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Sucrose Represses the Developmentally Controlled Transient Activation of the Plastocyanin Gene in *Arabidopsis thaliana* Seedlings

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The plastocyanin (PC) gene of *Arabidopsis thaliana* is activated independently of light during early seedling development. In etiolated seedlings, *PC* mRNA levels increase transiently and a maximum dark level is reached after 2 d of growth in darkness. In etiolated transgenic seedlings carrying a chimeric *PC*-promoter: luciferase fusion gene, luciferase activity is similarly increased after 2 d of growth. The transient increase in *PC* mRNA and luciferase activity levels can be repressed by sucrose. Nonmetabolizable sugars and polyethylene glycol do not have a major effect on *PC* gene expression. Also, light-grown seedlings show a similar transient and sucrose-sensitive increase in *PC* mRNA levels and luciferase activity, as in dark-grown seedlings, but here expression levels are 15-fold higher. These findings suggest the presence of a sucrose-sensitive, developmentally controlled expression mechanism that operates independently of light.

Light plays an important role in many aspects of plant development (Kendrick and Kronenberg, 1993). The perception of light by plants is mediated by at least three types of photoreceptors: phytochromes (PHY) and blue-light UV-A and UV-B receptors (Kendrick and Kronenberg, 1993). These photoreceptor molecules can activate signal-transduction processes and in this way induce light-dependent development. An attractive way to study these processes is to isolate and analyze mutants in which signaling events in transduction pathways are perturbed. The model plant *Arabidopsis thaliana* has been widely used for this purpose and several Arabidopsis genes have been identified and cloned whose products are essential for normal light-dependent development (Koornneef et al., 1980; Chory et al., 1989a, 1989b; Liscum and Hangarter, 1991; Deng et al., 1991, 1992; Ahmad and Cashmore, 1993; Pepper et al., 1994).

During germination the dehydrated and metabolically inactive seed undergoes dramatic changes. Germination in the absence of light results in a seedling with an etiolated phenotype, i.e. it has a long hypocotyl, an apical hook, and unexpanded cotyledons. In the light the seedling has a short hypocotyl with expanded cotyledons in which the plastids have developed into mature chloroplasts. Here, the PHYB photoreceptor is an important transmitter of the light signal, and one major function of this photoreceptor is the control of seedling cell elongation (Reed et al., 1993; Wester et al., 1994). Light-dependent development is associated with the modulated expression of many genes. The induction of nuclear-encoded photosynthesis genes has been studied in detail (for a review, see Thompson and White, 1991), and light-controlled regulatory genes, such as the Arabidopsis homeobox gene *ATH1* (Quaedvlieg et al., 1995), have also been identified.

The regulated expression of the *A. thaliana PC* gene has been studied in some detail in our laboratory (Vorst et al., 1988, 1993; Fisscher et al., 1994). The expression of this gene is light induced through modulation of promoter activity. Moreover, high levels of expression are observed only in chloroplast-containing cells. When chloroplast development is prevented by growing plants in the light in the presence of the herbicide norflurazon, *PC* gene expression is strongly decreased, suggesting that a signal from the chloroplast is necessary. The above-mentioned expression properties of the *PC* gene correspond to those of two other well-studied nuclear-encoded photosynthesis genes, *RBCS* and *CAB*, which encode the small subunit of ribulose-bisphosphate carboxylase and the Chl *a*/*b*-binding protein, respectively. Expression of these genes is light regulated and also requires a chloroplast factor (for a review, see Oelmüller et al., 1989). Moreover, GT-1-binding *boxII* elements, which are involved in light-controlled expression of *RBCS* genes (Lam and Chua, 1990), have been identified in the *PC* promoter (Fisscher et al., 1994).

In this paper we describe investigations of *PC* gene expression in wild-type and transgenic seedlings harboring a *PC* promoter-luciferase reporter chimeric gene. During the first days of seedling development a transient activation of the *PC* promoter was observed irrespective of the presence of light. This developmentally controlled activation can be efficiently repressed by Suc.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The pPCHPTLUC construct was created by cloning a *Hin*dIII-*Xba*I *PC* promoter fragment from pPCLUC (containing approximately 20 bp of 5′-untranslated region; Vorst et al., 1993) into pEMBL 18. A 1.4-kb *Sma*I fragment from pMOG22 (kindly provided by Mogen International,

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Abbreviation: PC, plastocyanin.

Leiden, The Netherlands), containing both the *hph* gene (Gritz and Davies, 1983) and the nopaline synthase polyadenylation site (NOS-t), was fused downstream of the *PC* promoter in the unique *Sma*I restriction site to create pPC342. pPCHPTLUC was created by cloning the 3.0-kb *Hin*dIII fragment from pPC342, containing the *PC* promoter fused to the *hph* gene and NOS-t, into the unique *Hin*dIII restriction site of pPCLUC. p35SLUC was created by exchanging the GUS gene in pBI121 (Jefferson et al., 1987) with a 1.8-kb *Pst*I-*Sac*I fragment containing the luciferase-coding sequence (De Wet et al., 1987).

The pPCHPTLUC and p35SLUC T-DNAs were transferred to Arabidopsis by *Agrobacterium*-mediated transformation (Valvekens et al., 1988) to create PC-LUC and 35S-LUC plant lines, respectively. The independent PC-LUC plant lines pc1A2.7 and pc1B80A.3 were homozygous as judged by luciferase activity segregation. The PC-LUC plant line pc2B9.6 and the 35S-LUC plant lines 35S3A and 35S11B were heterozygous for luciferase activity segregation.

For all experiments *Arabidopsis thaliana* C24 was used. Seeds were surface sterilized by washing for 5 min in commercial bleach and rinsed four times with sterile MilliQ water (Millipore). Seeds were sown onto germination medium (Valvekens et al., 1988) in 0.1% agarose. For mRNA analysis the surface-sterilized seeds were transferred to 10 mL of MilliQ and sown onto a filter paper that was placed on an agar plate. The water was then carefully removed. The plates containing the seeds were wrapped in aluminum foil, and the seeds were allowed to imbibe for 2 to 4 d at 4°C. After imbibition of the seeds, the plates were stored for 2 h in a light-tight chamber at room temperature. The seeds received a 45-min red light treatment to promote germination. The plates were wrapped in aluminum foil and stored in a light-tight box in a growth room at 23°C. For experiments with light-grown seedlings, the plates were transferred to continuous fluorescent light after the seeds imbibed. Where indicated, 1% (29 mM), 2% (59 mM), or 3% (88 mM) Suc was included in the germination medium.

Luciferase Assays

Luciferase activity measurements were performed essentially as described by Vorst et al. (1993) using the Promega luciferase assay system. Light- or dark-grown plants were removed from the agar and homogenized immediately in an ice-cold microtube containing extraction buffer. Protein extract (10 μL) was mixed with 50 μL of assay substrate and counted immediately in a liquid scintillation counter (Rackbeta 1209; LKB, Bromma, Sweden), with the coincidence circuit turned on.

RNA Analysis

Seedlings were frozen in liquid nitrogen and total RNA was isolated according to the method of Brusslan and Tobin (1992). Total RNA (7.5 μg) was electrophoresed and transferred to Hybond N (Amersham) as described by Fourney et al. (1988). Hybridization took place in hybridization buffer (Church and Gilbert, 1984) for 24 h at 65°C. After hybridization filters were washed two times at room temperature in 0.1% SDS, 0.5× SSC and two times at 65°C in the same buffer. The filters were subsequently used for autoradiography.

The mRNA abundance was measured using a laser densitometer (Ultroscan XL, LKB). A dilution series of a known RNA sample was included in each experiment to assure that the film was in the linear range of the x-ray films and for use as a standard. The major part of each experiment was repeated several times with similar results.

Carbohydrate Measurements

Etiolated seedlings were removed from the agar plate and washed briefly with MilliQ water to remove externally present sugars. Washed seedlings were frozen immediately in liquid nitrogen. The tissue was homogenized and sugar levels were determined using HPLC equipment attached to a refractive index detector as described by Ebskamp et al. (1994).

RESULTS

PC Promoter Activity during Etiolated Seedling Development

The *A. thaliana PC* promoter was fused to the firefly luciferase gene (Ow et al., 1986) to create a reporter system to monitor *PC* promoter activity. The advantage of luciferase as a reporter protein is that it can be detected in vivo without destroying the tissue. A construct was assembled that contained both the luciferase gene and the *hph* antibiotic resistance gene under control of the *PC* promoter (PC-LUC, Fig. 1). As a reference the luciferase gene was fused to the cauliflower mosaic virus 35S promoter (35S-LUC, Fig. 1). These constructs were introduced into Arabidopsis by *A. tumefaciens*-mediated DNA transfer (Valvekens et al., 1988). Several independent transgenic lines containing either the PC-LUC or 35S-LUC gene fusions were generated. From the independent plant lines a few were selected for further analysis.

In our experiments we noted that 6-d-old dark-grown seedlings already had surprisingly high levels of luciferase activity when compared to light-grown seedlings. To investigate this observation in more detail, seeds of PC-LUC line pc1A2.7 were germinated in the dark and luciferase activity was measured at several times afterward (Fig. 2A, No Suc). Luciferase activity increased approximately 6-fold between 2 and 4 d of growth in darkness. Similar results were obtained with two other independent PC-LUC lines, pc1B80A.3 and pc2B9.6 (results not shown).

To investigate whether the observed induction of luciferase activity in the dark is an intrinsic property of the *PC* promoter, *PC* mRNA abundance was measured in etiolated seedlings during successive developmental stages (Fig. 3, 0% Suc). The results showed that *PC* mRNA abundance increased in etiolated seedlings, reaching a maximum at d 2. On d 3 and 4 expression decreased again to reach a low level at d 5. This result indicated that the observed increase of luciferase activity in dark-grown seedlings was a result of activation of the *PC* promoter. The *PC* mRNA level

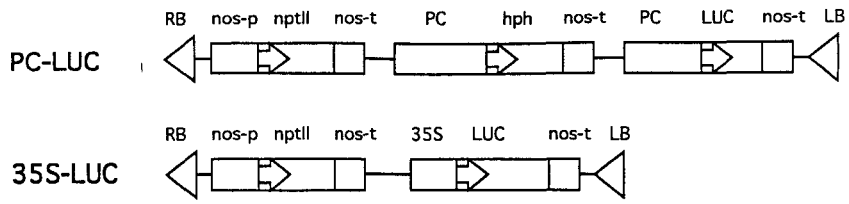

Figure 1. Constructs used to transform Arabidopsis plants. In the PC-LUC construct both the luciferase gene and the *hph* gene (the structural gene for hygromycin resistance) are driven by the *PC* promoter. The nopaline synthase terminator (nos-t) is downstream of the reporter genes. In the 35S-LUC construct, luciferase gene expression is driven by the cauliflower mosaic virus 35S promoter (35S). Each construct contains the structural gene for kanamycin resistance (*nptII*) fused to the nopaline synthase promoter (nos-p). The constructs are flanked by right (RB) and left border (LB) sequences. The arrows indicate the direction of transcription. Both the PC-LUC and the 35S-LUC constructs were transferred into Arabidopsis via *Agrobacterium*-mediated transformation.

returned to low levels on d 5, whereas luciferase activity remained high until at least 8 d of etiolation (Fig. 2A).

Two independent plants lines (35S3A and 35S11B) transformed with the 35S-LUC construct exhibited only a small increase in luciferase activity after 2 d of growth in darkness. Luciferase activity stayed fairly equal during the remainder of the experiment (Fig. 2B, line 35S3A). This expression pattern differs from the PC-LUC expression pattern.

The Transient Increase in *PC* Gene Expression Is Repressed by Suc

Suc was found to repress the increase in luciferase activity in etiolated PC-LUC seedlings. Addition of 3% Suc to the growth medium repressed the increase in luciferase activity, but lower Suc concentrations already resulted in a strong repression of luciferase activity (Fig. 2A). The same results were obtained with the independent transgenic PC-LUC plant lines pc1B80A.3 and pc2B9.6 (results not shown). As a control, the sensitivity of the cauliflower mosaic virus 35S promoter toward Suc was tested in two independent 35S-LUC plant lines (35S3A and 35S11B). During the first 4 d of growth, during which developmental stage the *PC* gene was most sensitive to Suc, no significant effect of Suc on luciferase activity could be detected (Fig. 2B, line 35S3A). After 5 d of growth, 35S-LUC seedlings grown on Suc-containing plates had lower luciferase activity levels than seedlings grown on plates without Suc. During this developmental stage the protein content in seedlings grown on plates without Suc rapidly declined, in contrast to seedlings grown on Suc-containing plates (results not shown). Since luciferase activity is expressed per amount of protein, this could explain why the luciferase activity was lower in seedlings grown on plates without Suc. Luciferase activity per plant remained higher in seedlings grown on Suc-containing plates (results not shown).

The experiments with the PC-LUC plants were complemented by measuring the effect of Suc on *PC* mRNA abundance in etiolated wild-type seedlings (Fig. 3). In the absence of Suc, *PC* mRNA levels were high on d 2 and then declined during a period of 5 d. The presence of increasing concentrations of Suc (1–3%) resulted in reduced *PC* mRNA levels and a 1 d delay in onset of mRNA increase. Considerable mRNA levels were reached in the presence of 1 or 2% Suc and these levels stayed relatively high during the course of the experiment. In the presence of 3% Suc only low levels of mRNA were found. Also in the transgenic plant line pc2B9.6 the endogenous *PC* mRNA levels were similarly affected by Suc (results not shown).

Growth in the presence of 3% Suc resulted in a number of morphological changes in the seedling. As in control seedlings, most seedlings grown on Suc emerged from the seed coat after 2 d of etiolation but subsequent hypocotyl elongation was reduced. Root growth, on the other hand, was stimulated by Suc treatment (Fig. 4). No obvious differences were present in seedlings germinated on 1% Suc compared to plants grown in the absence of Suc. When grown in the presence of other sugars such as trehalose (3%) the seedlings also were smaller (results not shown). Since trehalose cannot be metabolized efficiently, the decreased hypocotyl elongation observed in seedlings grown on 3% Suc could be due to an osmotic effect, resulting in an inhibition of normal elongation of hypocotyl cells. In conclusion, plants grown on 3% Suc have a shorter hypocotyl and longer roots, but gross morphology is not altered.

Neutral Sugars Can Accumulate during Seedling Development

In the absence of exogenous sugars the in planta Suc, Glc, and Fru levels remained low during etiolated seedling development (Fig. 5). However, the addition of Suc or Glc to the growth medium affected in planta neutral carbohydrates levels. The in planta levels of Suc, Glc, and Fru were especially high after 2 d of growth on either Suc or Glc, but these levels were strongly decreased on d 5 (Fig. 5). High in planta sugar levels on d 2 correlated with the delay of onset of expression of endogenous PC with 1 d (Fig. 3) and the repression of luciferase activity in PC-LUC plants (Fig. 2). The observed decrease in in planta sugar levels on d 3 and, especially, d 5 is surprising and is probably not due to exhaustion of the medium, since the volume of the seedlings represents only a minor fraction of the medium.

After Suc, other carbohydrates were tested for their ability to repress *PC* promoter activity in etiolated seed-

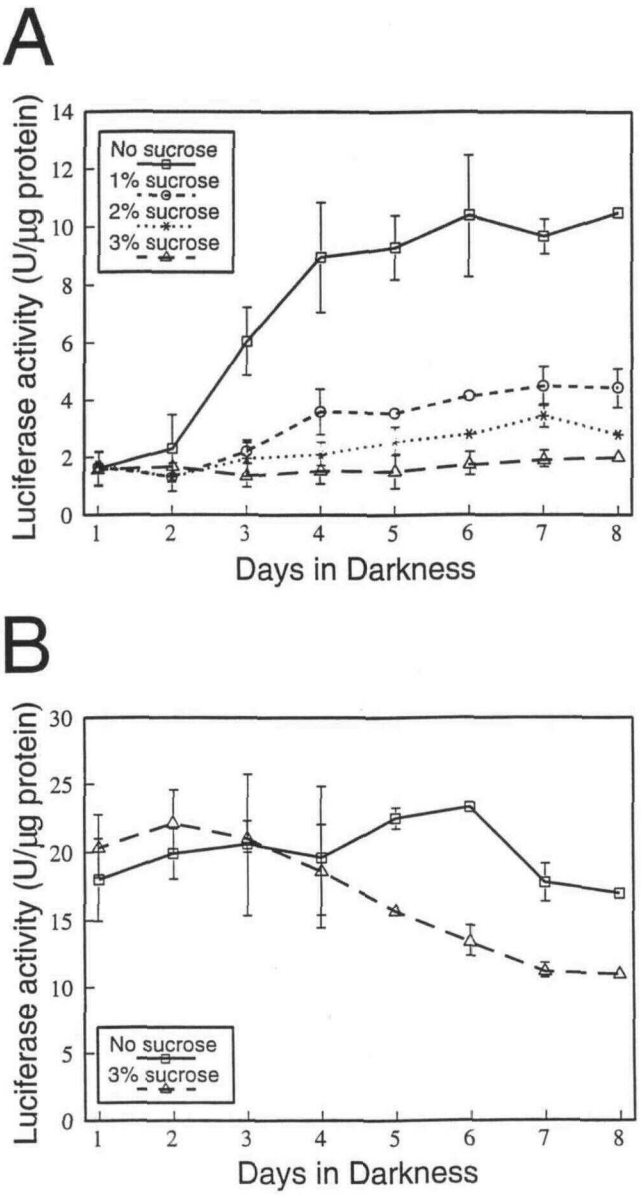

Figure 2. Effect of Suc treatment on luciferase activity in etiolated transgenic plants. A, Luciferase activity was measured in etiolated seedlings grown for 1 to 8 d in complete darkness. Plant line pc1A2.7 was grown in the absence (□) or in the presence of 1% (○), 2% (*), or 3% (△) Suc. Luciferase activity is expressed as arbitrary units (U) per microgram of protein. Each data point represents the average of three to five independent measurements. Error bars indicate the SDs. B, Plant line 35S3A, stably transformed with the 35S-LUC gene fusion, was grown in the absence (□) or in the presence of 3% (△) Suc. Luciferase activity was measured as described in A.

lings. Different sugars were included in the growth medium during etiolation of PC-LUC seedlings and after 5 d luciferase activity was measured (Table I). As shown above the presence of 3% (88 mM) Suc resulted in a 6- to 7-fold repression of luciferase activity to 15% of the activity in seedlings growing in the absence of Suc. The addition of Glc repressed luciferase activity 2-fold. In contrast, when seedlings were grown on equimolar amounts of Fru, luciferase activity remained high at a level comparable to the nonmetabolizable disaccharide sugar trehalose. Also, mannitol did not repress luciferase activity. As another control for possible effects of changed osmotic potential, seedlings were grown on 6.8% PEG 10,000. This polymer is not taken up by the root system (Lawlor, 1970) and a 6.8% solution gives a similar osmotic potential as 3% Suc. This treatment did not lead to repression of luciferase activity (Table I). These results indicate that osmotic pressure per se does not lead to repression of *PC* promoter activity. Similar results were obtained with plant line pc2B9.6 (results not shown). Luciferase activity in 35S-LUC plant lines 35S3A and 35S11B was not affected markedly by the metabolizable carbohydrates. Growth on trehalose, mannitol, and PEG resulted in a repression of luciferase activity to approximately 65% of the untreated control (Table I, plant line 35S3A), indicating that the 35S promoter is negatively influenced by osmotic stress.

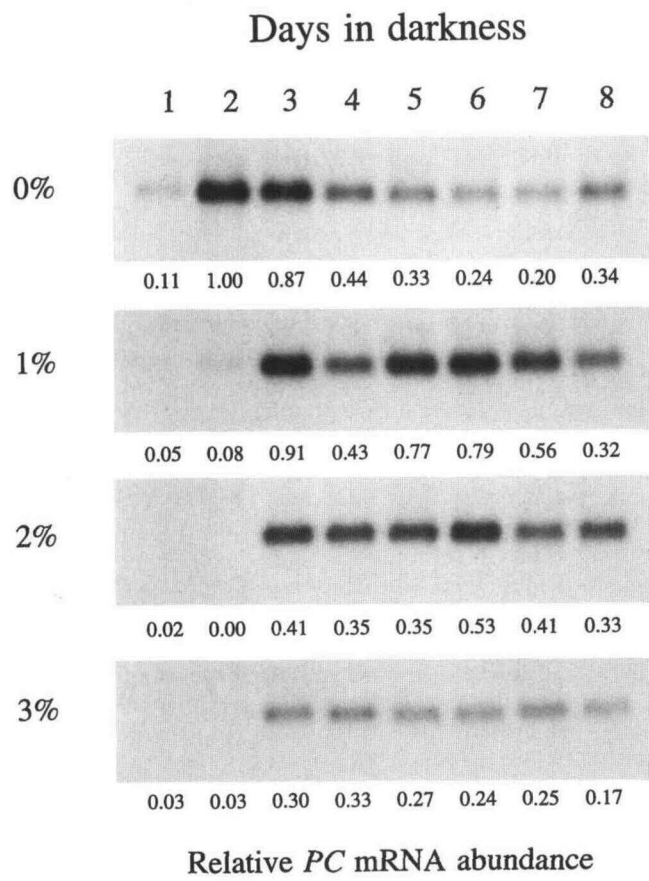

Figure 3. Effect of Suc on *PC* mRNA abundance in etiolated seedlings. A, Total RNA was isolated from wild-type Arabidopsis seedlings grown for 1 to 8 d in complete darkness. Plants were grown in the absence (0%) or in the presence of 1, 2, or 3% Suc. Total RNA (7.5 µg) was electrophoresed, blotted onto a nylon membrane, and hybridized with a 0.5-kb *PC* probe, labeled to the same specific activity in each experiment. A dilution series of a known RNA sample was included in each experiment and was used as a standard. Values below each lane represent the band intensity relative to the standard, quantified by laser scanning densitometry.

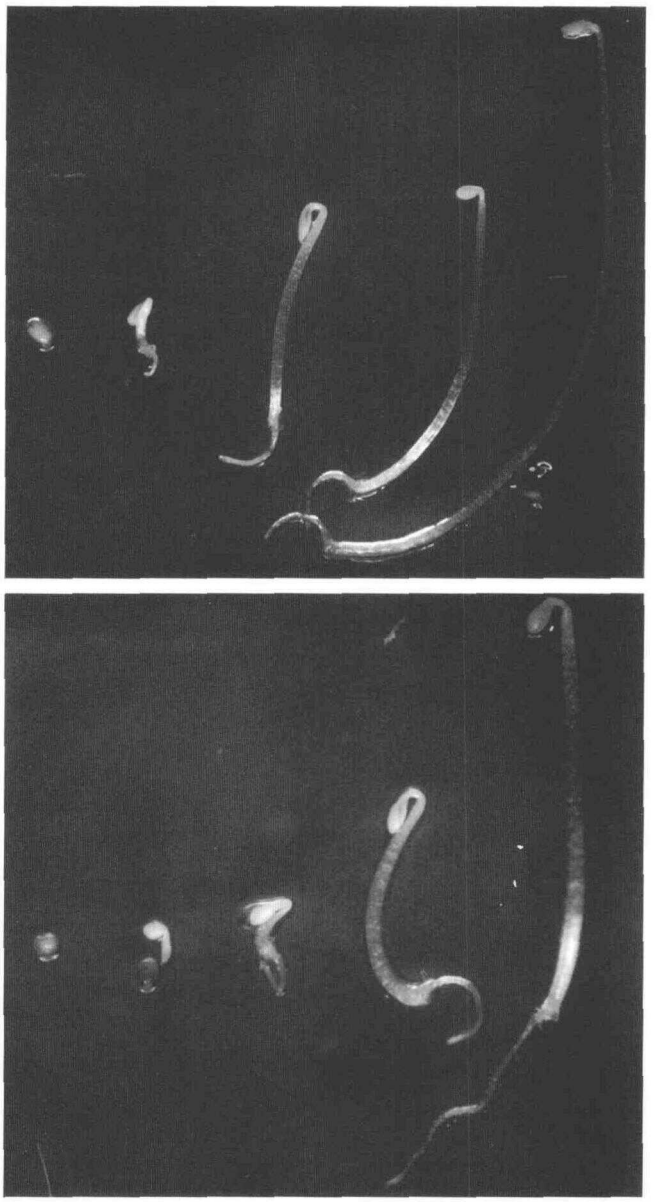

Figure 4. Effect of Suc on the appearance of etiolated seedlings. *A. thaliana* seedlings were etiolated for 1, 2, 3, 4, and 6 d in the absence (top) or presence (bottom) of 3% Suc and photographed.

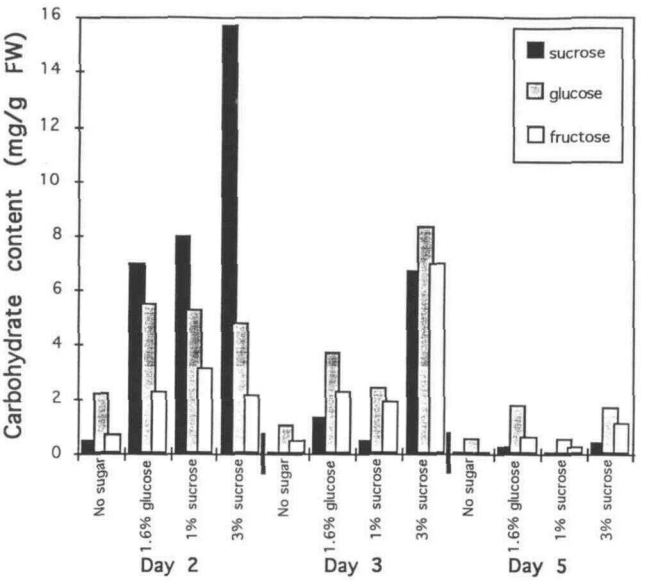

Figure 5. Effect of carbohydrates on sugar content in etiolated seedlings. Wild-type seedlings were grown for 2, 3, and 5 d in the dark, either in the absence of carbohydrates or in the presence of 1.6% (88 mM) Glc, 1% (29 mM) Suc, or 3% (88 mM) Suc. Carbohydrates were measured as described in "Materials and Methods." FW, Fresh weight.

Suc Represses *PC* Gene Expression in Light-Grown Seedlings

The transient activation of the *PC* promoter during etiolated seedling development can be a specific etiolation response or can be associated with development irrespective of the presence of light. Luciferase activity levels in light-grown transgenic pc1A2.7 seedlings increased 10-fold on d 2 and a large (>30-fold) increase was also found on d 2 for *PC* mRNA levels (Figs. 6A and 7). Both the increase in luciferase activity and mRNA level were transient. This transient activation of the *PC* promoter in the light could also be repressed by Suc as measured by luciferase activity in PC-LUC plants (Fig. 6A) and steady-state mRNA levels in wild-type plants (Fig. 7). *PC* mRNA abundance in light-grown plants was approximately 15-fold higher than in the dark (cf. Fig. 3 with Fig. 7), whereas luciferase-activity levels in the light were at most 2-fold higher than in the dark. From d 4 onward, no inhibitory effect of Suc on luciferase levels was observed. These experiments were also performed with plant line pc1B80A.3 with similar results (results not shown). In 35S-LUC control plants lines 35S3A and 35S11B luciferase activity increased 2- to 3-fold when germinated in the light but this increase was stimulated rather than repressed by the presence of Suc (Fig. 6B,

Table I. *Effect of carbohydrate treatment on luciferase activity in etiolated seedlings*

Luciferase activity was measured in etiolated seedlings grown for 5 d in complete darkness. Seedlings were grown in the absence of carbohydrates or in the presence of 88 mM Suc (3%), Glc, Fru, trehalose, mannitol, or 6.8% PEG 10,000. Transgenic plants transformed with either the PC-LUC construct (pc1A2.7) or the 35S-LUC construct (35S3A) were used. Luciferase activity is expressed as arbitrary units per microgram of protein. The amount of repression is expressed as the percentage of expression as compared to the untreated control.

Treatment	Plant Line pc1A2.7		Plant Line 35S3A	
	Luciferase activity	Percentage of control	Luciferase activity	Percentage of control
	units/μg protein		*units/μg protein*	
Control	8.1	100	20.2	100
Suc	1.2	15	17.1	85
Glc	3.8	47	22.4	111
Fru	6.0	74	18.6	92
Trehalose	6.3	78	13.4	66
Mannitol	7.3	90	11.9	59
PEG	7.1	88	13.3	66

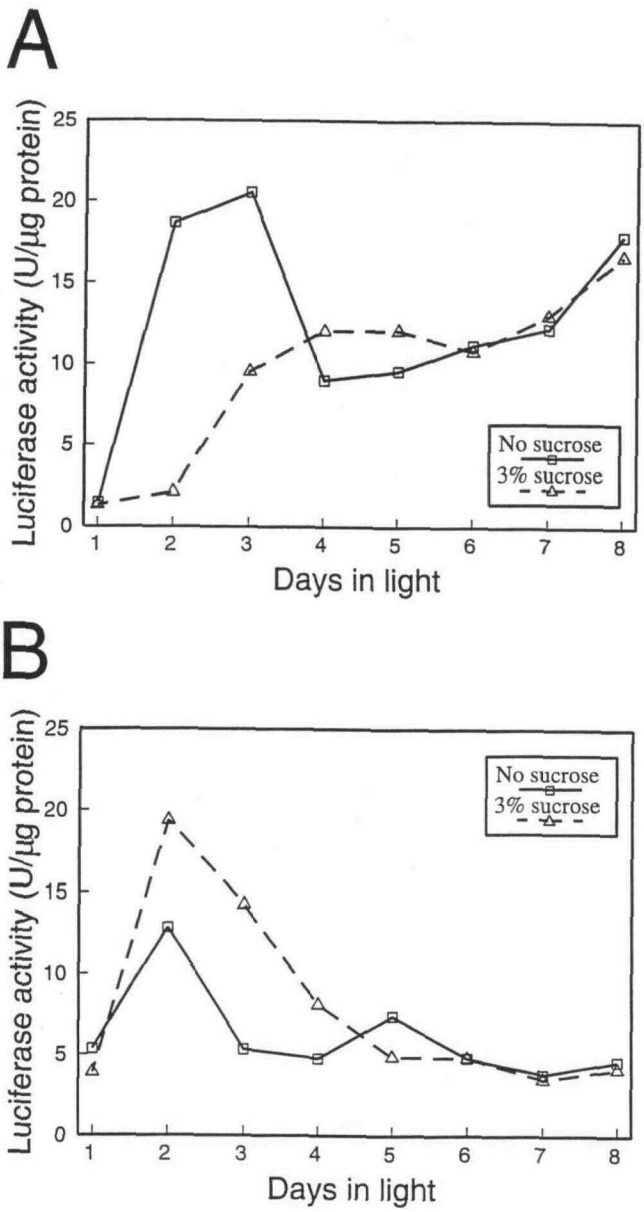

Figure 6. Effect of Suc on luciferase activity in light-grown transgenic plants. A, Luciferase activity was measured in seedlings grown for 1 to 8 d in continuous light. Plant line pc1A2.7, stably transformed with the PC-LUC gene fusion, was grown in the absence (□) or in the presence (△) of 3% Suc. Luciferase activity is expressed as arbitrary units (U) per microgram of protein. B, Plant line 35S3A, stably transformed with the 35S-LUC gene fusion, was treated as described in A.

line 35S3A). We conclude, therefore, that the transient activation of the *PC* promoter is developmentally controlled and probably independent of light.

DISCUSSION

Activity of the *PC* Promoter in Etiolated Seedlings

Germination is a major event in the plant life cycle and is associated with changes in gene expression. Here we describe a transient increase in *PC* gene expression during early postgerminative development. In green plants *PC* gene expression is light regulated (Vorst et al., 1993). The transient increase, however, was independent of light and was initiated when the cotyledons emerged from the seed coat. Transgenic PC-LUC lines also showed this increase in luciferase activity and we therefore conclude that the *PC* promoter can be activated in a light-independent way during early seedling development.

The transient increase in *PC* gene activity is not unique to this gene. In several plant species a similar increase has been found for other nuclear-encoded photosynthesis genes (Walden and Leaver, 1981; Fiebig et al., 1990). Also in Arabidopsis the nuclear-encoded photosynthesis genes encoding the Chl *a*/*b*-binding proteins (*CAB*) and the small subunit of ribulose-1,5-bisphosphate carboxylase (*RBCS*) were transiently expressed during etiolated seedling development (Brusslan and Tobin, 1992). These authors showed that this transient increase is not the result of the light treatment given to the seeds to promote germination, nor was a nonsynchronous circadian rhythm involved. A transient increase in expression of genes involved in photosynthesis could be part of a developmental program that is important in preparing the seedling for rapid biogenesis of the photosynthetic apparatus when the cotyledons emerge from the seed coat (Brusslan and Tobin, 1992).

Genetic analysis shows that for expression of many, if not all, light-regulated genes a derepression step is involved. Several genes have been identified that are involved in repression of light-induced genes, e.g. the *DET* and *COP* genes (Chory et al., 1989b, 1991; Deng et al., 1991; Wei and Deng, 1992; Hou et al., 1993). Such repressors may become active repressors of light-regulated genes relatively late during germination, thus allowing a brief period of activation of light-regulated genes. The transient activation is not confined to photosynthesis genes but is also observed in a *DET*1- and

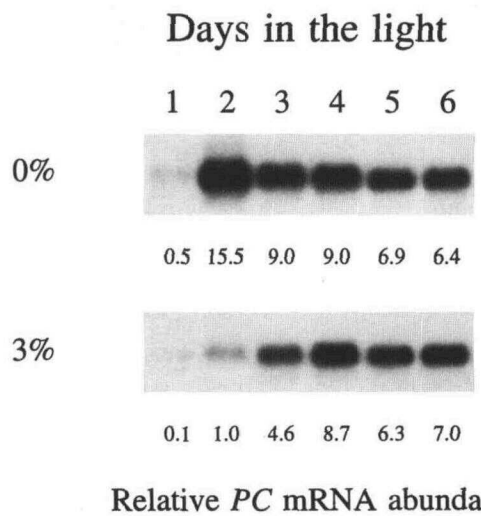

Figure 7. Effect of Suc on *PC* mRNA abundance in light-grown seedlings. Total RNA was isolated from wild-type Arabidopsis seedlings grown for 1 to 6 d in continuous light. Plants were grown in the absence (0%) or in the presence (3%) of 3% Suc. Values below each lane represent the band intensity. Total RNA (5 µg) was treated and band intensity was quantified as described in the legend to Figure 3.

*COP*1-repressed light-regulated homeobox gene that is expressed independently of chloroplasts (Quaedvlieg et al., 1995). However, the transient activation seems to be independent of the presence of light and could equally well be a transcriptional activator. This signal then gradually disappears after 3 d of growth both in the dark and in the light. Such a strategy would allow the plant to prepare optimally for growth in the light.

Repression of PC Promoter Activity by Suc

The developmentally controlled activation of the *PC* promoter in the PC-LUC plants was efficiently repressed by Suc in a concentration-dependent manner (Fig. 2A). The presence of 1% Suc was sufficient for repression but 3% Suc completely blocked an increase in luciferase activity. Between d 1 and d 4 *PC* mRNA levels also were repressed by Suc in a concentration-dependent manner. From d 5 to d 8, *PC* mRNA decreased to low levels in seedlings grown on a medium without Suc, which could be the result of energy depletion. When 1 to 3% Suc was present in the medium *PC* mRNA decreased with increasing Suc concentrations (Fig. 3). Thus, both luciferase activity and *PC* mRNA levels were repressed by Suc. Some differences in the luciferase and *PC* mRNA expression pattern, however, were observed (cf. Fig. 2A with Fig. 3). In PC-LUC seedlings, luciferase activity did not follow the observed decrease in *PC* mRNA abundance. This is probably due to the stability of the luciferase protein in the dark (Quandt et al., 1993). The substrate of luciferase, luciferin, has been shown to destabilize the luciferase protein (Millar et al., 1992). When the transgenic plants were germinated in the presence of luciferin, luciferase activity was transiently increased, like *PC* mRNA levels (results not shown). In light-grown plants luciferase activity more closely followed the pattern of *PC* mRNA levels even in the absence of luciferin (Figs. 6 and 7). Second, luciferase-activity levels were more sensitive to Suc than were *PC* mRNA levels. This indicates that not all control elements for the developmentally controlled increase of *PC* mRNA levels are present in the construct used. Such control elements may be present in the transcribed region or the 3′ UTR of the gene, which are absent in the PC-LUC construct. For example, Suc may stabilize *PC* mRNA and thereby decrease promoter effects.

Luciferase activity in PC-LUC seedlings was efficiently repressed by Suc and partially by equimolar amounts of Glc. Growth on Fru and the nonmetabolizable sugars trehalose and mannitol did not result in a major repression of *PC* promoter activity. A higher external osmotic pressure is not a signal in the regulation of *PC* gene expression, since PEG was not an effective repressor (Table I). Analysis of *PC* mRNA levels confirmed these conclusions, e.g. *PC* mRNA abundance was transiently increased in seedling grown on trehalose (results not shown). Analysis of in planta carbohydrate levels showed that Suc, Glc, and Fru can accumulate in the presence of external sugars (Fig. 5). The levels observed will mainly represent the intracellular location even though the precise location of maximal accumulation in the seedlings was not investigated. Addition of Suc or Glc to the growth medium led to high in planta levels of, especially, Suc and Glc. in planta sugar levels were highest on d 2 and decreased thereafter. On d 2 the seedlings emerged from the seedcoat and elongated rapidly in the following days. The increase in volume combined with biosynthetic activity may be responsible for this decrease in sugar levels. Seedlings possibly have insufficient sugar uptake capacity at this stage, since normally embryo-derived energy stores are used. Such selective or limited sugar uptake capacity may explain why Fru is not an effective inhibitor of luciferase activity in PC-LUC plants (Table I). Fru also did not efficiently repress an increase of *PC* mRNA levels on d 2 (results not shown).

The kinetics of *PC* mRNA accumulation were comparable in light- and dark-grown seedlings (Figs. 3 and 7), but in the light maximum mRNA levels were 15-fold higher than in the dark. The presence of Suc in the growth medium of light-grown plants repressed luciferase activity and *PC* mRNA levels during early seedling development (Figs. 6A and 7). We conclude, therefore, that Suc addition to the growth medium represses the developmentally controlled transient activation of *PC* promoter activity in a light-independent manner. It is unclear whether the light-derived signal and the Suc-derived signal interact in the control of *PC* gene expression.

In other systems sugars have been found to negatively affect expression of genes involved in photosynthesis (Sheen, 1990; Harter et al., 1993; Krapp et al., 1993; Jang and Sheen, 1994). In photomixotrophic cultures and protoplasts of rape, 2% Suc could repress light-induced *CAB* gene expression (Harter et al., 1993). Also, in autotrophic cell suspension cultures of *Chenopodium*, the addition of Glc led to a reversible decrease of the steady-state transcript levels of *RBCS*, *CAB*, and *ATP-δ* (Krapp et al., 1993). Treatments that increased the cytosolic carbohydrate content of intact tobacco and potato plants or spinach leaves led to a decreased *RBCS* transcript level (Krapp et al., 1993). Recently, an ethyl methanesulfonate-induced *Nicotiana plumbaginifolia* mutant was isolated, which was mapped to the *zea*3 complementation group that confers resistance to zeatine (Faure et al., 1994). This mutant requires a low carbon to nitrogen ratio for optimal growth. Growth of this mutant on 90 mM Suc (in addition to 10 mM nitrate) led to an approximately 5-fold higher in planta level of Suc, Glc, and Fru when compared to the wild type. Also here, higher carbohydrate levels in the seedlings resulted in a decreased *RBCS* mRNA level, whereas in the wild type the lower carbohydrate concentration coincided with an increased *RBCS* mRNA level.

It has been proposed that metabolic factors related to high carbohydrate content rather than carbohydrate concentration are the signal for repression of gene expression (Krapp et al., 1993; Graham et al., 1994; Sadka et al., 1994; Jang and Sheen, 1994). The expression of two genes that encode the glyoxylate cycle enzymes malate synthase and isocitrate lyase can be repressed by metabolizable carbohydrates in a cucumber cell culture (Graham et al., 1994). It is interesting that the nonmetabolizable carbohydrates Man and 2-deoxyglucose also repressed malate synthase and isocitrate lyase mRNA accumulation, whereas the non-

metabolizable sugars mannitol and 3-methylglucose did not repress gene expression. Since Man and 2-deoxyglucose can be phosphorylated and mannitol or 3-methylglucose cannot, it was proposed that intracellular concentration of hexose sugars or the flux of hexose sugars into glycolysis is the signal for regulating gene expression (Graham et al., 1994). In maize mesophyll protoplasts several promoters of photosynthesis genes were found to be repressed by various sugars in a transient expression system (Sheen, 1990; Jang and Sheen, 1994). Sugars that are a substrate for hexokinase caused repression of a maize *CAB* promoter, whereas Glc-6-P electroporated into protoplasts did not cause repression of promoter activity. Therefore, Jang and Sheen (1994) proposed that hexokinase may act as a key sensor and signal transmitter. For Suc-induced genes Sadka et al. (1994) proposed that sugar-responsive genes are activated in part by accumulation of sugar phosphates and the concomitant reduction of cellular phosphate levels. In this sugar-dependent, signal-transduction pathway, protein phosphatases also may be involved (Takeda et al., 1994).

Germination of Arabidopsis was strongly inhibited on medium containing 1 mM Man, irrespective of the presence of 5 mM phosphate (P.P. Dijkwel and S.C.M. Smeekens, unpublished data). In PC-LUC seedlings that were grown on Man concentrations that allowed germination (<0.5 mM), luciferase activity was not repressed (results not shown).

In conclusion, we show here that a developmentally regulated signal induces *PC* gene expression. This induction can be efficiently repressed by Suc. We are currently using a mutant approach to investigate whether the same mechanism that represses *PC* gene expression at the seedling stage also operates in controlling gene expression in mature plants. Similarly, we are interested in determining whether there is a relation between the transient activation of *PC* gene expression and light-controlled gene expression.

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