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Phenovariation induced in *Streptocarpus prolixus* (Gesneriaceae) by β-glucosyl Yariv reagent

Richard A. Rauh and Dominick V. Basile

Abstract: *Streptocarpus prolixus* C.B. Clarke is a species in the family Gesneriaceae characterized by an acaulescent vegetative body. Instead of a stem bearing leaves developing from a shoot apical meristem, its vegetative body derives from the continued, intercalary growth of one of its cotyledons. β-glucosyl Yariv phenyglycoside (β-D-Glc)$_3$ selectively binds a class of cell surface associated proteoglycans and glycoproteins known as arabinogalactan-proteins (AGPs). Treating seeds and seedlings of *S. prolixus* with (β-D-Glc)$_3$ induced phenovariants considered phyogenetically significant because they copied morphological features (phenocopied) characteristic of other, presumably more primitive species of *Streptocarpus* as well as species in other genera in the family to which it belongs. These results paralleled those obtained in earlier experiments with *S. prolixus* using antimetabolites of hydroxyproline-containing proteins (Hyp-proteins). Treatment with α-galactosyl phenylglycoside, a Yariv reagent that does not bind AGPs, did not induce phenovariants. The finding that (β-D-Glc)$_3$ produced the same "phyletic phenocopies" as Hyp-protein antagonists strongly suggests that the morphoregulatory Hyp-proteins that were antagonized in the earlier experiments were AGPs and that AGPs play a pivotal role in pattern formation and pattern change during plant morphogenesis.

Key words: Yariv phenyglycoside, arabinogalactan-protein, AGPs, *Streptocarpus*, phenovariation, morphogenesis.

Résumé : Le *Streptocarpus prolixus* C.B. Clarke est un espèce de la famille des Gesneriaceae caractérisée par un corps végétatif acaulescent. Plutôt que de porter des feuilles issues d'un méristème apical caulinaire, son corps végétatif dérive de la croissance intercalaire continue de l'un de ses cotylédons. Le β-glucosyl phénylglycoside de Yariv (β-D-Glc)$_3$ attache sélectivement une classe de protéoglycans et de glycoprotéines associés à la surface des cellules, et connues comme protéines arabinogalactaniques (AGPs). Lorsqu'on traite les graines et les plantules du *S. prolixus* avec le (β-D-Glc)$_3$, on induit des variations phénotypiques considérées comme phylogénétiquement significatives, parce qu'elles copient les caractères morphologiques (phénocopies) typiques de d'autres espèces de *Streptocarpus*, vraisemblablement plus primitives, ainsi que d'espèces de d'autres genres de la famille à laquelle il appartient. Ces résultats sont en parallèle avec ceux obtenus dans des expériences antécédentes sur le *S. prolixus*, en utilisant des antimétabolites des protéines contenant de l'hydroxyproline (Hyp-protéines). Un traitement avec l'α-galactosyl phénylglycoside, un réactif de Yariv qui n'attache pas les AGPs, n'induit pas de phénovariation. La constatation que le (β-D-Glc)$_3$ produit les même « copies phylétiques » que l'antagoniste des Hyp-protéines suggère fortement que les Hyp-protéines morphorégulatrices, antagonisées dans les expériences précédentes, étaient des AGP, et que les AGP jouent un rôle central dans le formation et la modification du patron, au cours de la morphogenèse de la plante.

Mots clés : phénylglycoside de Yariv, protéine arabinogalactanique, AGPs, *Streptocarpus*, phénovariation, morphogenèse.

[Traduit par la Rédaction]

Introduction

The genus *Streptocarpus* (Gesneriaceae) displays a wide range of vegetative morphologies. After exhibiting some degree of unequal development of their two cotyledons ("anisocotyly") typical of species in its subclass (Cyrtandroidae), individual species develop morphotypes that can be arranged in a series that range from the more familiar caulescent, node–internode type shoot organization (Fig. 1*g*) to a highly derived, unifoliate form (Fig. 1*g′*) (Hilliard and Burtt 1971). In the latter, the entire vegetative structure appears as a single leaf that arises from the extended development of one of the cotyledons and is known as a "phyllomorph" (Fig. 2). Between the caulescent and phyllomorph forms are many variations. All of the variations appear to be related to the relative differences in the time and (or) degree to which cell proliferation in the primary, embryonic shoot apical meristem is suppressed and desuppressed. In the unifoliate species, it was considered by some that the capacity to form a functional shoot apical meristem had been lost during the course of evolution and speciation within the Gesneriaceae.

In a recently completed study (Rauh and Basile 2000), *Streptocarpus prolixus* C.B. Clarke, a facultative unifoliate representing one extreme of the morphological series, was experimentally induced to express features typical of caulescent species at the other extreme as well as several morphotypes in between. The experimentally induced phenovarients could be categorized according to four morphotypes: (*i*) anisocotyly with premature formation of secondary phyllomorphs at the

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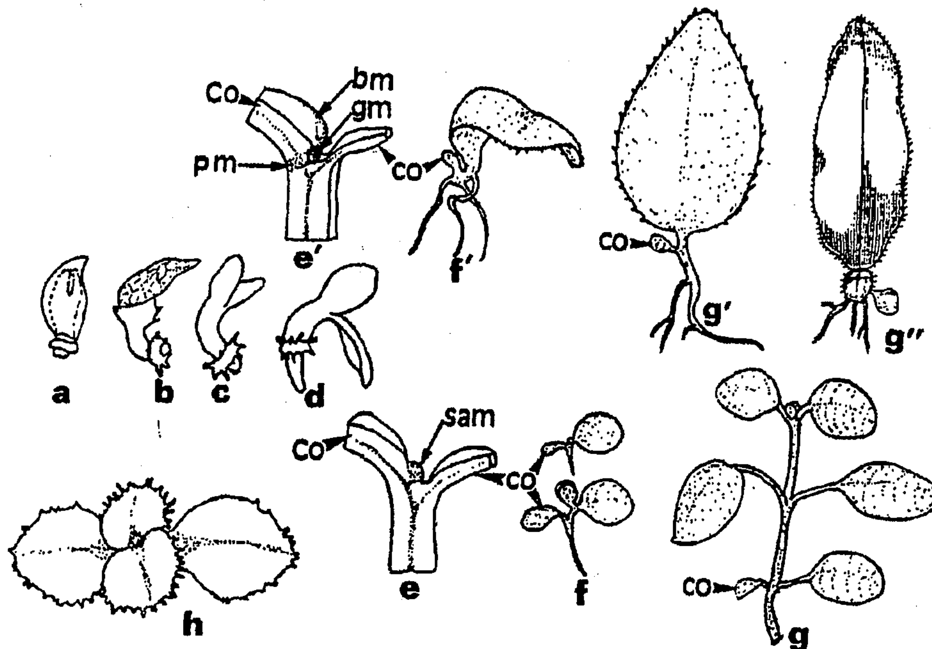
Fig. 1. Drawings showing the pertinent stages in the ontogeny of normal Gesneriaceae seedlings. (*a–g*) Seedlings of *Streptocarpus* in the subfamily Cyrtandroideae; (*h*) typical seedling from the subfamily Gesnerioideae. (*a–d*) Stages in seedling development that are essentially the same for all species. (*e* and *f*) Stages in the development of species assigned to the subgenus *Streptocarpella*, all members of which eventually manifest nodal organization (Fig. 1*g*) after passing through an anisocotylous stage (Fig. 1*f*) in which growth of one cotyledon (Co) is dominant over the other (co). A displacement of the shoot apical meristem (sam, Fig. 1*e*) is correlated with the accrescence of the larger "macrocotyledon" (Co). (*e′* and *f′*) Stages in the development of species assigned to the subgenus *Streptocarpus*, no member of which manifests true nodal organization. Rather, all members of this subgenus manifest a "phyllomorph"-type organization (Figs. 1*f′*, 1*g′*, and 1*g″*) after passing through a stage in which the embryonic shoot meristem is suppressed (Fig. 1*e′*), its position occupied by inactive meristematic-appearing cells, the groove meristem (gm). An intercalary meristem, the "petiolode meristem" (pm), extends across the hypocotyl from the groove meristem (Fig. 1*e′*). Cell maturation both proximal and distal to this meristem gives rise to the axial "petiolode" portion of the phyllomorph. Another zone of intercalated meristematic cells, the basal meristem (bm), develops across the basal, proximal portion of the macrocotyledon (Fig. 1*e′*). All tissues of the lamina portion of the phyllomorph, exclusive of the midrib, are derived from the basal meristem. (*g″*) An early stage of plants with unifoliate or plurifoliate habits. Figure 1*g″* shows an early stage of *S. primufolius*, a plant with the rosulate habit. Figure 1*g′* shows an early stage of phyllomorph organization. The smaller cotyledon will soon fall off, and the mature plant will consist of the axis the petiolode, which is a combination of stem and petiole, and the lamina, which develops from the macrocotyledon. The seedlings of the subfamily Gesnerioideae exhibit isocotyly and the activity of the shoot apical meristem is manifest in the formation of paired stem leaves (Fig. 1*h*).



base of the macrocotyledon (*Streptocarpus*, plurifoliate type) (Figs. 3*a* and 4*a*), (*ii*) anisocotyly with multiple phyllomorphs (*Saintpaulia* type) (Figs. 3*b* and 4*b*), (*iii*) anisocotyly with plumular development (*Streptocarpella* type) (Figs. 3*c* and 4*c*), and (*iv*) isocotyly with plumular development (Gesneriodeae type) (Figs. 3*d* and 4*d*). The first three types have features characteristic of different species placed in the subfamily Cyrtandroidae. The fourth type is characteristic of species placed in a different subfamily of the Gesneriaceae, the Gesneriodeae. The phenovariant morphologies that were induced experimentally thereby phenocopied features of other species in the genus and other genera in the Gesneriaceae. It must be emphasized that although the phenovariants manifested some features typical of other species, they were not phenocopies of any particular species. Furthermore, the phenovariant patterns induced were transitory, presumably persisting only as long as *S. prolixus* seedlings were exposed to "effective" concentrations of the antimetabolites of Hyp-protein synthesis, 3,4-dehydroproline, 2,2′-dipyridyl, or hydroxy-L-proline. Apparently, locally suppressed cell proliferation in *S. prolixus* was prevented ("desuppressed") by temporarily antagonizing the synthesis of some Hyp-proteins. Essentially the same results were obtained in earlier experiments with leafy liverworts (Basile and Basile 1990, 1993, 1994; Basile et al. 2000). The similar results from studies conducted with liverworts and a flowering plant suggest that the molecular basis for the correlative inhibition ("suppression") of localized growth processes may be the same in all land plants.

Since the antagonists used in the studies cited above were capable of interfering with the normal synthesis of any Hyp-protein, it was not possible to identify the specific Hyp-protein in which the altered synthesis resulted in the wide range of phenovariation observed. Evidence from studies conducted with bryophytes (Basile and Basile 1990, 1993; Basile et al. 2000), however, suggested that one or more arabinogalactan-proteins (AGPs) were likely candidates.

AGPs constitute a large family of glycoproteins and proteoglycans associated with the plasma membranes and cell walls of all plants investigated for their presence (Clarke et al. 1978; Basile and Basile 1987; inter alia). For more than 30 years, both experimental and inferential evidence has been accumulating implicating AGPs in a wide range of processes underlying plant development such as cell–cell recognition, cell proliferation, cell fate determination, and

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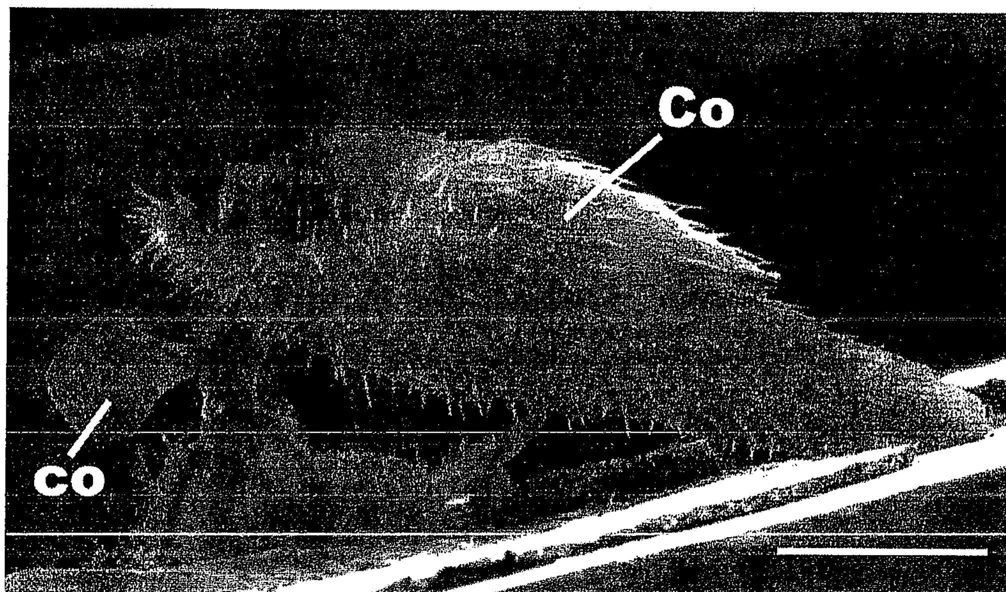
Fig. 2. Micrograph of a control *Streptocarpus prolixus* plant (phyllomorph) at the same period of growth as the phenovarients shown in Figs. 3 and 4. Note the macrocotyledon (Co) and the microcotyledon (co) with no indication of any additional plumular or phyllomorphic growth. Because of the large size of the lamina (5.1 mm), the minimal enlargement possible on the scanning electron microscope (25×) did not allow for a complete picture of the plant, and this is an amalgam of two micrographs. Bar = 1.2 mm.



organogenesis (for reviews, see Clarke et al. 1979; Fincher et al. 1983; Keuger and van Holst 1996; Majewska-Swaka et al. 2000; Nothnagel 1997; Pennell 1992; Serpe and Nothnagel 1999; Showalter and Varner 1989; Sommer-Knudsen et al. 1998). Of the papers reviewed, most germane to the present study were those that provided evidence to indicate that experimentally induced changes in the size, shape, and (or) distribution of organs (i.e., leaves and branches) could be correlated with changes in AGPs (Basile and Basile 1990, 1993; Basile et al. 2000). Also relevant is the evidence that some AGPs may mediate changes in morphogenesis by virtue of locally suppressing cell enlargement and (or) cell proliferation (Ding and Zhu 1997; Jauh and Lord 1996; Serpe and Nothnagel 1994; Thompson and Knox 1998; Willats and Knox 1996). These studies involved the use of Yariv phenylglycosides. One of these, β-D-glucosyl phenylglycoside $(\beta\text{-D-Glc})_3$, is widely considered to be diagnostic for AGPs. It reversibly binds to and precipitates most AGPs in the presence of low concentrations of salts (Nothnagel 1997). Other Yariv phenylglycosides such as $(\beta\text{-D-Man})_3$ and $(\alpha\text{-D-Gal})_3$ do not bind AGPs and were used as controls in the binding experiments cited above.

The first to find that $(\beta\text{-D-Glc})_3$, when added to a medium of actively growing cell suspension cultures, could suppress cell proliferation were Serpe and Nothnagel (1994). The $(\beta\text{-D-Man})_3$ and $(\alpha\text{-D-Gal})_3$ phenylglycosides had no discernible effects. When the $(\beta\text{-D-Glc})_3$ was washed out of the cells, proliferation resumed. Subsequently, others used this approach to perturb the function of AGPs, thereby altering different aspects of seedling development (Ding and Zhu 1997; Thompson and Knox 1998; Willats and Knox 1996) and pollen tube growth (Jauh and Lord 1996). It was concluded from these experiments that the modified developmental patterns observed resulted from the capacity of $(\beta\text{-D-Glc})_3$ to perturb AGPs.

In the study reported here, seedlings of *S. prolixus* were treated with Yariv $(\beta\text{-D-Glc})_3$ or $(\alpha\text{-D-Gal})_3$ phenylglycosides to determine whether AGPs could be similarly implicated in mediating altered patterns of development, particularly altered patterns of organogenesis.

Materials and methods

Plant material

The seeds of *S. prolixus* used in this study were from plants grown by the authors, hand-pollinated, harvested, and kept at 5–6°C. The stock plants themselves were grown from seeds obtained through the Seed Fund of the American Gloxinia-Gesneriad Society.

Preparation of inocula

Seeds were surface sterilized using the "washing machine" method of Basile (1973) in 3% Clorox for 4 min.

Culture media

For the axenic culture of the seeds, a basal medium of Knops macronutrients (0.125 g of KNO_3/L, 0.5 g of $Ca(NO_3)_2{\cdot}4H_2O$/L, 0.125 g of $MgSO_4{\cdot}7H_2O$/L, 0.125 g of KH_2PO_4/L) supplemented with Hutner's (1972) "metals 49" adjusted to a pH of 5.8 was used. It was solidified with Gellan (Research Organics, Cleveland, Ohio) at 4.5 g/L. Seeds were sown in a proscribed pattern in each Petri dish and sealed with Parafilm.

Application of antagonists

3,4-Dehydroproline was obtained from Sigma Chemical Co. (St. Louis, Mo.), α-galactosyl and β-glucosyl Yarif phenylglycosides had been prepared by Dr. D.V. Basile and Dr. I. Ganjian (Ganjian and Basile 1997). The reagents were filter sterilized in a laminar flow hood using Pro-X Filter units. The reagents were added to the medium to produce working concentrations of 2.5, 5, 10, 20, and 30 μM in the case of the β-glucosyl Yariv reagent, 5 and 30 μM in the case

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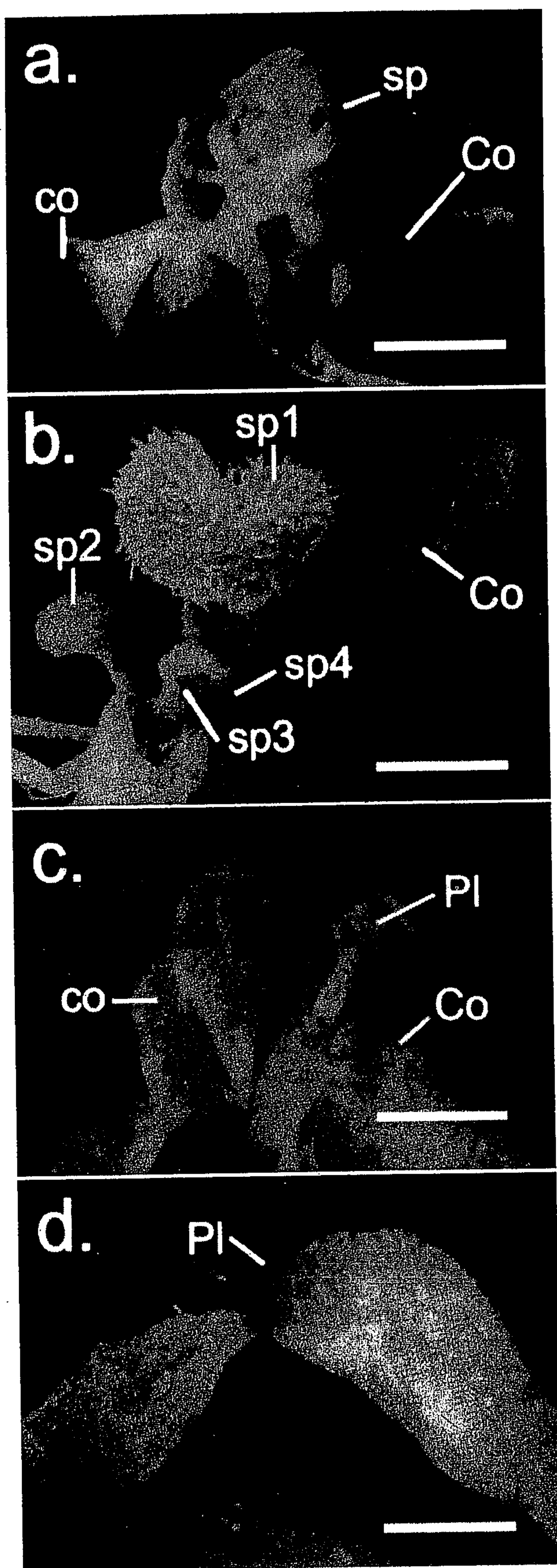

Fig. 3. Photomicrographs of phenovarients of *Streptocarpus prolixus* induced by β-glucosyl Yariv phenylgylcoside ((β-D-Glc)$_3$) taken at 85 days after germination showing the same four types induced in earlier experiments by antagonists of Hyp-proteins. For comparison, both Figs. 1*f′* and 1*g′* show the normal phenotype at a corresponding age and Fig. 2 is a micrograph of a control phenotype at 85 days after germination. The types are (*a*) anisocotyly with a secondary phyllomorph (*Streptocarpus*, plurifoliate type) induced by 5 μM (β-D-Glc)$_3$, (*b*) multiple phyllomorphs (*Saintpaulia* type) induced by 30 μM (β-D-Glc)$_3$, (*c*) anisocotyly with a plumule (*Streptocarpella* type) induced by 5 μM (β-D-Glc)$_3$, and (*d*) isocotyly with a plumule (Gesnerioideae type) induced by 20 μM (β-D-Glc)$_3$. co, microcotyledon; Co, macrocotyledon; sp, secondary phyllomorph; sp1–sp4, additional phyllomorphs; Pl, plumule. Bar = 500 μm.

of the α-galactosyl Yariv reagent, and 50 μM in the case of 3,4-dehydroproline.

Incubation of cultures

Cultures were maintained at 18 ± 2°C under cool white fluorescent lamps at 3000 lx at a 14-h photoperiod in a Percival growth chamber.

Double diffusion assay

Ten-millilitre gels composed of 1% w/v agarose dissolved in 0.15 M NaCl were made up in 60 × 15 mm plastic Petri dishes. Five wells in a quadrangonal were punched in the gels with a No. 1 cork borer. A 10-μL aliquot of *S. prolixus* AGPs at a concentration of 0.5 mg/mL was placed in the center well and 10-μL aliquots of α-galactosyl Yariv reagent and β-glucosyl Yariv reagent at concentrations of 1 mg/mL were placed in opposite wells.

Extraction of AGPs from *S. prolixus*

Plant material from a phyllomorph of *S. prolixus* was ground in Barbitol buffer (pH 8.6) containing 0.2 M $CaCl_2$, 5 mM EDTA, 3.2 mM DTT (dithiothreitol), 0.05% w/v CHAPSO, and 0.02% w/v NaN_3. Extract was filter centrifuged at 3000 rpm for 15 min through two layers of Miracloth and again at 25 000 × *g* for 1 h at 4°C. The supernatants were concentrated and desalted using Amicon Centricon 30 tubes following the manufacturers instructions. The concentrate of AGP is made to 80% v/v ethanol to precipitate AGPs.

Measurements

Measurements were made under a Bausch and Lomb binocular microscope along the midrib of each cotyledon. Observations of the presence of phenovariation were made at the same time. Phenovariation was noted with the appearance of a third or more leaflike forms and (or) the appearance of a plumule.

Documentation

Microphotographs were made under a Bausch and Lomb binocular microscope using a Polaroid MicroCam. Drawings were made from samples using the dissecting scope. A sheet of 1-mm^2 graph paper was placed beneath the Petri dishes for accuracy of measurement. Microphotographs were traced using Micron technical pens on clear acetate sheets to clarify the photographs. For the scanning electron micrograph, the

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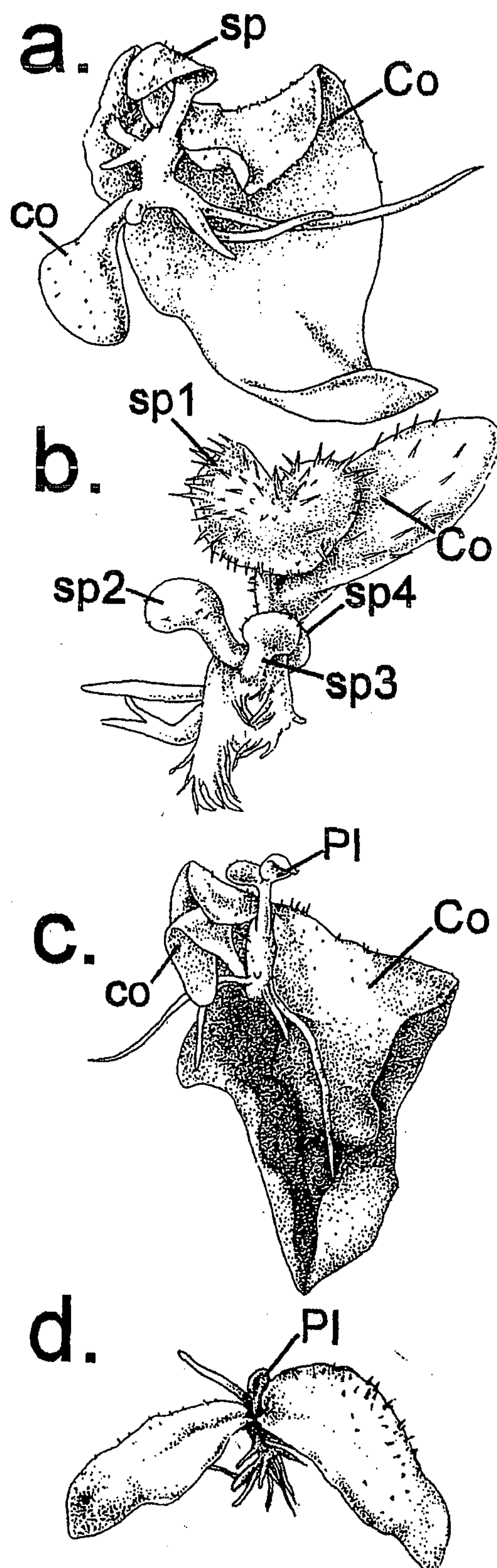
Fig. 4. Drawings of the whole *Streptocarpus prolixus* plants shown in Fig. 3 in an attempt to clarify the phenovariation shown in the micrographs. The drawings have been oriented so that the primary axis (the petiolode) is vertical. co, microcotyledon; Co, macrocotyledon; sp, secondary phyllomorph; sp1–sp4, additional phyllomorphs; Pl, plumule. Bar = 500 µm.



control seedling was fixed, dehydrated, critical point dried in a Tousimis Samdri-790 (Rockville, Md.), and sputter coated with gold using a Technics Hummer II (Technics Corp., Alexandria, Va.). The seedling was photographed in a Hitachi S-2700 scanning electron microscope with a Polaroid camera attachment. Data were entered into the JMP3.2.2 Statistical software program of SAS Institute Inc. (Cary, N.C.). Polaroid images were scanned into Adobe Photoshop 5.5 to remove unnecessary artifacts from the background, and Adobe Illustrator 9.0 was used to add scale bars and figures. Graphs were made using the figures obtained from the JMP statistical program and created using the Deneba Canvas 6.0 graphics program.

Results

Three separate experiments were conducted using (β-D-Glc)$_3$ to perturb AGPs. In the first, the effects of (β-D-Glc)$_3$ on organogenesis in *S. prolixus* were compared with those of a prolyl hydroxylase inhibitor, 3,4-dehydroproline (Cooper and Varner 1983; Nolan et al. 1978). In the second, the effects of additional concentrations of (β-D-Glc)$_3$ were determined. In the third, the influence of (β-D-Glc)$_3$ was compared with that of (α-D-Gal)$_3$, a Yariv reagent that does not bind AGPs (Serpe and Nothnagel 1994).

In the first experiment, 5 and 30 µM concentrations of the β-glucosyl Yariv reagent and a 50 µM concentration of 3,4-dehydroproline were used. Germination was observed taking place in all dishes from a period of 6 to 15 days after seeding, with the majority of germination occurring on day 8. Day 8 was taken as the experimental starting point. Observations and measurements of the lengths of the cotyledons were made periodically over the following 85 days. At the 18th day after germination (DAG), most of the cotyledons were still equal in length (isocotyly). By the 28th DAG, the number of plants showing unequal growth of the cotyledons (anisocotyly) had increased substantially, and the measurements of the macrocotyledon indicated that the mean of the control-grown seedlings was significantly longer than any of the seedlings grown in the antagonists (Fig. 5). The number and types of phenovariant plants were recorded at 85 DAG. Phenovariation was apparent not only due to differences in cotyledon length but more importantly due to the number of plants with distinctive changes in morphology. Statistical analysis of the amount of phenovariation observed at this time showed that the amount of phenovariation attributable to either the perturbation of AGPs or the antagonism of Hyp-protein synthesis was in each case highly significant ($P = 0.01$). Data from this analysis, as well as from the other experiments, are given in Table 1.

In a second experiment in which only (β-D-Glc)$_3$ was used to perturb AGPs, the range of concentration of the (β-D-Glc)$_3$ was increased to include concentrations of 2.5, 10, and 20 µM (β-D-Glc)$_3$. Observation of the plants 63–69 DAG indicated that phenovariation had already taken place (see Figs. 3 and 4). Statistical analysis of the results of this experiment corroborated the results of the previous experiment ($P = 0.01$).

A third experiment was conducted in which the effects of 5 and 30 µM (β-D-Glc)$_3$ and (α-D-Gal)$_3$ on morphogenesis of *S. prolixus* were compared. Measurements and observations were made at 20, 40, 65, and 90 DAG. Statistical analysis of

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the number of phenovariant plants at 90 DAG showed that no significant phenovariation was induced by (α-D-Gal)$_3$ (Table 1). Phenovariation induced by (β-D-Glc)$_3$ in this third experiment, on the other hand, copied the changed morphotypes induced in the previous two experiments with the same reagent as well as with antagonists of Hyp-proteins. A double diffusion assay testing AGPs extracted from *S. prolixus* confirmed this result. The β-glucosyl Yariv phenylgylcoside formed a precipitate with the *S. prolixus* AGPs, while the α-galactosyl one did not (Fig. 6).

To reiterate, in three separate experiments, (β-D-Glc)$_3$, like the previously tested Hyp-protein antagonists, induced *S. prolixus* to develop plumules with or without equal cotyledon growth, secondary phyllomorphs without equal cotyledon growth, or multiple phyllomorphs (Figs. 3 and 4).

Discussion

As stated earlier, the primary objective of this study was to determine whether by using Yariv phenylglycosides to perturb the function of AGPs, phenovariation comparable with that previously induced by Hyp-protein antagonists could be induced in *S. prolixus*. A qualitative and quantitative analysis of three separate experiments indicated that (β-D-Glc)$_3$ could (Table 1). The morphological changes induced by (β-D-Glc)$_3$, but not by (α-D-Gal)$_3$, exactly phenocopied the morphological changes induced by three different acting antagonists of Hyp-proteins. These results strongly suggest two things. One is that suppression and desuppression of the development of the organs affected are mediated by (β-D-Glc)-binding AGPs. Not all AGPs react with (β-D-Glc)$_3$ (Nothnagel 1997). The other is that the AGPs mediating suppression and desuppression contain Hyp, even though Hyp is not a component of all AGPs (Baldwin et al. 1993). Because there were no discernible relationships between the phenovariants produced and the concentration of Yariv phenylglycoside supplied, the results of these experiments indicate that it was differences in the developmental stage of the individual plants rather than a specific concentration of (β-D-Glc)$_3$ that was the most important in inducing the range of different phenotypes observed.

How exposure of newly germinating seeds to (β-D-Glc)$_3$ permitted the continued development of organs in which continued development is normally suppressed while simultaneously suppressing development in parts of the plants not normally suppressed could not be learned from these experiments. A reasonable explanation can be offered based on two assumptions. The first is that (β-D-Glc)$_3$ binds to and thereby impairs the functions of a wide range of AGPs. The second is that AGPs have evolved to serve a variety of functions in plants. Some AGPs function directly or indirectly to promote growth and development. Perturbation of these AGPs results in impaired growth and (or) development. Other AGPs apparently function directly or indirectly to suppress growth and (or) development. Perturbation of these growth-suppressing AGPs has the reciprocal effect. It results in continued growth and (or) development.

It seems that the AGPs that mediate phenovariation in plants do so by virtue of their capacity to suppress cell proliferation. How AGPs suppress growth cannot be known from these experiments. It is assumed, however, that their capacity to mediate growth processes is related to the fact that they are cell surface associated, either bound to the cell membrane or as components of the extracellular matrix (Nothnagel 1997). AGPs are therefore in a position to interact with other molecules at the surface of the individual cell with which they become associated as well as with molecules emanating from other cells.

In this study, the appearance of organs where they are normally suppressed is the result of perturbing AGPs by a reagent well known to bind to them. Since most AGPs characterized thus far contain a high percentage of Hyp (Clarke et al. 1979; Fincher et al. 1983; Showalter and Varner 1989), it seems reasonable to assume that the Hyp-protein hypothesized to be mediating suppression and desuppression as a result of earlier experiments belongs to the class of

Fig. 5. Graph showing the mean of the macrocotyledon length along the midrib of the untreated and treated seedlings of *Streptocarpus prolixus* from the first experiment at 20, 28, 36, 65, and 85 days after germination (DAG). The treated plants were grown in solutions of 5 and 30 μM β-glucosyl Yariv phenylgylcoside and 3,4-dehydroproline. The bars show a combined mean of the length of their macrocotyledons. The treated seedlings showed significant phenovariation, but also a significant reduction in overall growth, which had occurred in earlier experiments when antagonists of Hyp-proteins were tested.

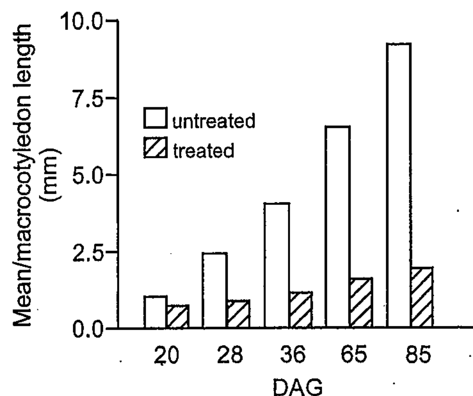

Table 1. Comparison of the numbers and percentages of samples showing phenovariation that developed in untreated and 3,4-dehydroproline- and Yariv-phenylglycoside-treated cultures of *Streptocarpus prolixus* seedlings at 85 days after germination.

Treatment (μM)	*N*	%	*P*
0	30	3	
3,4-dehydroproline			
50	35	29	0.0036
(β-D-Glc)$_3$			
2.5	19	79	<0.0001
5	55	31	0.0009
10	4	75	<0.0001
20	19	26	0.0191
30	58	31	0.0008
(α-D-Gal)$_3$			
5	64	9	0.2664
30	48	8	0.3593

Note: (β-D-Glc)$_3$, β-D-glucosyl Yariv phenyglycoside; (α-D-Gal)$_3$, α-D-galactosyl Yariv phenyglycoside; *P*, probability based on a χ^2 analysis.

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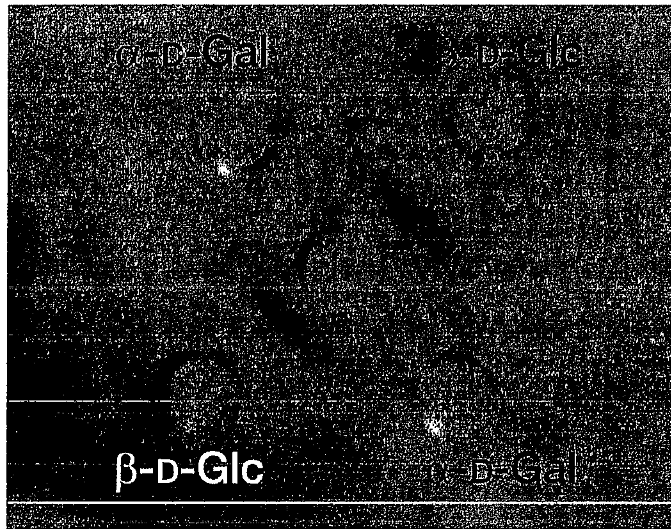

Fig. 6. Results of a double diffusion assay. As the *Streptocarpus* arabinogalactan-proteins and the Yariv phenylglycosides diffused towards each other, strong precipitation lines formed with the β-D-glucosyl Yariv reagent but not with the α-D-galactosyl Yariv phenylgylcoside.

glycoproteins known as AGPs. This was previously found to be the case in experiments with leafy liverworts and mosses. This is the first time that the Hyp-proteins affecting this type of morphoregulation in a flowering plant can be confidently identified as AGPs.

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