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Green Roots: Photosynthesis and Photoautotrophy in an Underground Plant Organ[1]

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The potential for photosynthetic and photoautotrophic growth was studied in hairy root cultures of Asteraceae and Solanaceae species. Upon transfer to light, initially heterotrophic root cultures of *Acmella oppositifolia* and *Datura innoxia* greened rapidly, differentiated chloroplasts, and developed light-dependent CO_2 fixation in the cortical cells. Photosynthetic potential was expressed in root cultures of all the Asteraceae genera examined (*Acmella, Artemisia, Rudbeckia, Stevia,* and *Tagetes*). Hairy roots of *A. oppositifolia* and *D. innoxia* were further adapted to photoautotrophy by growing in the presence of light and added CO_2 (1–5%) and by direct or sequential transfers into media containing progressively lower sugar concentrations. The transition to photoautotrophy was accompanied by an increase in CO_2 fixation and in the specific activity of 1,5-ribulose-bisphosphate carboxylase/ oxygenase (Rubisco). During the adaptation of *A. oppositifolia* roots to photoautotrophy, the ratio of Rubisco to phospho*enol*pyruvate carboxylase increased significantly, approaching that found in the leaves. The levels and patterns of alkaloids and polyacetylenes produced by Solanaceae and Asteraceae hairy roots, respectively, were dramatically altered in photomixotrophic and photoautotrophic cultures. Photoautotrophic roots of *A. oppositifolia* have been maintained in vitro for over 2 years.

Plant roots usually grow underground as heterotrophic organs, depending on the shoot and leaves for their energy source. Roots may become green when exposed to light or when they develop as adventitious organs (Torrey and Clarkson, 1975). In roots of the epiphytic Orchidaceae (Benzing et al., 1983) and in the aerial roots of mangroves (Gill and Tomlinson, 1977), photosynthesis by this organ does, in fact, contribute to the carbon economy of the whole plant. The expression of photosynthetic capacity in aerial roots is accompanied by remarkable biochemical and morphological changes (Torrey and Clarkson, 1975; Gill and Tomlinson, 1977; Benzing et al., 1983; Hew, 1987). In most higher plants, however, we know very little about root photosynthetic potential, the conditions favoring its expression, or how widespread this metabolic capacity may be. This applies particularly to roots that normally grow underground. Although many roots, in vivo or in vitro, can become green when grown under light, this ability appears to vary widely among plant species (H. Flores, unpublished observations). It is also well known that isolated root cultures are dependent on vitamins such as thiamine for continuous growth in vitro (White, 1937). The tap roots of weeds such as dandelions (Peterson, 1975) and many in vitro root cultures can regenerate green shoots, suggesting that the root cells remain totipotent. However, we do not know to what extent the root, as an organ, has retained its potential for photosynthesis and photoautotrophy, a property assumed to be common to all plant organs.

The in vitro culture of isolated plant roots dates back to the pioneering work of P.R. White and was for several decades a widely used experimental system (White, 1934). The availability of hairy root cultures obtained by genetic transformation with *Agrobacterium rhizogenes* (Tepfer, 1984) has renewed interest in this system. Hairy root cultures can grow as fast as unorganized plant cell suspensions while maintaining a stable differentiated phenotype. Furthermore, hairy roots can express root-specific metabolic pathways as efficiently as normal root cultures or roots in vivo (Flores and Filner, 1985; Flores and Curtis, 1992). In contrast with the instability and variability commonly observed in plant cell suspensions, hairy roots have shown long-term, stable production of alkaloids, polyacetylenes, sesquiterpenes, naphthoquinones, and many other plant secondary metabolites (Flores et al., 1988; Signs and Flores, 1990). We have also shown that hairy roots are responsive to stimuli such as elicitors and changes in the physical environment and are capable of transforming inert xenobiotics into bioactive metabolites (Flores and Curtis, 1992). During these studies, we frequently observed that root cultures derived from evolutionarily distinct taxa (Asteraceae, Solanaceae, Cucurbitaceae, etc.) had the ability to become green when grown in the light. As we report below, the potential for photosynthesis and photoautotrophy can be expressed by isolated roots under appropriate conditions and may be widespread.

MATERIALS AND METHODS

Establishment of Hairy Root Cultures

Hairy roots of *Acmella oppositifolia* were obtained by transformation with *Agrobacterium rhizogenes*. Briefly, the stems

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Abbreviations: PEPCase, phospho*enol*pyruvate carboxylase; TEM, transmission electron microscopy.

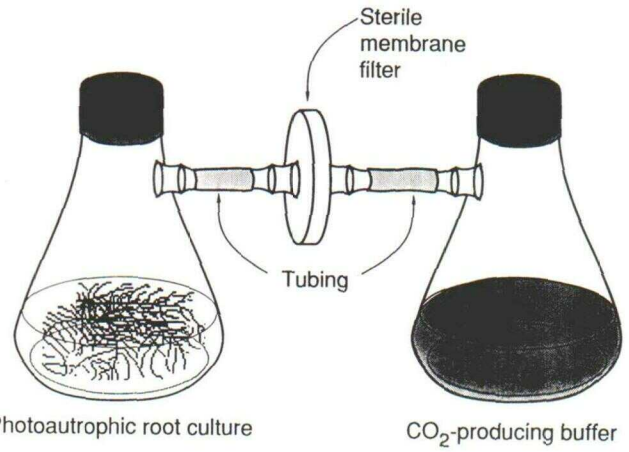

Figure 1. Scheme of the two-flask apparatus used to develop photoautotrophic root cultures.

of in vitro-grown seedlings of *A. oppositifolia* were inoculated with a 3- to 5-d-old culture of *A. rhizogenes* strain 15834 American Type Culture Collection (ATCC); adventitious roots emerging at the infection site were transferred to basal solid medium containing 100 to 200 mg/mL of carbenicillin. The hairy root phenotype was verified by opine assays and comparison with growth rates of normal root cultures. Some root cultures of *A. oppositifolia* were also obtained from Dr. Martin Hjortso (Louisiana State University). The hairy root culture of *Datura stramonium* was established in the same way using *A. rhizogenes* strains TR-105 or ATCC 15834 for infection. Root cultures of *Bidens sulphureus* and other Asteraceae genera (*Artemisia, Carthamus, Rudbeckia, Tagetes, Stevia*) were established and maintained as stocks in solid medium as described previously (Flores et al., 1988).

Establishment of Photoautotrophic Root Cultures

All experiments described in this paper were performed on roots grown in liquid media. Photomixotrophic stock cultures of *A. oppositifolia* and *D. stramonium* were maintained on either Murashige and Skoog's (1962) or Gamborg's B5 (Gamborg et al., 1968) medium containing 3% Suc. They were cultured under continuous light (70 μE m^{-2} s^{-1}) on a gyratory shaker (100 rpm) at 25 ± 1°C.

To develop photosynthetic root cultures, we designed a horizontal closed two-flask system, modified from the two-tiered culture apparatus of Husemann and Barz (1977). Roots were cultured on Gamborg's B5 medium containing 3%, 0.3%, or 0% Suc in a sidearm flask connected by a sterile Gelman membrane filter or a sterile cotton-filled tube to another flask containing a CO_2-generating buffer (from 2 M $KHCO_3$/2 M K_2CO_3, 79:21, v/v) to provide a 1 to 5% CO_2 atmosphere (Fig. 1). This system simplified the dispensing of the CO_2 buffer. The CO_2 generated by the buffer was transferred by diffusion to the flask containing the root culture. At the end of 3 weeks, the average CO_2 concentration in the ambient treatment was 0.03% in the light and 0.04% in the dark, whereas that in the enriched treatment was 4.54% in the light and 4.99% in the dark. To establish photoautotrophic cultures, root cultures on a medium containing 3% Suc were transferred either directly into sugar-free medium or sequentially into media containing progressively lower sucrose levels (1, 0.3, and 0%). Cultures were kept under continuous light (70 μE m^{-2} s^{-1}) on a gyratory shaker (100 rpm) at 25 ± 1°C and transferred every 3 to 4 weeks. Once the photoautotrophic root cultures were established, they were routinely maintained by subculturing them in the light in 125-mL flasks and bubbling 5% CO_2 directly into the medium at 1 mL/s (Fig. 2). A scaled-up culture of photoautotrophic roots was performed using a 10-L bioreactor in a 5% CO_2 atmosphere on sugar-free Gamborg B5 medium at room temperature.

Rubisco and PEPCase Assays

Rubisco and PEPCase activities were assayed by a procedure modified from Seemann et al. (1985). One hundred milligrams of root tissue were homogenized in a prechilled Wheaton tissue grinder with 1.2 mL of 25 mM Bicine buffer with 1 mM EDTA, 10 mM DTT, 20 mM $MgCl_2$, 10 mM $NaHCO_3$ (pH 8.0) and centrifuged at 16,000*g* for 1 min. Total Rubisco activity was determined in the supernatant; the assay buffer

Figure 2. Photoautotrophic root cultures of *A. oppositifolia* grown in 125-mL Erlenmeyer flasks in basal medium with an external source of CO_2 (2–5%).

consisted of 100 mM Bicine, 1 mM EDTA, 20 mM $MgCl_2$, 5 mM DTT, and 20 mM $NaHCO_3$ (pH 8.2). Fifty microliters of enzyme extract and 400 μL of assay buffer were incubated at room temperature for 10 min. The assay was started by adding 25 μL of 10 mM 1,5-ribulose bisphosphate and 25 μL of $NaH^{14}CO_3$ (ICN, 2.5 μCi per assay vial, 56 mCi/mmol) and run at room temperature for 2 min. The assay was stopped by adding 100 μL of 2 N HCl. One hundred microliters of the reaction mixture were removed and placed in a scintillation vial. After drying the sample, 3 mL of scintillation solution (Eco Scint, National Diagnostics) was added to the vial and counted in a Beckman model LS 5000TA. The assay for PEPCase was run as above, with the following changes: (a) the assay buffer was 100 mM Bicine, 1 mM EDTA, 10 mM $MgCl_2$, 5 mM Gln, 5 mM DTT, 20 mM $NaHCO_3$, pH 8.1; (b) 25 μL of 10 mM PEP was added as substrate to the assay mixture, and no preincubation was necessary.

CO_2 Fixation

One hundred milligrams of root tissue were incubated in the light (100 μE m^{-2} s^{-1}) or darkness for 2 h (in a 100 rpm shaker) in 10 mL of a sugar-free B5 medium containing 5 mM $NaH^{14}CO_3$ (ICN, 0.035 mCi/mmol). The reaction mixtures were shaken at 100 rpm for 2 h. After they were rinsed with deionized H_2O and extracted overnight in 1 mL of 0.1 N HCl, roots were homogenized and centrifuged at 15,850*g* for 5 min. Two portions of 50-μL aliquots of the supernatant were mixed with 3.5 mL of Eco Scint cocktail (National Diagnostics) and counted as above. The pellet was treated with TS-1 tissue solubilizer (Research Products International Corp.) at 50°C for 2 h, neutralized with acetic acid, and counted as above.

Light and Electron Microscopy

Samples of light- and dark-grown hairy roots of *A. oppositifolia* and *B. sulphureus* were taken from the mature portions of exponential-phase cultures growing in liquid medium. For light microscope observations, free-hand cross-sections were prepared. Sections were examined and photographed on a Nikon Diaphot fluorescence microscope (excitation, 470–490 nm; emission, 560 nm). For the TEM study, root tissues were fixed in glutaraldehyde (3% in 0.15 M sodium cacodylate buffer, pH 7.1) for 3 h and then incubated in 1% osmium tetroxide buffer for 1 h at room temperature. Tissues were prestained with 0.5% uranyl acetate overnight. After dehydration, samples were infiltrated with Firm Spurr (10.0 g of vinylcyclohexane, 6.0 g of diglycidyl ether of polypropylene glycol, 26.0 g of nonenyl succinic anhydride, 0.4 g of dimethylaminoethanol) before embedding. Thin sections (60–90 nm) were prepared, stained with 2% uranyl acetate, and examined at 80 kV accelerating voltage in a JEOL 1200 EX11 transmission electron microscope.

Alkaloid Analysis

Tropane alkaloids were analyzed in *D. stramonium* root cultures following a recently published procedure (Monforte-Gonzalez et al., 1992). Briefly, roots were freeze-dried after harvesting; 0.5 g of dried root tissue was homogenized in 30 mL of methanol at 50 to 55°C for 2 h. After filtration, the extract was evaporated to dryness, resuspended in 2.5% (v/v) H_2SO_4 (15 mL), and washed three times with 15 mL of ethyl acetate. The aqueous phase was neutralized with 28.4% NH_4OH and adjusted to pH 9.5, and the alkaloids were extracted in ethyl acetate. Samples were concentrated, spotted on TLC plates (Whatman LK 60), and run in chloroform:acetone:methanol:28.4% NH_4OH (75:10:15:2). Chromatograms were sprayed with Dragendorff reagent, and alkaloid spots were quantified in a Shimadzu densitometer (A_{515}). Standard curves were obtained using atropine and scopolamine standards (Sigma).

Polyacetylene Extraction and Analysis

Polyacetylenes were analyzed as described before (Flores et al., 1988). Briefly, a 0.5-g (fresh weight) sample of *A. oppositifolia* roots was immersed in dichloromethane for 7 d at 5°C in complete darkness. A small aliquot of anhydrous $MgSO_4$ was added and mixed with the extraction mixture, and the roots were filtered out through Whatman No. 1 paper. The solvent was evaporated to dryness under N_2 and the sample was resuspended in HPLC-grade chloroform. Samples were prepared for HPLC by refiltering the extract into 250-μL limited-volume inserts through 0.2-μm Nalgene cellulose acetate HPLC syringe filters. The chloroform solvent was then evaporated to dryness under N_2 and the extract resuspended in 250 μl of HPLC-grade methanol. Polyacetylenes were separated isocratically in a Multisolvent Delivery System 600E (Waters) equipped with a photodiode array detector. Twenty-microliter samples were run through a μBondapak C_{18} column using a mobile phase of acetonitrile:water (60:40), a flow rate of 1.00 mL/min, and a run time of 25 min. Polyacetylenes were identified based on their characteristic UV spectra (200–400 nm) and after comparison with a spectral library (Bohlman et al., 1973). For routine analysis, the resulting chromatograms were monitored at 245 and 270 nm.

Chl and Protein Determination

Chl was extracted with 80% acetone. Samples were analyzed in a Beckman DU 65 spectrophotometer and total Chl was estimated following a modification of the procedure by Arnon (1949) as described by Harborne (1984). Protein concentration was measured according to the method of Bradford (1976) using α-globulin as a standard.

RESULTS AND DISCUSSION

We have previously observed (Flores et al., 1988) that many hairy root cultures, especially those of Asteraceae species, are capable of greening when exposed to light. Photomixotrophic root cultures of *B. sulphureus* retained their typical root anatomy while showing the characteristic fluorescence associated with Chl (Fig. 3). *B. sulphureus* roots carried out light-dependent CO_2 fixation, showed Rubisco activity, and grew significantly better in the light than dark-grown cultures in the same medium, suggesting that photo-

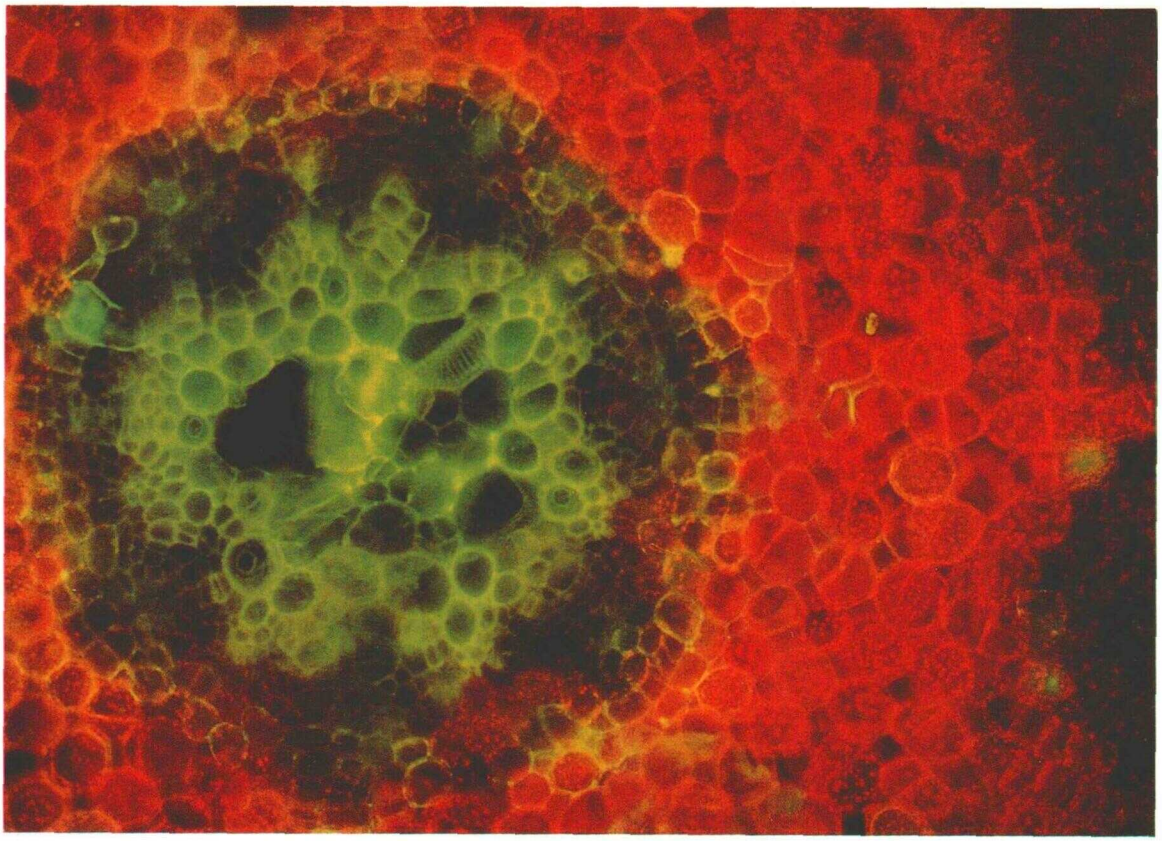

Figure 3. Cross-section of a photosynthetic root culture of *B. sulphureus*, showing green autofluorescence of the vascular tissues and red fluorescence of chlorophyllous cortical cells (excitation, 470–490 nm; emission, 560 nm). 130×.

synthesis does contribute to the growth of these green roots (Flores et al., 1988). Root cultures of all the other Asteraceae genera in our collection showed greening and photosynthetic ability when grown in the light. Root cultures of another Asteraceae species, *A. oppositifolia*, showed even faster greening when transferred to light, and were used in the studies described below.

Hairy roots of *A. oppositifolia* were obtained by transformation with *A. rhizogenes*. These cultures show much higher growth rates than normal roots (Flores et al., 1988) and have been maintained for over 2 years in a simple culture setup (Fig. 2). Cultures were originally maintained in Murashige and Skoog (1962) basal medium supplemented with 3% Suc under continuous light (50–100 μE m^{-2} s^{-1}). Later we found that growth of these photomixotrophic cultures (utilizing both Suc from the medium and ambient or fed CO_2) was significantly better in Gamborg's B5 medium (Gamborg et al., 1968) as measured by increased fresh weight, higher CO_2 fixation rates, and higher Rubisco and PEPCase activities. Therefore, all subsequent experiments were done in the latter medium.

Photoautotrophic hairy root cultures of *A. oppositifolia* were established by sequential transfer of photomixotrophic roots to progressively lower concentrations of Suc while maintaining light and feeding with CO_2 (2–5%). Roots adapted to photoautotrophy using this simple procedure were routinely maintained by subculturing into Suc-free medium in the presence of light and an enriched CO_2 atmosphere. Photoautotrophy is defined in this report in the context used in all previous work with plant cell cultures (Husemann et al., 1984; Husemann, 1985; Widholm, 1989), that is, the ability to grow continuously in the absence of Suc or other organic carbon source and to fix CO_2 in a light-dependent manner. Media formulations for photoautotrophic cell cultures vary widely and include vitamins and/or plant hormones (Husemann et al., 1984; Widholm, 1989).

In our studies, we have used the basal formulations of Murashige and Skoog (1962) and Gamborg et al. (1968) without hormones because it is well established that hairy roots can show fast growth rates independent of exogenous growth regulators (Rhodes et al., 1990). In contrast, most photoautotrophic callus or cell suspensions reported so far need plant hormones (Husemann, 1985; Widholm, 1992). Vitamins are present in very small amounts (0.1–10.0 mg/L) and thus cannot account for the long-term growth of our root cultures in the presence of light and CO_2. Recent results in our laboratory suggest that *A. oppositifolia* photosynthetic roots do not have an obligate requirement for thiamine (J. Cuello and H. Flores, unpublished observations), a vitamin previously thought to be required by heterotrophic roots (White, 1937; Bonner and Bonner, 1948). Light was absolutely required for photoautotrophic growth of *A. oppositi-*

Table I. *Light and CO_2 requirements for growth and Chl content of photoautotrophic roots of A. oppositifolia*

Roots were cultured for 3 weeks in the two-flask system described in "Materials and Methods" and shown in Figure 1. Chl analysis was performed as described in "Materials and Methods." For CO_2-enriched cultures, an initial concentration of 2% CO_2 was provided by a CO_2-generating buffer. For ambient CO_2 cultures, the attached flask contained distilled water (CO_2 is supplied by trapped air and that produced by the photosynthesizing culture). Values represent the average ± SE of two experiments done in duplicate.

Culture Conditions	Initial Fresh Weight	Net Fresh Weight Increase	Chl
	g	*g*	*μg g⁻¹ fresh weight*
Light-grown			
Ambient CO_2	10.18 ± 0.4	1.87 ± 0.4	85.7 ± 8.3
Enriched CO_2	7.36 ± 0.7	4.35 ± 0.2	178.6 ± 8.6
Dark-grown			
Ambient CO_2	8.57 ± 0.7	No growth	ND[a]
Enriched CO_2	10.46 ± 0.7	0.35 ± 0.1	

[a] ND, Not detectable.

folia, and CO_2 enrichment was necessary for optimal growth (Table I). Although dark-grown cultures showed some residual growth in the presence of CO_2, these roots failed to grow in subsequent transfers. Throughout their adaptation to Suc-limiting conditions, light-grown roots fed with CO_2 showed 100 to 300% higher biomass increase than roots grown in ambient CO_2 (Table I).

As shown in Table II, light-grown cultures of *A. oppositifolia* exhibited light-dependent CO_2 fixation and Rubisco activity. As expected, the rate of CO_2 fixation increased in parallel with Rubisco activity during adaptation to photoautotrophy. Dark-grown root cultures, which had no detectable Rubisco activity, also showed a significant amount of CO_2 fixation correlated with the presence of PEPCase activity, suggesting the operation of an anaplerotic pathway for CO_2 fixation (data not shown). PEPCase activity in light-grown roots, however, was much higher than in dark-grown cultures. The specific activities of both enzymes are within the ranges reported previously for photoautotrophic cell cultures (Husemann, 1982, 1985; Widholm, 1989). It is interesting that as the root cultures became adapted to photoautotrophy, the specific activity of Rubisco increased, whereas PEPCase showed the opposite trend. As a result, the Rubisco/PEPCase ratio of photoautotrophic roots became similar to that found in leaves of *A. oppositifolia* (Table II). Similar changes have been observed during the transition from photomixotrophy to photoautotrophy in plant cell suspensions (Husemann, 1985; Neumann 1989).

The morphology of photosynthetic and photoautotrophic roots of *B. sulphureus* and *A. oppositifolia*, respectively, was studied by light microscopy and TEM (Figs. 3 and 4). As seen previously with *B. sulphureus* roots (Fig. 3), photoautotrophic cultures of *A. oppositifolia* maintain their typical root anatomy, and the cortical cells develop the fluorescence characteristic of Chl. The plastids found in the cortical cells of both species have well-differentiated thylakoids and starch grains and are overall very similar to the chloroplasts found in leaves (Fig. 4, A and B). In contrast, the plastids of cortical cells from dark-grown roots remained largely undifferentiated and in some cases formed starch granules (Fig. 4C).

In addition to Asteraceae roots, we have been able to establish photosynthetic and photoautotrophic cultures of Solanaceae (Table III). We have previously observed that root cultures of this family have a reduced capacity for greening when grown photomixotrophically (Flores and Filner, 1985; Flores et al., 1988; Y-R. Dai and H. Flores, unpublished data). However, following the protocol established for *A. oppositifolia* roots, we were able to establish hairy root cultures of *D. stramonium* (Flores et al., 1988) and adapted them to photoautotrophy as shown in Table III. In contrast with Asteraceae, roots of *D. stramonium* showed little greening and had no detectable Rubisco activity when grown with as little as 0.3% Suc under light and ambient CO_2. Upon supplementation with CO_2, however, Chl and Rubisco activity were expressed. Chl levels of photoautotrophic root from both species ranged from 80 to 120 μg/g fresh weight. In fact, Chl levels exceeded those of leaves when expressed on a protein basis. Thus, our results show that photoautotrophy can be expressed in root cultures under appropriate conditions and suggest that this metabolic potential may be of widespread occurrence.

Table II. *CO_2 fixation and Rubisco and PEPCase activity in photomixotrophic and photoautotrophic roots of A. oppositifolia cultured under light with 2% CO_2*

Data for roots represent the average ± SE of three experiments with duplicate samples per treatment; data for leaves represent the average ± SE of a representative experiment (duplicate samples from leaves of young seedlings).

Organ	Sucrose Concentration	CO_2 Fixation		L/D[a]	Rubisco	PEPCase	Rubisco/PEPCase
		Light incubation	Dark incubation				
	%	*μmol CO_2 mg⁻¹ Chl h⁻¹*			*μmol CO_2 mg⁻¹ Chl h⁻¹*		
Roots	3.0	21.6 ± 0.7	7.7 ± 0.9	2.8	31.5 ± 3.6	69.7 ± 7.7	0.45
	0.3	36.0 ± 3.0	7.7 ± 0.6	4.7	36.0 ± 7.4	42.5 ± 6.6	0.85
	0.0	38.1 ± 4.0	6.8 ± 0.6	5.6	54.5 ± 4.4	41.2 ± 6.2	1.32
Leaves		19.2 ± 4.3	1.1 ± 0.3	18.2	14.5 ± 1.6	4.8 ± 0.7	2.95

[a] L/D, Light rate/dark rate.

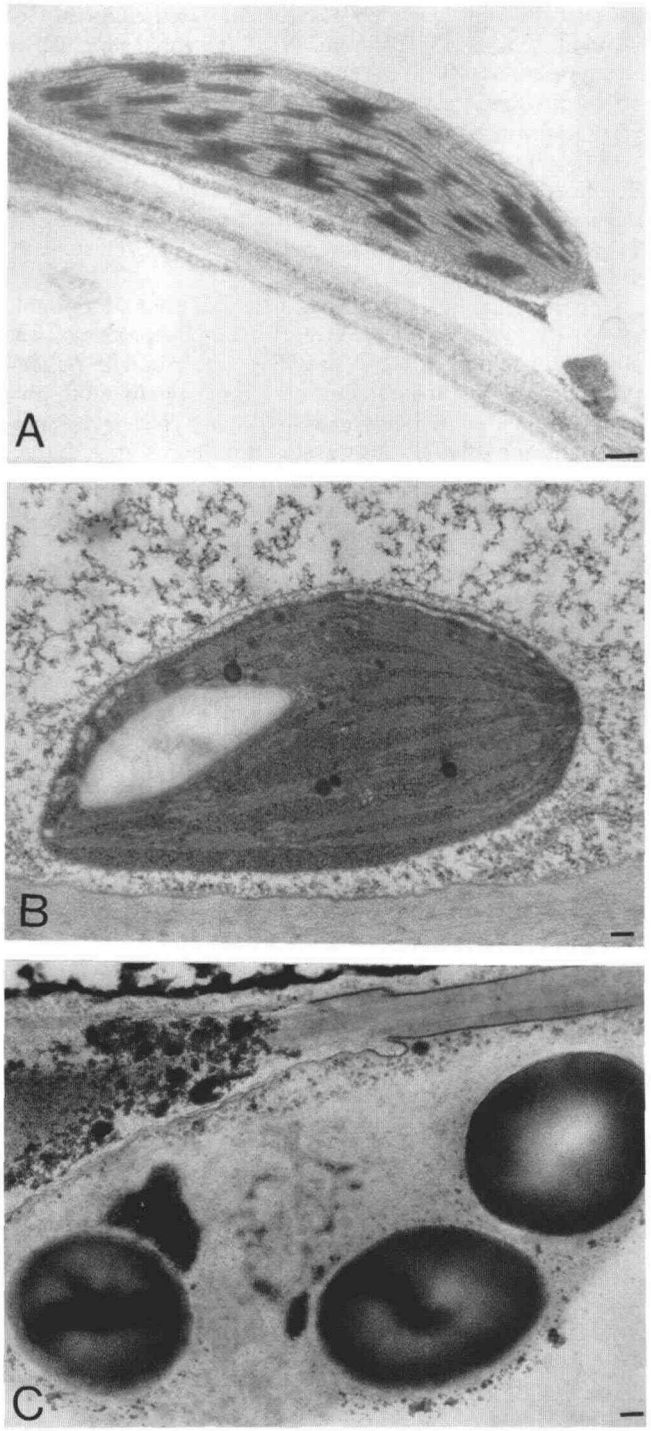

Figure 4. Plastid structure in photosynthetic and heterotrophic root cultures of Asteraceae species. Tissues were fixed and observed as described in "Materials and Methods." A, Chloroplast in cortical cell of photoheterotrophic roots of *B. sulphureus* (35,000×). B, Chloroplast in cortical cell of photoautotrophic roots of *A. oppositifolia* (31,000×). C, Plastid in cortical cell of dark-grown roots of *A. oppositifolia* (29,000×). Bars represent 0.1 μm.

Photoautotrophic plant cell cultures were first developed by Bergmann (1967) and have now been reported in 24 species of higher plants (Husemann, 1985; Widholm, 1992). In most cases, these cultures have been obtained from stem or leaf cells. The establishment of photoautotrophic plant cell cultures is usually time-consuming and difficult, involving repeated selection and subcloning of cells lines with enhanced ability to become photosynthetic (Husemann et al., 1984). Most photoautotrophic cell lines grow at slower rates than heterotrophic cultures, and the reason for this is still unknown. In contrast, the root cultures described in this report can be readily adapted to photoautotrophic growth. The doubling rate that we have obtained for *A. oppositifolia* photoautotrophic roots cultured in flasks bubbled with 5% CO_2 was 9.8 d. This is comparable with those reported for photoautotrophic cell cultures. We also have observed that root cultures grown under submerged conditions develop aerenchyma-like tissue (H.E. Flores, unpublished data), suggesting that these cultures may be oxygen limited and at least partially anaerobic. It is likely that our light-grown root cultures are similarly limited for CO_2. Bioreactors for plant cell and organ culture, which deliver nutrients via a mist or nutrient "shower," have been recently developed (Wilson et al., 1990). We project that the development of such "gas-phase" reactors should improve the growth rates of photoautotrophic cell/organ cultures, and this work is currently in progress in our laboratories (Flores and Curtis, 1992).

Root cultures can produce a wide range of plant secondary metabolites and provide a useful alternative to cell cultures as an experimental system and for the large-scale production of useful plant chemicals (Flores et al., 1987, 1988; Flores, 1992). In contrast with most cell cultures, in which somaclonal variation also affects the patterns of secondary metabolites, in many cases adversely, normal and transformed root cultures show very stable production. For example, hairy root cultures of *Hyoscyamus muticus* established in 1983 (Flores and Filner, 1985) still grow and produce the tropane alkaloid hyoscyamine at their original rate (Flores and Curtis, 1992) after about 100 monthly subcultures. However, metabolite production in heterotrophic root cultures has so far been restricted to those chemicals that are normally synthesized in the underground (i.e. dark-grown) root.

As shown in Table IV, dark-grown roots of *D. stramonium* produced the tropane alkaloid hyoscyamine, which is well known to be synthesized in the root in vivo (Waller and Nowacki, 1977). In contrast, photomixotrophic cultures showed increased levels of hyoscyamine and, in addition, produced scopolamine, a hyoscyamine derivative that is not normally found in roots but is usually extracted from leaves. Previous biosynthetic studies suggest that hyoscyamine is synthesized in the root and transported as hyoscyamine or a closely related metabolite to the shoot, where it is finally converted to scopolamine in the leaf (Waller and Nowacki, 1977; Hashimoto et al., 1991). Photosynthetic root cultures thus display characteristics of roots and shoots vis-a-vis tropane alkaloid patterns, and consistent with this trend, root cultures adapted to photoautotrophy showed the highest levels of scopolamine (Table IV). Strikingly, the level of total alkaloids in photomixotrophic and photoautotrophic root

Table III. *CO_2 fixation and Rubisco and PEPCase activity in photomixotrophic and photoautotrophic roots of D. stramonium*

Experimental conditions and analysis as described for Table II.

Organ	Suc Concentration	CO_2 Fixation		L/D[a]	Rubisco	PEPCase	Rubisco/PEPCase
		Light incubation	Dark incubation				
	%	*nmol CO_2 g fresh wt h^{-1}*			*nmol CO_2 mg^{-1} protein h^{-1}*		
Roots	1.0[b]	4.49 ± 0.0	3.4 ± 0.1	1.3	1.85 ± 0.1	0.18 ± 0.01	10.1
	0.3[b]	1.95 ± 0.10	1.3 ± 0.1	1.0	6.2 ± 0.1	0.16 ± 0.01	37.6
	0.3[c]	1.64 ± 0.10	0.9 ± 0.0	1.83	9.3 ± 0.1	0.24 ± 0.02	37.8
	0.0[c]	0.79 ± 0.0	0.5 ± 0.0	1.6	6.3 ± 0.8	0.12 ± 0.01	55.2
Leaves		0.2 ± 0.0	0.03 ± 0.0	6.0	3.14 ± 0.3	0.02 ± 0.003	136.5

[a] Light rate/dark rate. [b] With ambient CO_2. [c] With 2% CO_2.

cultures was over 2-fold that found in heterotrophic roots.

We have also observed dramatic changes in polyacetylene patterns in photosynthetic roots of *A. oppositifolia*. Roots of Asteraceae species such as *A. oppositifolia* characteristically produce a complex pattern of fatty acid-derived polyacetylenes, many of which have nematicidal and fungicidal activities (Flores, 1992). Figure 5 shows the levels of polyacetylenes in *A. oppositifolia* root as a function of light, Suc, and CO_2 concentrations. Heterotrophic root cultures grown with the standard Suc concentration (3%) showed the highest levels of polyacetylenes. As shown in Table I, these cultures grew faster in the light, and this was correlated with higher levels of polyacetylenes than in dark-grown roots. Furthermore, photosynthetic cultures grown in an enriched CO_2 atmosphere showed a pronounced increase in polyacetylenes compared with their dark-grown counterparts. This difference was not as pronounced for root cultures grown under ambient CO_2. Roots grown at 0 and 0.3% Suc showed little response in their polyacetylene levels as a function of light and enriched CO_2. HPLC analysis has also revealed marked qualitative changes in root polyacetylenes. Although the effects of light and carbon source may be rather complex, it is apparent from these results that the expression of photosynthesis and photoautotrophy by roots can have important consequences for secondary metabolic pathways. It has been reported that the transition of photoautotrophy in cell cultures has significant effects on plastid-associated pathways (Seemann et al., 1985). For example, anthraquinone pigments are confined to heterotrophic cultures of *Morinda lucida*, whereas photoautotrophic cells produce only lipoquinones (Igbavboa et al., 1985). Thus, we propose that the expression of photosynthesis and photoautotrophy may be used to expand the range of bioactive metabolites produced in roots.

Root photoautotrophy has been previously reported only in the "shootless" epiphytic species of Orchidaceae (Benzing et al., 1983). In this very specialized case, the shoot is reduced to a rudimentary organ until the time of flowering, and the root assumes the function of CO_2 assimilation for the whole plant during most of its life cycle. To our knowledge, this report provides the first demonstration that roots that normally grow underground can express their capacity for carbon autotrophy. Because it occurs in at least two taxonomically distinct and evolutionarily advanced families of higher plants (Cronquist, 1988), our results suggest that the potential for photoautotrophy in roots may be of general occurrence. Characterization of photosynthesis and photoautotrophy in roots could provide new insights into the biochemical and molecular regulation of nutrient assimilation and partitioning and suggest new ways for manipulating and utilizing the vast biosynthetic potential of this rather neglected plant organ.

Table IV. *Alkaloid content of photomixotrophic and photoautotrophic root cultures of D. stramonium*

Growth conditions were as described in Table II. Tropane alkaloids were analyzed as described in "Materials and Methods." Values represent the means ± SD of triplicate samples from three experiments.

Culture Conditions	Total Alkaloids	Hyoscyamine	Scopolamine	Scopolamine/ Hyoscyamine
	% dry wt	*% dry wt*	*% dry wt*	
Light grown				
1% sucrose[a]	1.69 ± 0.5	0.29 ± 0.1	0.014 ± 0.01	0.05
0.3% sucrose[a]	1.65 ± 0.08	0.16 ± 0.01	0.013 ± 0.003	0.09
0.3% sucrose[b]	2.83 ± 0.03	0.19 ± 0.06	0.049 ± 0.011	0.27
0% sucrose[b]	2.89 ± 0.02	0.26 ± 0.003	0.033 ± 0.007	0.13
Dark grown				
3% sucrose[a]	1.2 ± 0.2	0.15 ± 0.04	ND[c]	0.00

[a] In ambient CO_2. [b] With 2% CO_2. [c] ND, Not detectable.

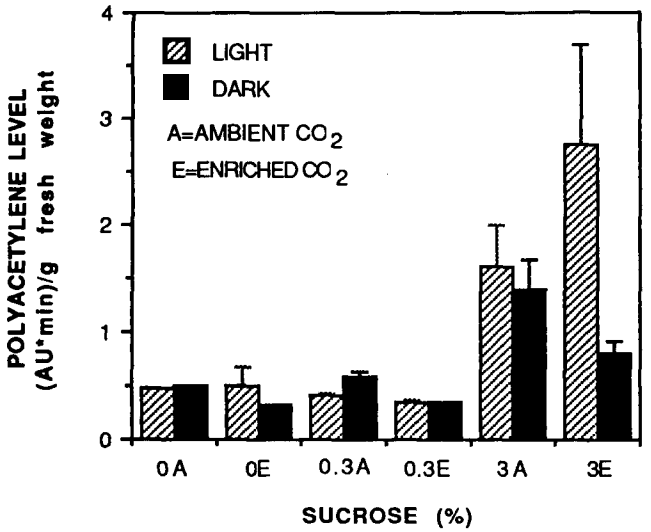

Figure 5. Effects of light and CO_2 on polyacetylene patterns of photosynthetic and photoautotrophic hairy root cultures of *A. oppositifolia*. Polyacetylenes were analyzed as described in "Materials and Methods."

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