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Characterization and Light-Induced Expression of the *S*-Adenosylmethionine Decarboxylase Gene from *Ipomoea nil*

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We screened a genomic library of Japanese morning glory (*Ipomoea nil*) and isolated the *MGSDCg1* clone (GenBank Accession Number U64927). The genomic clone comprised four exons and three introns. *MGSDCg1* contained a 660-bp 5'-untranslated region (UTR), a 1089-bp coding region of 362 amino acids, and a 209-bp 3'-UTR. A TATA box sequence (TATATAA) was found at position −29. The transcript leader of this *SAMDC* gene contained a short, upstream open reading frame (uORF) of 51 amino acids, which was interrupted with a 107-bp intron. Two other introns were positioned upstream of the uORF in the 5'-UTR. Six-day-old seedlings were grown under a 12-h light/dark cycle, then treated with white light. The amount of *SAMDC* transcript dramatically increased from the first hour after light exposure, then returned to a basal level. *SAMDC* mRNA was accumulated more under high rather than low irradiance. These results suggest a relationship between photo-response and the physiological role of polyamines (PAs). The level of *SAMDC* mRNA was increased only by brassinosteroids, not by any other plant hormone such as kinetin, IAA, ABA, and GA. This hormonal response may have replaced the effect of light on *SAMDC* gene expression in the dark. PAs decreased the *SAMDC* mRNA level, which could then be restored by their inhibitors. This indicates that *SAMDC* gene expression is regulated by its cellular contents.

Keywords: brassinosteroids, light, polyamines, S-adenosylmethionine decarboxylase

The cellular polycationic polyamines (PAs) -- putrescine, spermidine, and spermine -- are ubiquitous in nature and are absolutely required for eukaryotic cell growth (Ha et al., 1998; Yamakawa et al., 1998). These small, basic molecules are thought to promote plant growth and development by activating the synthesis of nucleic acids and proteins (Walden et al., 1997). Different mechanisms maintain tight regulation of the PA cellular levels, and prevent its excessive accumulation in many cells and tissues. These controls include regulation at the level of gene expression; enzymatic activity at several steps in PA biosynthesis; enzyme stability; and PA degradation and excretion (Lee et al., 1997).

S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) is a key enzyme in PA biosynthesis. Its product, decarboxylated *S*-adenosylmethionine (SAM), serves as an aminopropyl donor in spermidine and spermine syntheses. Because the concentration of decarboxylated SAM is very low under physiological conditions, SAMDC probably constitutes a rate-limiting factor, as its availability may actually control the rate of PA formation (Marié et al., 1992). The SAMDC protein has a rapid turnover rate and represents a minor component of the cellular protein pool. Its expression is highly regulated by a variety of physiological, hormonal, and developmental stimuli (Lee et al., 1997).

With some photoperiodically sensitive plants, increases in PA titers occur very soon after an inductive cycle, well ahead of any morphogenetic changes. This is usually indicated by a titer increase (Galston et al., 1997). PAs are present in chloroplasts, thylakoid membranes, photosystem II membranes, and light-harvesting complex (Galston et al., 1997), suggesting a possible relationship between photosynthetic activity and a physiological role for PAs.

Several plant *SAMDC* genes have been cloned from potato (Taylor et al., 1992; Mad Arif et al., 1994), spinach (Bolle et al., 1995), *Catharanthus roseus* (Schröder and Schröder, 1995), *Tritordeum* (Dresselhaus et al., 1996), and carnation (Lee et al., 1997). SAMDC activity increases dramatically in the leaves of *Pharbitis nil* when lights are turned on (Hirasawa and Shimada, 1994; Kamachi and Hirasawa, 1995). A partial *SAMDC* gene isolated from *P. nil* has shown that this light-dependent increase in activity is concomitant with changes in the levels of *SAMDC* mRNA after lights-on, suggesting that the photo-response is regulated primarily at the transcriptional level (Yoshida and Hirasawa, 1998).

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To better understand the regulation of the *SAMDC* gene, we isolated and analyzed a genomic *SAMDC* clone from *Ipomoea nil*. We also examined whether this gene was regulated by light or by plant hormones.

MATERIALS AND METHODS

Plant Material

Seeds of Japanese morning glory (*I. nil* Choise cv Violet) were treated with concentrated sulfuric acid for 30 min, then washed extensively in running tap water. The seeds were allowed to swell in a flask containing distilled water while being stirred for two days. Afterward, they were germinated in vermiculite. The seeds were incubated in the dark for one day, then placed in a growth chamber at 25°C under a 12-h light/12-h dark photoperiod. Fully expanded leaves of plants grown under greenhouse conditions were used for extracting genomic DNA. To analyze gene expression under various conditions, hypocotyls were harvested from six-day-old, greenhouse-grown seedlings. After the plants were treated for certain time periods, the tissues were collected, frozen immediately in liquid nitrogen, and stored at −70°C until RNA extraction.

RNA Extraction and Analysis

Total RNA was extracted from the leaves with guanidine HCl, using the method described by Sambrook et al. (1989). Twenty micrograms of the total RNA was separated electrophoretically on 1% agarose gels containing 2.2 M formaldehyde, then transferred to a positively charged nylon membrane (Hybond-N+, Amersham, UK), and alkaline-fixed with 0.05N NaOH. The membranes were prehybridized at 42°C for 4 h in a solution containing 50% formamide, 0.25 M $NaHPO_4$, 10 mM EDTA, 0.25 M NaCl, 7% SDS, and 100 μg mL^{-1} of denatured salmon sperm DNA. Hybridization was carried out for 18 h in the same prehybridization solution after adding 5×10^5 cpm mL^{-1} of denatured ^{32}P-labeled cDNA probe. The morning glory *SAMDC* cDNA fragment was labeled using a Random Priming Labeling kit (Boehringer Mannheim, Germany) with [α-^{32}P] dCTP (3000 Ci $mmol^{-1}$), and was used as a probe. Following hybridization, the membranes were washed twice with 2× SSC containing 0.1% SDS for 15 min, and twice with a solution containing 25 mM $NaHPO_4$, 1 mM EDTA (pH 8.0), and 0.1% SDS for 15 min. Finally, the membranes were exposed to FUJI X-ray film with an intensifying screen at −70°C to provide an appropriate exposure. All washes were carried out at 52°C. Equal loading was tested by ethidium bromide staining, and major segments of each experiment were replicated.

Cloning of Partial *SAMDC* cDNA with PCR

Total RNA, isolated from the hypocotyls of six-day-old seedlings, was used for synthesizing the first strand of cDNA. PCR amplification performed using synthetic, degenerated oligonucleotide primers (forward primer, 5′-aa(GA)AC(GATC)TG(TC)GG(GATC)AC-3′; reverse primer, 5′-TTACTI(GC)(AT)(GA)TA(IATC)CC(GA)CA(GATC)GG-3′). These were designed with the region conserved among the *SAMDC* genes in carnation, potato (Taylor et al., 1992; Mad Arif et al., 1994), spinach (Bolle et al., 1995), and *C. roseus* (Schröder and Schröder, 1995). PCR was performed in a total volume of 100 μL that contained 1 μL of the first-strand cDNA reaction mixture, 1 μM of each primer, and 2.5 units of *Taq* polymerase. Thirty-five cycles were carried out, each comprising 1 min at 94°C, 1 min at 48°C, and 1 min at 72°C, in an automatic thermal cycler (Perkin-Elmer/Cetus, Norwalk, CT, USA). PCR products were separated on an agarose gel, and the expected 462-bp band was eluted and cloned into the pGEM-T vector. The insert-DNA sequence was analyzed to confirm the identity of this clone.

Cloning and Sequence Analysis of *SAMDC* Genomic Clone

A genomic library was constructed with the genomic DNA of fully expanded leaves, using a Lambda DASH II vector digested with BamHI (Stratagene, USA). To isolate the SAMDC clone, approximately 3×10^5 plaques were screened with the ^{32}P-labeled, randomly primed *Ipomoea* cDNA fragment. After the third round of screening, two clones (*p16-2* and *p4-4*) were isolated and subjected to gene mapping. Because the *p16-2* clone (3.5 kb) was overlapped with the 7.0-kb *p4-4* clone, the latter was identified as *MCSDCg1*. The 7.0-kb fragment, digested with XbaI and XhoI, was subcloned into the XbaI and XhoI sites of pBluescript KS. DNA sequencing was conducted according to the dideoxy chain termination method, using a Sequenase 2.0 DNA sequencing kit (US Biochemicals, USA). A series of nested deletions was created by the ExoIII/SI nuclease method (Erase-a-Base System, Promega, USA). Sequence analysis and computation were carried out with the DNAsis DNA connection program. The transcription start site was

determined via a primer extension experiment, in which the oligonucleotide 5′-GGAATAGCTTCTCA-GAGGTTCAC-3′ was used for reverse transcription against total RNA from the leaves. This transcription start site was also confirmed with the resulting 5′ RACE (Rapid Amplification of cDNA Ends) products (5′ RACE kit; Boehringer Mannheim, Germany). The promoter sequence was analyzed for putative *cis*-acting elements with the PLACE Signal Scan program.

Determination of SAMDC Activity

SAMDC activity was measured according to the procedure described by Park and Lee (1994). Crude tissue extract or the translated products served as enzyme sources, and was reacted with 0.1 μL of [carboxyl-^{14}C]SAM for SAMDC enzyme activity.

RESULTS

Isolation and Structural Characterization of *MGSDCg1* Gene

A genomic library from fully expanded *Ipomoea* leaves was screened with the probe of the PCR product, *MGSAMDC*. For further analyses, we chose the 7-kb *MGSDCg1* clone (GenBank Accession Number U64927). By comparing the sequence of *MGSDCg1* with the results of primer extension and RACE we determined that the genomic clone consisted of four exons and three introns (Fig. 1). The transcription initiation site (+1) also was identified through primer extension and 5′RACE, which defined the size of the 5′-untranslated region (UTR) of *MGSDCg1* as 660 nt long. In addition, the clone contained a 1089-bp coding region of 362 amino acids and a 209-bp 3′-UTR (Fig. 1). A TATA box sequence (TATATAA) was found at position −29 in the 5′ flanking region.

The 1089-bp coding region for SAMDC was not interrupted by introns. The transcript leader of this *SAMDC* gene contained a short uORF (upstream open reading frame) of 51 amino acids at position +1015 to +1277, which was interrupted with a 107-bp intron between +1150 and +1256. Another two introns were positioned upstream of the uORF in the 5′-UTR. The deduced amino acid sequence of *MGSDCg1* shared 68% identity with the *SAMDC* gene from carnation (GenBank Accession No. U38527), 65% with potato (GenBank Accession No. S74514) and 45% with *Tritordeum* (GenBank Accession No. X83881). However, the identity of the uORF at the amino acid sequence level, compared with uORFs from other species, was 80.4 to 96.1%.

Information about the sequence for the 1697-bp promoter was obtained from the 7.0-kb genomic DNA. The promoter region of *gMGSDCg1* was analyzed for plant *cis*-acting regulatory DNA elements using PLACE, a database of motifs for these elements in vascular plants. We found many putative light-responsive elements, e.g., GATA box, GT1 consensus motif, RBCS consensus motif, and I box core motif. In addition, the promoter region contained several putative *cis*-acting elements that induce responses to such environmental stresses as water, metal ions, low temperatures, and elicitors.

Light-Regulated Gene Expression of *SAMDC*

When six-day-old seedlings, grown under a 12-h light/dark cycle, were treated with white light, the amount of *SAMDC* transcripts was dramatically increased from the first 1 h after light exposure, then returned to a basal level after 8 h (Fig. 2A). The level of chlorophyll a/b-binding protein (*Cab*) transcripts also increased in the first 4 h after light exposure. Amounts of *SAMDC*

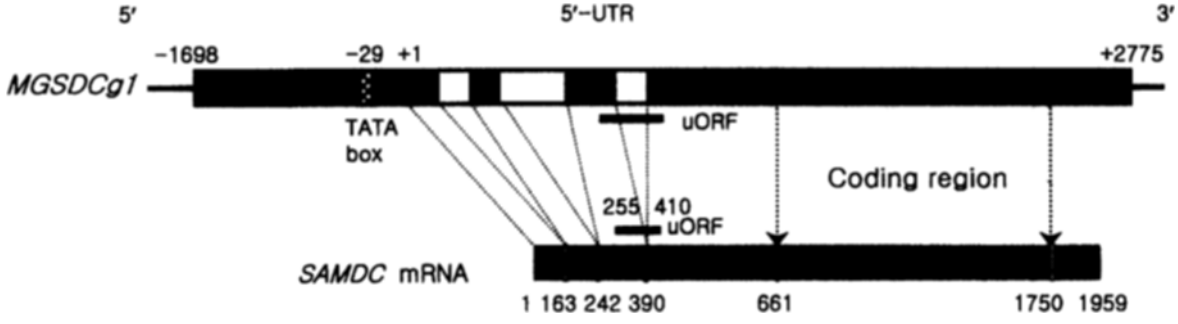

Figure 1. Structure of *SAMDC* genomic DNA and mRNA from *I. nil*. The exon and intron regions of *MGSDCg1* are presented as closed and open bars, respectively. +1 indicates a transcription start site.

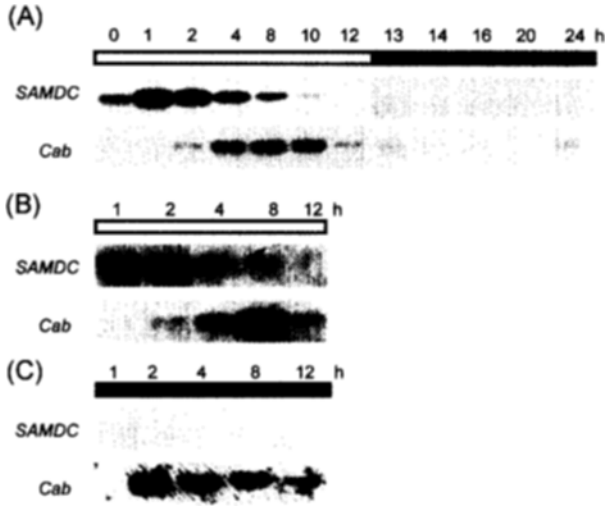

Figure 2. Changes in *SAMDC* mRNA accumulation in hypocotyls of *I. nil*. Six-day-old seedlings grown under a 12-h dark/light cycle were used for total RNA extraction **(A)**. Next day after dark period, seedlings were transferred to lights-on **(B)** or to continuous darkness **(C)**. Total RNA was extracted at pointed time intervals. Northern blot analyses were performed using ^{32}P-labeled probe of *MGSAMDC* and *Cab*, which was amplified with PCR primers based on the nucleotide sequence of Cab protein in GenBank database.

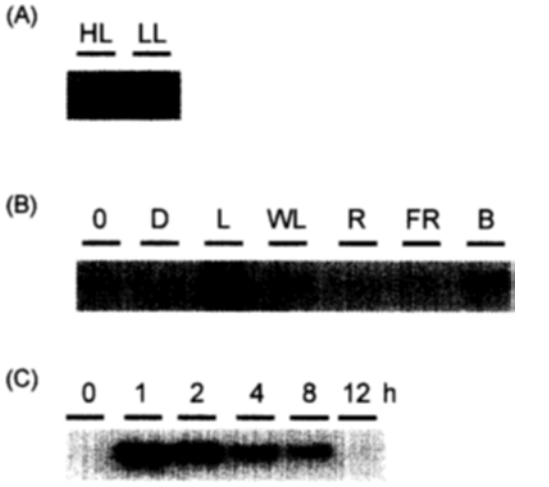

Figure 3. Effects of different wavelengths of light on *SAMDC* gene expression in hypocotyls of *I. nil*. **(A)** Six-day-old seedlings grown under 12-h dark/light cycle were irradiated for 1 h with high light (HL) and low light (LL), then harvested for total RNA extraction. **(B)** Six-day-old seedlings grown under 12-h dark/light cycle were exposed for 15 min to red light (15.5 W m^{-2}), far-red light (6.2 W m^{-2}), or blue light (2.2 W m^{-2}). White light was supplied by fluorescent lamps. Red light (R) was transmitted through 3-mm acrylic red filter, far-red light (FR) through 3-mm acrylic far-red filter, and blue light (B) through blue acrylic filter. Halogen lamps for red and far-red light were filtered with a 10-cm water layer. **(C)** Blue-light treatment using blue fluorescent lamp for 12 h. Total RNA was extracted at pointed time intervals after light exposure, then subjected to northern blot analysis.

and *Cab* transcripts did not change during the dark part of the cycle. The next day, these seedlings were treated with either darkness or light. Transcript levels of *SAMDC* and *Cab* accumulated dramatically after lights-on during the next day (Fig. 2B), but those for *SAMDC* did not increased in seedlings kept in darkness (Fig. 2C). However, the level of *Cab* transcript, which follows a circadian rhythm, accumulated during the first 2 h after the dark period. Under those conditions, the *Cab* transcript seemed to oscillate with a short period (Fig. 2C).

To determine the effect of light intensity, six-day-old seedlings were irradiated with either high (32.4 W m^{-2}) or low (5.8 W m^{-2}) light for 1 h. High irradiance caused more *SAMDC* mRNA to accumulate (Fig. 3A). The effect of a particular wavelength also was examined by exposing seedlings to far-red, red, or blue light. Hypocotyls were harvested after 15 min of light exposure, and total RNA was subjected to northern blot analysis. After blue-light exposure, the level of *SAMDC* transcripts increased to that found in seedlings treated with white light (Fig. 3B). Lengthy exposure to blue light at high irradiance caused the level of *SAMDC* mRNA in hypocotyls to increase rapidly, peaking at 1 h (Fig. 3C).

BR-Induced Gene Expression of *SAMDC*

To determine whether hormones induce *SAMDC* gene expression, six-day-old seedlings were treated in the dark for 1 h with 10^{-6} M of BR, kinetin, IAA, ABA, or GA_3. Northern blot analysis showed that only BR caused the level of SAMDC *mRNA* to increase (Fig. 4A). This accumulation was also more effective in light than in darkness (Fig. 4B), indicating a synergistic effect between BR and light. In fact, treating with BR in the darkness caused the *SAMDC* transcript to accumulate to the same level as that found with previously described light treatments (Fig. 4C). Therefore, BR may have replaced the effect of light on *SAMDC* gene expression when seedlings were kept in the darkness.

Effect of PA on Gene Expression of *SAMDC*

PA biosynthesis is regulated by feedback inhibition (Galston et al., 1997). To examine the effect of PA on

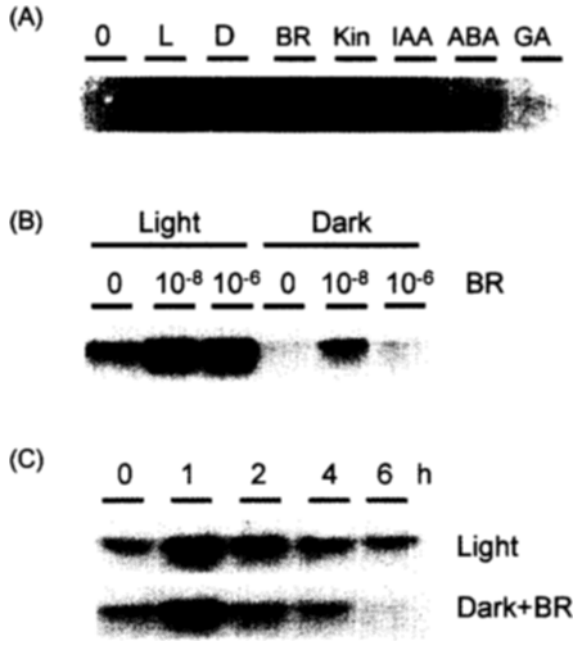

Figure 4. Effects of plant hormones on *SAMDC* gene expression in hypocotyls of *I. nil*. **(A)** Six-day-old seedlings grown under 12-h dark/light cycle were treated with plant hormones (BR, kinetin, IAA, ABA, and GA), for 1 h in darkness. **(B)** BR treatment for six-day-old seedlings with light or darkness. **(C)** Amount of *SAMDC* transcript determined for BR-treated six-day-old seedlings in darkness versus those seedlings treated in light without BR. Total RNA was extracted after 1h of hormone treatment, then subjected to northern blot analysis.

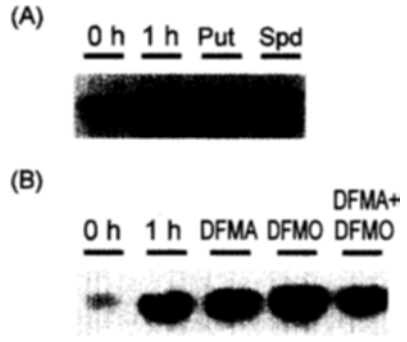

Figure 5. Effects of PAs on *SAMDC* gene expression in hypocotyls of *I. nil*. **(A)** Six-day-old seedlings grown under 12-h dark/light cycle were treated with 0.1 mM putrescine or spermidine. **(B)** Six-day-old seedlings grown under 12-h dark/light cycle were treated with 1 mM of inhibitors of PA biosynthesis -- difluoromethylarginine (DFMA) or difluoromethylornithine (DFMO). All seedlings were treated with the chemicals prior to 30 min of light exposure. Total RNA was extracted after 1 h of light exposure, then subjected to northern blot analysis.

the regulation of *SAMDC* gene expression, we treated six-day-old seedlings for 1 h with 0.1 mM of putrescine or spermidine. Both PA types caused a decrease in *SAMDC* mRNA levels compared with the controls (Fig. 5A). In addition, the irreversible inhibitors of PA biosynthesis, i.e., 2-difluoromethylarginine (DFMA) and 2-difluoromethylornithine (DFMO) (Lee et al., 1996), restored the amount of *SAMDC* transcript to that seen for control seedlings that had been treated with light for 1 h (Fig. 5B).

DISCUSSION

When six-day-old seedlings of *I. nil* were treated with white light, transcripts levels for *SAMDC* increased transiently after 1 to 2 h of exposure (Fig. 2A). This suggests that the enzymes for PA biosynthesis in plants may respond to light as an environmental factor. However, light-induced expression of the *SAMDC* gene could also be replaced by treating seedlings with BR in darkness (Fig. 4C). The light response of SAMDC activity produced a transient accumulation of PAs, especially spermidine (Yoshida and Hirasawa, 1998).

Cold temperatures can also affect SAMDC levels. Ndoye et al. (1994) showed that circadian regulation of PA levels in tomato was controlled primarily by temperature and only secondarily by light. Transcription of the *SAMDC* gene cloned from *P. nil* and from *Tritordeum* was also under circadian control. In the current study, however, the *MGSDCg1* cloned from morning glory did not exhibit circadian regulation under constantly dark conditions. It may be possible that another *SAMDC* gene exists for circadian regulation. In our Southern blot analysis, the *SAMDC* gene belonged to a small gene family comprising at least two genes (data not shown).

Many environmental stresses, e.g., UV, ozone, aluminum, air pollutants, low temperatures, salt, drought, heat shock, wounding, or pathogens, cause plants to produce of active oxygen species. Endogenous levels of PAs may also be induced by high osmotic pressure, low temperature, and low pH (Yamakawa et al., 1998). PAs can act as free radical scavengers, or they may interact with other molecules such as free ferulic and caffeic acids (Ye et al., 1997). Injury or other visible symptoms that result from low pH (Velikova et al., 2000), osmotic stress (Borrell et al., 1996), ozone (Ormrod and Beckerson, 1986), or pathogens (Yamakawa et al., 1998) could be prevented by supplying the plants with exogenous putrescine, spermidine, or spermine.

Therefore, the transient induction of *SAMDC* gene expression by light might, as an example, be responsible for preventing photo-oxidative damage in plants.

In our system, only blue light induced the accumulation of *SAMDC* transcript in *Ipomoea* seedlings (Fig. 3). Yoshida et al. (1999) have reported that short-term exposure to red light increases expression of the *SAMDC* gene; this induction is reversed by subsequent far-red light irradiation. Illuminating with blue light immediately after the initial red-light exposure can further increase *SAMDC* mRNA levels. Therefore, both the blue light photoreceptor- and the phytochrome-mediated pathways are involved in light regulation of the *SAMDC* gene. Although both pathways influence *SAMDC* gene expression, blue light is the dominant factor in modulating this expression (Yoshida et al., 1999). Because individual members of a gene family are differentially regulated by various mechanisms (Gao and Kaufman, 1994), it is possible that one clone, *MCSDCg1*, is responsible for the blue-light response, while another clone, perhaps present in morning glory, regulates the blue/red-light effect.

In the light, BR-deficient *Arabidopsis* mutants, such as *det2* and *cpd*, show strong dwarfism, with curly, dark green leaves. In the darkness, they have a de-etiolated phenotype with short hypocotyls and open cotyledons, characteristic of light-grown plants. Clouse and Sasse (1998) have shown that this phenotype can be rescued by applying brassinolide; other plant hormones, such as auxin and GA, have no effect. Likewise, BR-deficient mutants can accumulate light-regulated genes in the dark (Chory et al., 1991). For example, Asami et al. (2000) showed that in dark-grown *Arabidopsis*, morphological characteristics that were previously modified by brassinazole (an inhibitor of BR biosynthesis) were restored nearly to those of the wild type simply through brassinolide treatment. They also demonstrated that expression levels of light-regulated genes were significantly higher than those in dark-grown, untreated seedlings. In the light, however, levels did not differ between the brassinazole-treated and the untreated seedlings. A mutation that can abolish in-vivo activity of Det2 and lead to defects in light-regulated development may be ameliorated by application of BR (Li et al., 1996). In the current study, light-induced *SAMDC* gene expression was restored by applying BR in the dark (Fig. 4C); auxin, GA, cytokinin, and ABA had no such effect (Fig. 4A). Therefore, we suggest that BR biosynthesis is involved in the light-regulated pathway of *SAMDC* gene expression.

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LITERATURE CITED

Asami T, Min YK, Nagata N, Yamagishi K, Takatsuto S, Fujioka S, Murofushi N, Yamaguchi I, Yoshida S (2000) Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. Plant Physiol 123: 93-99

Bolle C, Herrmann RG, Olemüller R (1995) A spinach cDNA with homology to *S*-adenosylmethionine decarboxylase. Plant Physiol 107: 1461-1462

Borrell A, Besford RT, Altabella T, Masgrau C, Tiburcio AF (1996) Regulation of arginine decarboxylase by spermine in osmotically-stressed oat leaves. Physiol Plant 98: 105-110

Chory J, Nagpal P, Peto CA (1991) Phenotypic and genetic analysis of det2, a new mutant that affects light-regulated seedling development in *Arabidopsis*. Plant Cell 3: 445-459

Clouse SD, Sasse JM (1998) Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49: 427-451

Dresselhaus T, Barcelo P, Hagel C, Lörz H, Humbeck K (1996) Isolation and characterization of a *Tritordeum* cDNA encoding S-adenosylmethionine decarboxylase that is circadian-clock-regulated. Plant Mol Biol 30: 1021-1033

Galston AW, Kaur-Sawhney R, Altabella T, Tiburcio AF (1997) Plant polyamines in reproductive activity and response to abiotic stress. Bot Acta 110: 197-207

Gao J, Kaufman LS (1994) Blue-light regulation of the *Arabidopsis thaliana Cab1* gene. Plant Physiol 104: 1251-1257

Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero Jr RA (1998) The natural polyamine spermine functions directly as a free radical scavenger. Proc Natl Acad Sci USA 95: 11140-11145

Hirasawa E, Shimada A (1994) The photoresponse of S-adenosylmethionine decarboxylase activity in leaves of *Pharbitis nil*. Plant Cell Physiol 35: 505-508

Kamachi S, Hirasawa E (1995) Sensitivity to humidity and temperature in darkness of the photoinduction of S-adenosyl-L-methionine decarboxylase activity in leaves of *Pharbitis nil*. Plant Cell Physiol 36: 915-917

Lee MM, Lee SH, Park KY (1997) Characterization and expression of two members of the *S*-adenosylmethionine decarboxylase gene family in carnation flower. Plant Mol Biol 34: 371-382

Lee SH, Kim YB, Lee MM, Park KY (1996) The Stimulation of Arginine Decarboxylase Activity by α-Difluoromethyl Ornithine in Tobacco Suspension Cultured Cells. J Plant Biol 39: 107-112

Li J, Nagpal P, Vitart V, McMorris TC, Chory J (1996) A role for brassinosteroids in light-dependent development of *Arabidopsis*. Science 272: 398-401

Mad Arif SA, Taylor MA, George LA, Butler AR, Burch LR, Davies HV, Stark MJR, Kumar A (1994) Characterization of the S-adenosylmethionine decarboxylase (SAMDC) gene of potato. Plant Mol Biol 26: 327-338

Marié SC, Crozat A, Jänne OA (1992) Structure and organism of the human *S*-adenosylmethionine decarboxylase gene. J Biol Chem 267: 18915-18923

Ndoye M, Millet B, Paynot M, Martin-Tanguy J (1994) Diurnal modulation of polyamine content in seedlings of *Lycopersicon esculentum* Mill cv, Ace 55 by temperature and light. Plant Sci 104: 11-15

Ormrod DP, Beckerson DW (1986) Polyamines as antiozonants for tomato. Hort Sci 21: 1070-1071

Park KY, Lee SH (1994) Effects of ethylene and auxin on polyamine levels in suspension-cultured cells. Physiol Plant 90: 382-390

Sambrook, J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schröder G, Schröder J (1995) cDNAs for *S*-adenosylmethionine decarboxylase from *Catharanthus roseus*, heterologous expression, identification of proenzyme-processing site, evidence for the presence of both subunits in the active enzyme, and a conserved region in the 5′ mRNA leader. Eur J Biochem 228: 74-78

Taylor MA, Mad Arif SA, Kumar A, Davies HV, Scobie LA, Pearce SR, Flavell AJ (1992) Expression and sequence analysis of cDNAs induced during the early stages of tuberisation in different organs of potato plant (*S. tuberosum* L.). Plant Mol Biol 20: 641-651

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. Plant Sci 151: 59-66

Walden R, Cordeiro A, Tiburcio AF (1997) Polyamines: small molecules triggering pathways in plant growth and development. Plant Physiol 113: 1009-1013

Yamakawa H, Kamada H, Satoh M, Ohashi Y (1998) Spermine is a salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. Plant Physiol 118: 1213-1222

Ye B, Müller HH, Zhang J, Gressel J (1997) Constitutively elevated levels of putrescine and putrescine-generating enzymes correlated with oxidant stress resistance in *Conyza bonariensis* and wheat. Plant Physiol 115: 1443-1451

Yoshida I, Hirasawa E (1998) Photoinduction of arginine decarboxylase activity in leaves of *Pharbitis nil*. Phytochem 49: 2255-2259

Yoshida I, Yamagata H, Hirasawa E (1999) Blue- and red-light regulation and circadian control of gene expression of S-adenosylmethionine decarboxylase in *Pharbitis nil*. J Exp Bot 50: 319-326