yariv from the media, growth inhibition of roots (and stems) is reversed, and normal growth is reestablished within hours (Figs 4A and 5A). Such reversibility is also exhibited with respect to root cell elongation, root hair formation, and root cell proliferation (Fig. 5).

A number of studies using Yariv reagent have implicated AGPs in various aspects of plant growth and development. Yariv treatment inhibits cell proliferation in suspension-cultured rose cells (Serpe and Nothnagel 1994) and cell elongation in suspension-cultured carrot cells (Willats and Knox 1996). While the growth of carrot seedlings is not affected by the Yariv treatment, Yariv-treated Arabidopsis seedlings show reduced root growth, accompanied with bulging epidermal cells and reduced root cell elongation (Willats and Knox 1996, Ding and Zhu 1997). Furthermore, Yariv inhibits growth of pollen tubes in lily but not some other species (Lord et al. 1996). Most recently, programmed cell death is induced in Arabidopsis cell suspension cultures by Yariv treatment, implicating AGP involvement (Gao and Showalter 1999).

Clearly, tomato seedlings respond to Yariv in a generally similar manner as that noted in the two Arabidopsis seedling studies, showing reduced root length and cell elongation, but with some notable distinctions and additions. First, no appreciable root epidermal cell bulging is observed in tomato seedlings. Second, cell proliferation is inhibited in tomato seedling roots. This phenomenon is observed in rose suspension-cultured cells (Serpe and Nothnagel 1994), but was not investigated in Arabidopsis seedlings. Third, a markedly reduced number of root hairs are present in tomato seedlings; this observation was not found in the Arabidopsis seedlings (Willats and Knox 1996, Ding and Zhu 1997, our observation). Like the growth of pollen tubes, root hair elongation also results from tip growth of individual cells (Heath 1990, Berger et al. 1998). Interestingly, the growth responses of pollen tubes and root hairs to the treatment of Yariv reagent differ in various plants. An inhibitory effect is seen in some plants while no effect is seen in others. Very likely, the inhibition of growth with the treatment of Yariv reagent reflects the amount and/or types of AGPs on the cell surface of pollen tube and root hair that are important for the two cell growth processes in some

Fig. 6. (β-D-Gal)₃ Yariv treatment blocks water uptake in tomato seedlings. (A) Ten-day-old seedlings were transferred to MS medium and MS medium containing (α-D-Gal)₃ or (β-D-Gal)₃ Yariv for 6 days and then photographed. (B) Water uptake recorded for each of the treatment groups; values were determined by subtracting the amount of water present at a given time in the experiment from that at the beginning.
plants. However, more plants need to be studied with respect to the effect(s) of Yariv reagent on pollen tube and root hair growth before a general conclusion can be reached.

AGPs may function in tomato seedling growth and development, and more specifically in root cell elongation and proliferation, and root hair formation. Given the inherent limitations of studies using Yariv reagent, the degree to which one or more AGP family members are involved in these processes awaits further examination and will require other approaches, perhaps involving mutants with altered root phenotypes or anti-sense plants with reduced expression of a particular AGP.

### Yariv reagent inhibits water uptake in tomato seedlings

Water uptake in tomato seedlings is progressively reduced to approximately 60% of control values in the presence of (β-D-Gal), Yariv reagent after 6 days (Fig. 6). Some chlorosis of leaves in (β-D-Gal), Yariv-treated seedlings also occurs and may indicate a nutrient deficiency as would be expected with inhibition of water uptake. Binding of (β-D-Gal), Yariv occurs largely in the outermost one or two root cell layers (Fig. 4C), and it is likely that multimeric Yariv-AGP complexes are formed at the plasma membrane/cell wall interface, as observed in rose suspension-cultured cells (Serpe and Nothnagel 1994). Such complexes may impede water uptake directly by forming a physical barrier or indirectly by substantially altering plasma membrane properties, which would in turn impair the normal function of root hairs and other root tissues in water uptake. It is also interesting to note that AGPs are extremely hydroscopic molecules and thought to serve as humectants (Fincher et al.

### Mechanism of action

The precise mechanism by which (β-D-Gal), Yariv induces its various responses in these and other related studies is unknown. However, given its ability to bind and aggregate AGPs, Yariv would serve to disrupt the function of AGPs at the cellular level. Coupled with knowledge of AGP localization at the cellular level, such disruption may involve one or more of the following: (1) altering AGP connections associated with the plasma membrane-cell wall network, (2) altering interactions between AGPs and other cell wall components, (3) preventing AGPs from acting as signal molecules, and (4) preventing AGPs from acting as signal receptors. Clearly, the challenges that now lie ahead are to demonstrate the unequivocal function of a particular AGP and to describe its mode of action.

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